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Transcriptome analysis reveals the gene expression changes in postharvest goji berry (*Lycium barbarum* L.) in response to hydrogen sulfide treatment

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Abstract

Hydrogen sulfide (H_2S) is recognized for its beneficial role in postharvest fruit and vegetable preservation. Postharvest goji berries are easy to mildew and rot which reduce the nutritional value, flavor, and shelf life. Hydrogen sulfide treatment could improve the quality attributes of postharvest goji berry (*Lycium barbarum* L.); however, it is not clear that the effects of H_2S at the transcriptional level in postharvest goji berries. Our data revealed that the differentially expressed genes (DEGs) of fresh goji fruits after H_2S treatment were different with different storage time. There were 523 DEGs in the three comparison groups after H_2S treatment on the 2nd, 4th and 6th day of storage. In the pathway of KEGG metabolic function, most of the DEGs were conserved in carbohydrate metabolism, secondary metabolites biosynthesis, amino acid metabolism, lipid metabolism, energy metabolism, the metabolic pathways of terpenoids and polyketones. The genes in the phenylpropanoid biosynthesis, flavonoid biosynthesis, starch, and sucrose metabolism pathway were selected and validated by quantitative real-time PCR (qRT-PCR). Our results provide insights into the effect of H_2S on postharvest goji berries at the transcriptional level and provide the basis for application of H_2S as gas regulator for preservation of goji berry.

Keywords: goji berry; hydrogen sulfide; transcriptome; DEGs; expression pattern.

Practical Application: Present results demonstrated that the mRNA expression of genes related to carbohydrate metabolism, secondary metabolites biosynthesis, amino acid metabolism, lipid metabolism, energy metabolism, and the metabolic pathways of terpenoids and polyketones in postharvest goji berry were regulated by H_2S treatment, which provided the basis for application of H_2S as gas regulator for preservation of goji berry.

1 Introduction

Goji berry (*Lycium barbarum* L) is a medicinal fruit, rich in many bioactive substances such as polysaccharides, carotenoids, betaine and phenolic compounds (Ma et al., 2022b), high in antioxidant active substances such as vitamin C and flavonoids (Shang et al., 2022). The effects of goji berry include promoting blood production, anti-aging, anti-cancer, regulating blood sugar and blood lipids, and protecting the liver, kidneys and eyesight (Jiang et al., 2021; Ma et al., 2022a; Yang et al., 2022). Goji berry is mainly produced in the northwest of China (Fan et al., 2023), due to the high temperature in summer, fresh goji fruit thin skin and juicy is not easy to store and limit the promotion of fresh goji fruit consumption (Elam et al., 2022; Xing et al., 2022).

H_2S is a novel gas transmitter involved in signal transduction (Wang et al., 2021). In recent years, H_2S has received increasing scholarly attention in the postharvest physiology of fruits and vegetables. H_2S has been applied to more than 20 kinds of fruits and vegetables such as peaches (Wang et al., 2022a), persimmon fruit (Niazi et al., 2021), sweet peppers (Muñoz-Vargas et al., 2020), tomatoes (Liu et al., 2020), and avocados (Joshi et al., 2020) for postharvest storage and preservation to extend shelf life (Lata et al., 2022). In our previous study, we found that H_2S retarded postharvest fruit and vegetable ageing mediated by various mechanisms such as activation of antioxidant enzyme, inhibition of fungal growth, inhibition of cell wall degradation, regulation of genes related to senescence, ethylene biosynthesis, ethylene signal transduction and respiratory and energy metabolism (Wang et al., 2022b; Wang et al., 2023).

RNA-seq techniques are widely used in food research to investigate the physiological and biochemical changes in transcript levels under different conditions (Xu et al., 2022; Wang & Zhao, 2022). Postharvest goji berries are easy to mildew and rot due to thin and juicy skin which reduce the nutritional value, flavor, and shelf life of fresh goji berries. Hydrogen sulfide treatment could improve the quality attributes of postharvest goji berry (*Lycium barbarum* L.) (Wang et al., 2023); however, there are no reports on the effects of H_2S at the transcriptional level in postharvest storage of goji berries. We aimed to investigate the effect of H_2S treatment on changes in gene expression levels in postharvest storage of goji berry. The transcriptomic and bioinformatics analyses revealed a significantly different response to H_2S in fresh postharvest goji fruit and lay a basic understanding of effects of H_2S treatment in fresh postharvest goji fruit.

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2 Materials and methods

2.1 Experimental design

Goji berries (*L. barbarum* L) were harvested from Ningxia Academy of Agriculture and Forestry Sciences, Goji Research Institute in Yinchuan, Ningxia, China. The treatment method of goji berries was referred to the previous study (Wang et al., 2023). Treatment with distilled water on different days 0, 2, 4 and 6 were recorded as CK0, CK2, CK4, and CK6, respectively, and treatment with 1.4 mM NaHS on day 2, 4 and 6 were recorded as T2, T4, and T6, respectively. After removal of the fruit stalks, the samples were stored at -80 °C until the next use, with three replicates of each treatment.

2.2 RNA extraction

For Library preparation and RNA-Seq technique, samples were outsourced at Hangzhou LianChuan Biotechnology (Hangzhou, China). After the extraction of total RNA of goji berries using Trizol reagent (thermofisher), its quantity and purity were analyzed through Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit (Agilent, CA, USA).

2.3 Sequencing, data processing, and assembly

RNA-seq was performed based on the method of Zhang et al. (2021). After the construction of cDNA library, the ends were repaired, A-tailed, and ligated to sequencing connectors. The purified cDNAs of 250-300 bp were screened with AMPure XP beads, amplified and purified again to obtain the libraries and the size of the latter was checked using an Agilent 2100 bioanalyzer. The selected libraries were pooled according to the effective concentration and the target downstream data volume and then sequenced by Illumina.

For the optimal quality and reliability of the data analysis, reads with joints, containing N, and low-quality reads were removed and the Q20, Q30, and GC content were calculated on the clean data. All subsequent analyses are based on the clean data for high quality analysis. The clean reads were compared and analyzed with the reference genome of goji using the HISAT package to obtain mapped reads for subsequent expression calculation. RSEM software was used to quantify gene expression. The FPKM could evaluate the abundance of genes and DEGs analysis was performed by DESeq2 software between two different groups (and by edgeR between two samples). The genes with the parameter of false discovery rate (FDR) below 0.05 and absolute fold change ≥ 2 were considered DEGs.

2.4 Enrichment and analysis of DEGs by GO and KEGG

All the DEGs were mapped to each GO database term and then significantly enriched GO entries in DEGs were identified compared to the whole genomic background. Corresponding DEGs to the KEGG database by KEGG enrichment analysis, significantly enriched pathways were identified in DEGs compared to the whole genomic background.

2.5 Expression validation of DEGs from RNA-Seq by qRT-PCR

After the cDNA was synthesized, the qRT-PCR was performed in 96-well plates using a LightCycler (Roche Diagnostics, Indianapolis, IN). For quantitative real-time PCR (qRT-PCR), the primers (Table 1) were designed using primer 5.0 software (Premier Biosoft International, Palo Alto, CA) and DNAMAN (Lyn nonBioSoft) according to the rigorous principle and the target gene expression (flavonoid biosynthesis, phenylpropane biosynthesis, starch and sucrose metabolism pathways) was normalized with actin 3 as reference gene. The forward primer sequence was: CCATCTACGAGGAGGGTTACGCTTTG; the reverse primer sequence was: AGTCAAGAGAGCCACATAGGCAAGC. The qRT-PCR procedure was as followed: pre- denaturation at 95 °C for 5 min, denaturation at 95 °C for 40 times for 10 s, annealing at 60 °C for 30 s, extension at 72 °C for 32 s, 40 cycles. The reaction system constituted of 20 μL, containing 1 μL cDNA, 0.5 μL forward and reverse primers (10 μmol), 10 μL UItraSYBR mixture and 8 μL ddH_2O. Relative expression was calculated using (Jiang et al., 2020). The $2^{-\Delta\Delta Ct}$ method for different storage periods of postharvest goji berries. Data are expressed as mean ± standard error (SE) (n = 3).

Table 1. List of primers used in the present study.

Gene ID	Gene Name	Forward	Reverse
CCG051659	*FLS*	ATCCAGCATGTCCTAGACCTGACC	GCCAACTGAGCTGCCTTTACCC
CCG010964	*ANS*	TGCCCTCAACCTGAACTTACAATGG	TGCCTTGATGCTGAAGTTGGAGAC
CCG020767	*CYP75B1*	CGGCTCGTTATGGAATCTGACCTG	CGTTGATCTCACAGCTCTCGGATG
CCG019005	*HCT*	CCACTGTTCTGTGTCTCGATCAAGG	CGTCTCGTCCACATCCATAATACCC
CCG036842	*CCOMT*	ATCAGCAGATGAAGGGCAGTTCTTG	GAATAGCAAGGGCAGTAGCAAGGAG
CCG035502	*4CL*	CTTTCAGGTCGCACCAGCAGAG	ACCTCACCAGCCTCAGCATCAG
CCG052659	*CAD*	GGGGACACAGTTGGAGTTGGATTG	GGCTTGCCGTCAGTGTAGACATC
CCG029725	*PAL*	TCAAGAACACAGTGAGCCAAGTAGC	AATAGGTATTCACGGTCCACGATGC
CCG030749	*CCR*	CATTTGACGCTGTGGTTGATGGATG	TGGCACATGAACCGAGAAGATTGAG
CCG035385	*bamyL*	CACTGGCACTACGGAACAAGGTC	AGCATCTGAGCAATGGGAAGGTAAC
CCG002534	*GN1_2_3*	GAAGATCAGGCTCATCCAACACCAG	ATTGTGCGTCCGTTGCTTCTACC
CCG049222	*otsB*	CACAAATGACATCACCGGAGCAAAG	GCAGGCGTGGGTAGTGTCTTAAC
CCG037659	*bglX*	GTGTCAGCCGATGGTCTAGTCATTG	AAGAAGCGTGCCCACCAAGATG
CCG044234	*malZ*	GGGCAATTAAAGGAGGTGGTGGTG	ACAACAGCGTCGGTAGCATTCTG

3 Results

3.1 Transcriptome sequencing and assembly of goji berry after H_2S treatment

To investigate the effect of H_2S treatment on postharvest goji berries, we extracted fresh fruit RNA and constructed cDNA libraries, which were sequenced by Illumina Hiseq 4000 sequencer with high throughput. The average sample yielded approximately 4.5 × 107 valid reads per set, with an efficiency rate of 96.81%. 99.95%, 97.91%, and 42.00% for Q20, Q30 and GC content (%) were obtained (Table 2). The data indicated that the samples were sequenced with good transcriptome quality and the data quality met the criteria for subsequent transcriptome expression profiling.

3.2 Identification and analysis of DEGs of goji berry after H_2S treatment

To examine the overall DEGs in fresh goji fruit after H_2S treatment, we examined the number of DEGs in three different groups, T2 vs CK2, T4 vs CK4 and T6 vs CK6. In the pre-harvest period (T2 vs CK2), there were a total of 2919 DEGs in T2 vs CK2 (Figure 1A, 1D), of which 1288 were up-regulated and 1631 were down-regulated. In the middle of the storage period (T4 vs CK4), DEGs in T4 vs CK4 were the lowest, with 1884 DEGs (Figure 1B, 1D), of which 379 were up-regulated and 1505 were down-regulated. The highest number of DEGs was found at the later stage of storage of fresh goji fruit after H_2S treatment (T6 vs CK6), with a total of 4339 DEGs in the T6 vs CK6 group after H_2S treatment (Figure 1C, 1D), of which 1543 were up-regulated and 2796 were down-regulated. Our data speculated the higher number of down-regulated genes compared to up-regulated genes in all the three groups, with a total of 523 DEGs (Figure 1E); this is consistent with the pattern of regulation of secondary metabolite accumulation and metabolism in postharvest storage of goji fruit treated with H_2S. This implied that the main accumulation periods of H_2S regulated secondary metabolite products was in the early and late stages of storage.

3.3 GO analysis of DEGs of goji berry after H_2S treatment

GO mainly represents the gene functions covering the biological, cellular and the molecular processes. We presented the top 25, 15, and 10 GO terms with the highest abundance of DEGs in these three categories, respectively. In T2 vs CK2 group (Figure 2A), T4 vs CK4 group (Figure 2B), T6 vs CK6 group (Figure 2C), and three comparison groups (Figure 2D), the representive biological subcategories included transcription regulation, DNA-templated, transcription, DNA-templated, defense response, protein phosphorylation, oxidation-reduction process, and secondary metabolite biosynthetic process. The major cellular subcategories included nucleus, plasma membrane, cytoplasm, integral component of membrane, membrane,and extracellular region. The major molecular subcategories included protein serine/threonine kinase activity, protein binding, DNA-binding transcription factor activity, ATP binding, DNA binding, and oxidoreductase activity. The GO enrichment analysis of the DEGs in goji fruit on days 2, 4, and 6 after H_2S treatment showed slight differences in the number of DEGs in each comparison group, but the gene enrichment results were generally consistent.

Table 2. Statistical analysis of the transcriptome sequence data of goji berry after H_2S treatment.

Sample	Raw reads	Valid reads	Valid ratio (%)	Q20 (%)	Q30 (%)	GC content (%)	Total mapped	Mapped ratio (%)
CK0_1	49589754	47909512	96.61	99.95	98.00	42.00	44161857	92.18
CK0_2	44772464	43484110	97.12	99.97	97.93	42.00	40368845	92.84
CK0_3	45217248	43847274	96.97	99.94	97.76	42.00	40572152	92.53
CK2_1	47331324	45958042	97.10	99.97	98.76	42.00	43645325	94.97
CK2_2	40815696	39694690	97.25	99.96	97.77	42.00	37146225	93.58
CK2_3	46333056	44847222	96.79	99.96	97.90	42.00	41966195	93.58
CK4_1	47693246	46278796	97.03	99.95	97.8	42.00	42638140	92.13
CK4_2	46862762	45440726	96.97	99.95	97.88	42.00	41884355	92.17
CK4_3	48386096	46880154	96.89	99.95	97.81	42.00	43165260	92.08
CK6_1	48662408	46857934	96.29	99.95	98.00	42.50	43869436	93.62
CK6_2	43664202	41917970	96.00	99.96	98.04	42.00	39238448	93.61
CK6_3	43823028	42227484	96.36	99.94	97.83	42.00	39454114	93.43
T2_1	52140198	49622438	95.17	99.94	97.54	42.00	45998900	92.70
T2_2	38432558	37369670	97.23	99.94	97.82	42.00	34901884	93.40
T2_3	44988316	43697602	97.13	99.94	97.80	42.00	40803991	93.38
T4_1	37791828	36658076	97.00	99.94	97.80	42.00	34253749	93.44
T4_2	40776912	39538372	96.96	99.94	97.78	42.00	36911315	93.36
T4_3	54880618	53278578	97.08	99.95	97.96	42.00	49881127	93.62
T6_1	46359194	44885504	96.82	99.94	98.01	42.00	41194007	91.78
T6_2	45716090	44415766	97.16	99.96	97.91	42.00	40754109	91.76
T6_3	40239906	39068242	97.09	99.94	98.04	42.00	35843214	91.75

Q20 and Q30 respectively represent 1% and 1 ‰ of base error probability; GC content represents the proportion of base G and C.

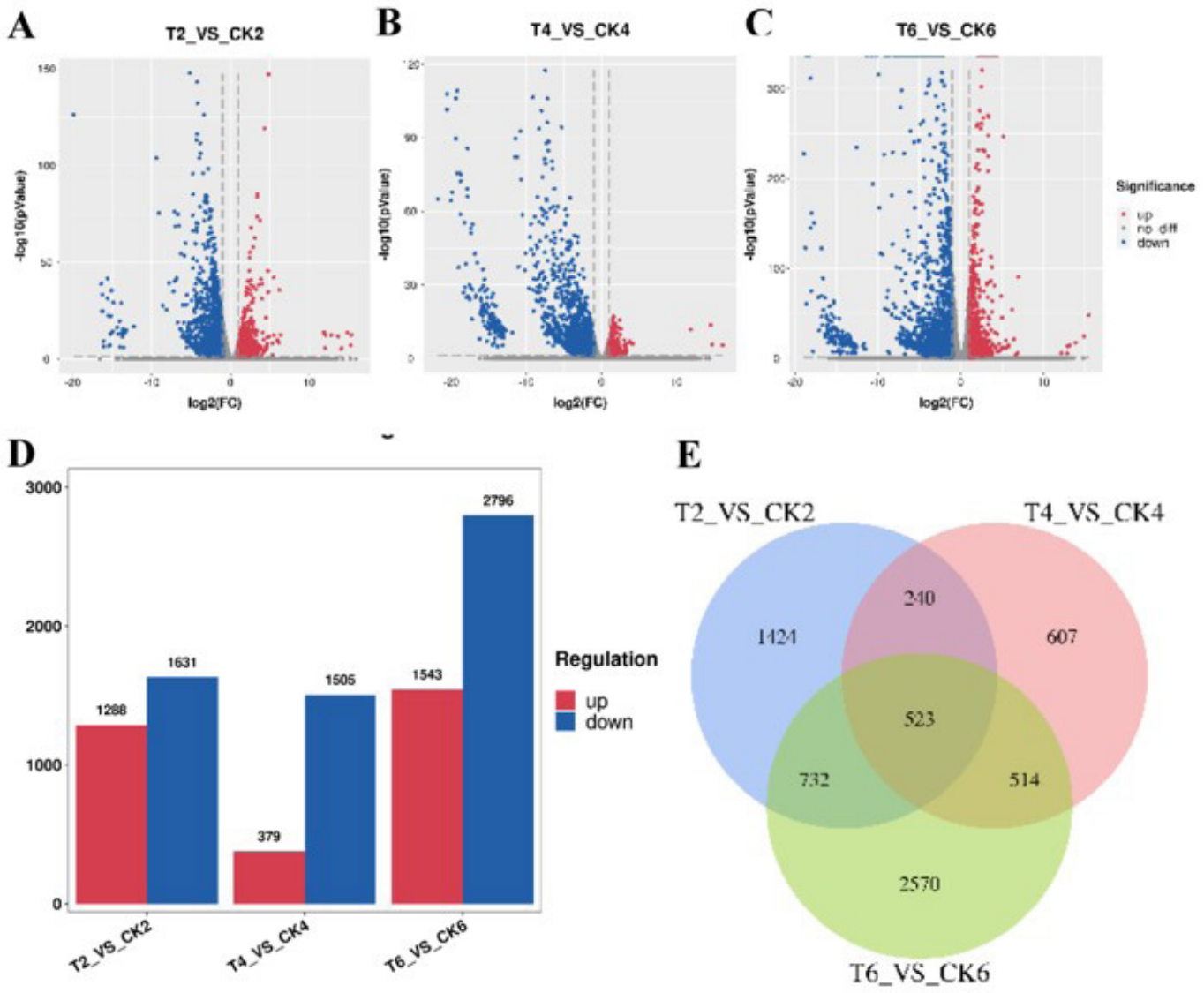

Figure 1. Volcano plot of DEGs in T2 vs CK2 (A), T4 vs CK4 (B), T6 vs CK6 (C), DEGs histogram (D), and venn diagram of shared DEGs (E) of goji berry after H_2S treatment. The horizontal ordinate (fold change of gene expression) and the vertical ordinate (Statistical significance of the change). The up-regulated genes are represented by red point and the down-regulated genes are represented by blue points; whereas, the grey points represent the genes without differential expression. Q20 and Q30 respectively represent 1% and 1 ‰ of base error probability; GC content represents the proportion of base G and C.

3.4 KEGG pathway analysis of DEGs of goji berry after H_2S treatment

KEGG pathway analyses provide databases of gene function and integrated metabolic pathway queries. To gain insight into gene function, DEGs were annotated into metabolic pathways for analysis. In the T2 vs CK2 group (Figure 3A), T4 vs CK4 group (Figure 3B), T6 vs CK6 group (Figure 3C), and three comparison groups (Figure 3D), the number of DEGs in the metabolic pathways varied at different days of storage, but the classification of metabolic pathways was generally consistent. Among the pathways of metabolic function, most of the genes were differentially expressed in carbohydrates metabolism, secondary metabolites biosynthesis, amino acid, lipid, and energy metabolism, and terpenoids and polyketides; among environmental signaling processes, the most DEGs were differentially altered in signal transduction; and among genetic information transfer processes, the most DEGs were associated with folding, rearrangement, and degradation.

3.5 KEGG pathway enrichment analysis of DEGs of goji berry after H_2S treatment

Pathway significant enrichment analysis uses pathway as the unit in the KEGG database to analyze whether *DEGs* are significantly different in metabolic pathways. The most influential metabolic pathways were screened according to the proportion of DEGs in the metabolic pathways in goji fruit after H_2S treatment, and the most influential pathways differed according to the number of days of treatment. On day 2 of H_2S

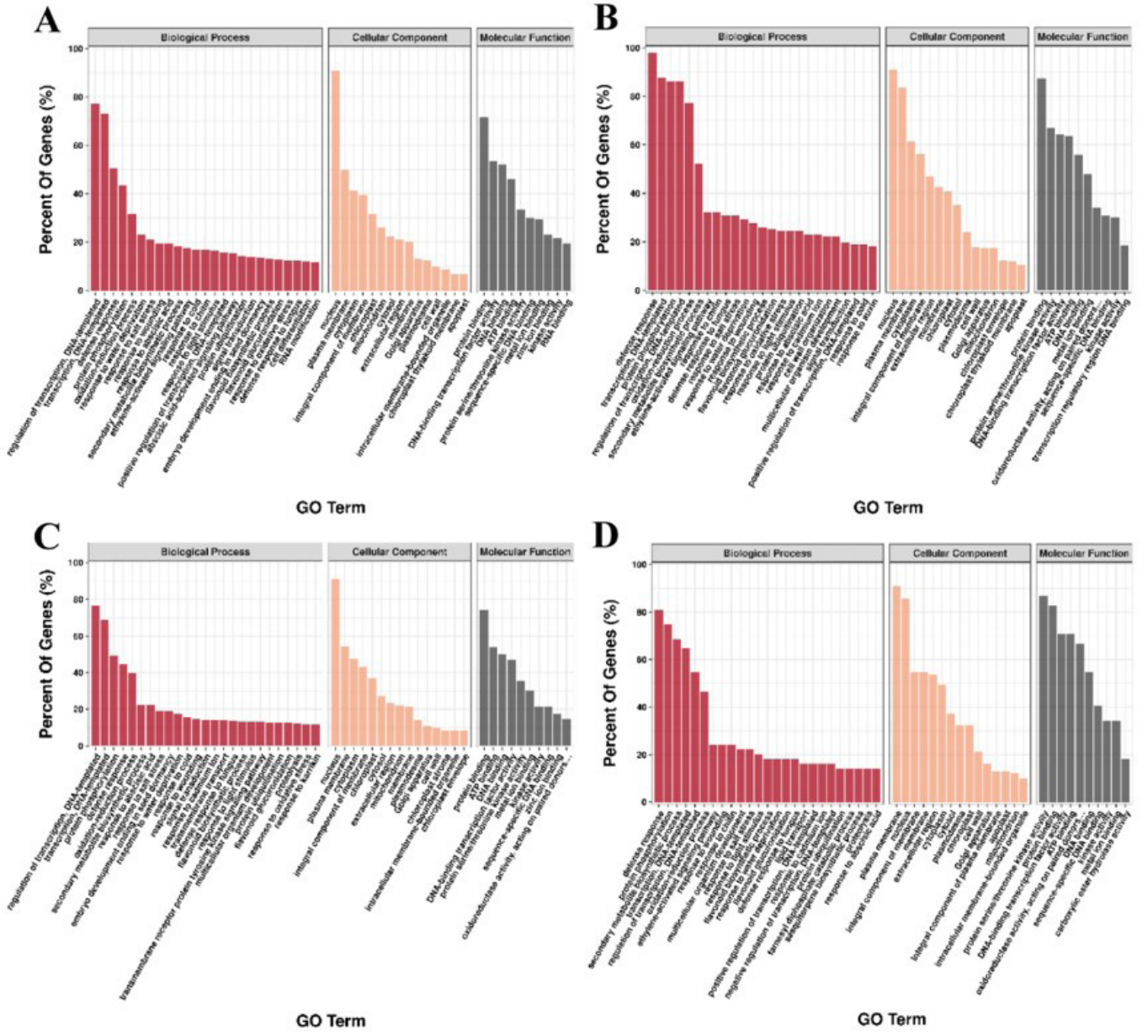

Figure 2. DEGs of Gene Ontology (GO) enrichment of H_2S treatment on the postharvest goji berries transcriptome in groups T2 vs CK2 (A), T4 vs CK4 (B), T6 vs CK6 (C), and three comparison groups (D). The X-axis correlates to various gene function, and the Y-axis depicts the number of DEGs.

treatment, in T2 vs CK2 group (Figure 4A), the pathways of photosynthesis, photosynthetic antenna protein, carotenoid biosynthesis, α-linolenic acid metabolism and phenylpropanoid biosynthesis metabolism DEGs accounted for a larger proportion of genes in the pathway after H_2S treatment; On day 4 of H_2S treatment, in the T4 vs CK4 group (Figure 4B) isoflavone biosynthesis, photosynthetic antenna protein, photosynthesis, phenylpropanoid biosynthesis, and phenylalanine metabolism pathways differentially expressed genes accounted for a greater proportion of genes in the pathway after H_2S treatment; At day 6, in the T6 vs CK6 group (Figure 4C), isoflavone flavonoid biosynthesis, carotenoid biosynthesis, flavonoid biosynthesis, starch and sucrose metabolism, and unsaturated fatty acid biosynthesis pathways accounted for a greater proportion of genes in the pathway after hydrogen sulfide treatment. In the three comparison groups on days 2, 4, and 6 after H_2S treatment (Figure 4C), isoflavone biosynthesis, carotenoid biosynthesis, flavonoid biosynthesis, starch and sucrose metabolism, unsaturated fatty acid biosynthesis, galactose metabolism, plant-pathogen interactions, and butyrate metabolism accounted for a larger proportion of genes in the pathway.

3.6 Verification of DEGs of goji berry after H_2S treatment with qRT-PCR

To verify the reliability of the RNA-seq data from goji fruit after H_2S treatment, 14 candidate DEGs were selected for qRT-

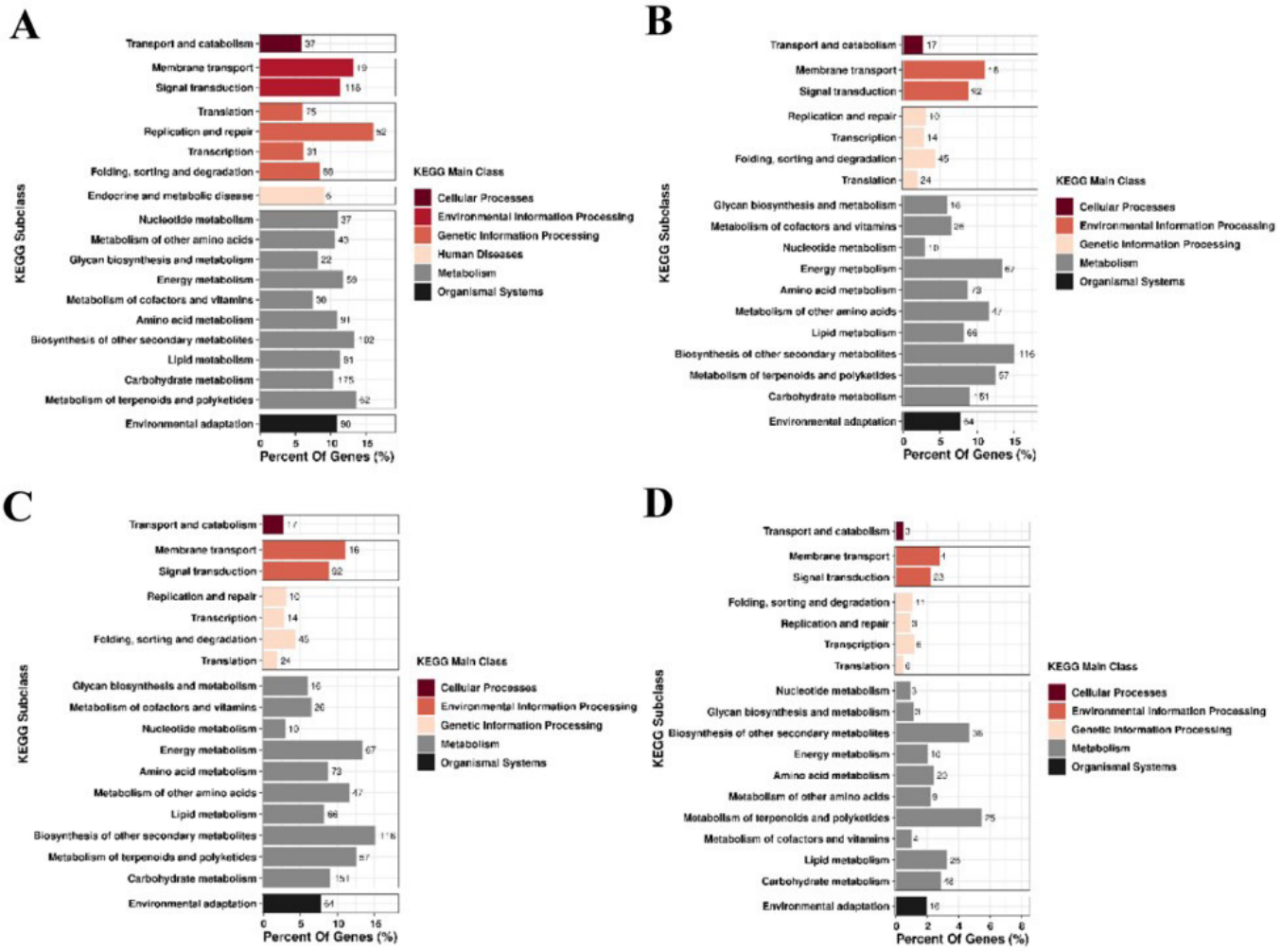

Figure 3. The KEGG classification of DEGs, H_2S treatment on the postharvest goji berries transcriptome in groups T2 vs CK2(A), T4 vs CK4(B), T6 vs CK6(C), and three comparison groups (D). The number of genes annotated and proportion of the number of DEGs annotated to the total number of genes into the pathway are represented by x-axis whereas, the name of the enriched KEGG pathways are represented by y-axis. KEGG analysis was performed using KEGG database (Kyoto Encyclopedia of Genes and Genomes, 2022).

PCR analysis. The selected DEGs were mainly associated with the phenylpropanoid biosynthesis, flavonoid biosynthesis and starch and sucrose metabolism pathways. These results revealed the consistency of qRT-PCR results with the transcriptome, confirming the accuracy and reliability of the sequencing data and revealing significant differences in these DEGs in fresh goji fruit after H_2S treatment (Figure 5).

FLS (flavonol synthase), *ANS* (anthocyanin synthase), *CYP75B1* (flavonoid 3'-monooxygenase), *HCT* (mangiferyl o-hydroxycinnamoyltransferase), *CCOMT* (caffeoyl coenzyme A O-methyltransferase), *4CL* (4-coumaric acid-CoA ligase), *CCR* (cinnamoyl coenzyme A reductase), *CAD* (cinnamyl alcohol dehydrogenase) and *PAL* (phenylalanine deaminase) genes in the flavonoid biosynthesis and phenylpropane biosynthesis pathways were all upregulated following H_2S treatment.4CL, PAL, FLS, and ANS are key enzymes in the synthesis of benzene is anthocyanins and flavonoids (Sharma et al., 2019), and H_2S treatment up-regulated the expression of key genes in phenylpropanoid biosynthesis and flavonoid biosynthesis in fresh goji fruit after harvest, thereby increasing the antioxidant activity of goji berries.

Five genes in the starch and sucrose metabolic pathways, *bglX* (β-glucosidase), *malZ* (α-glucosidase), *bamyL* (β-amylase), *GN1_2_3* (glucan endo-1,3-β-glucosidase) and *otsB* (alglucose-6-phosphate phosphatase), were all down-regulated in gene expression following H_2S treatment. And *bglX* and *bamyL* are key enzymes for starch and cell wall metabolism. H_2S treatment down-regulated the expression of key enzymes for starch and cell wall metabolism in postharvest goji berries, thereby inhibiting starch and cell wall metabolism and delaying postharvest fruit softening.

3.7 Correlation analysis of DEGs transcription of goji berry after H_2S treatment

The transcriptional correlation between the H_2S treatment of postharvest goji (Figure 6) showed that the expression of

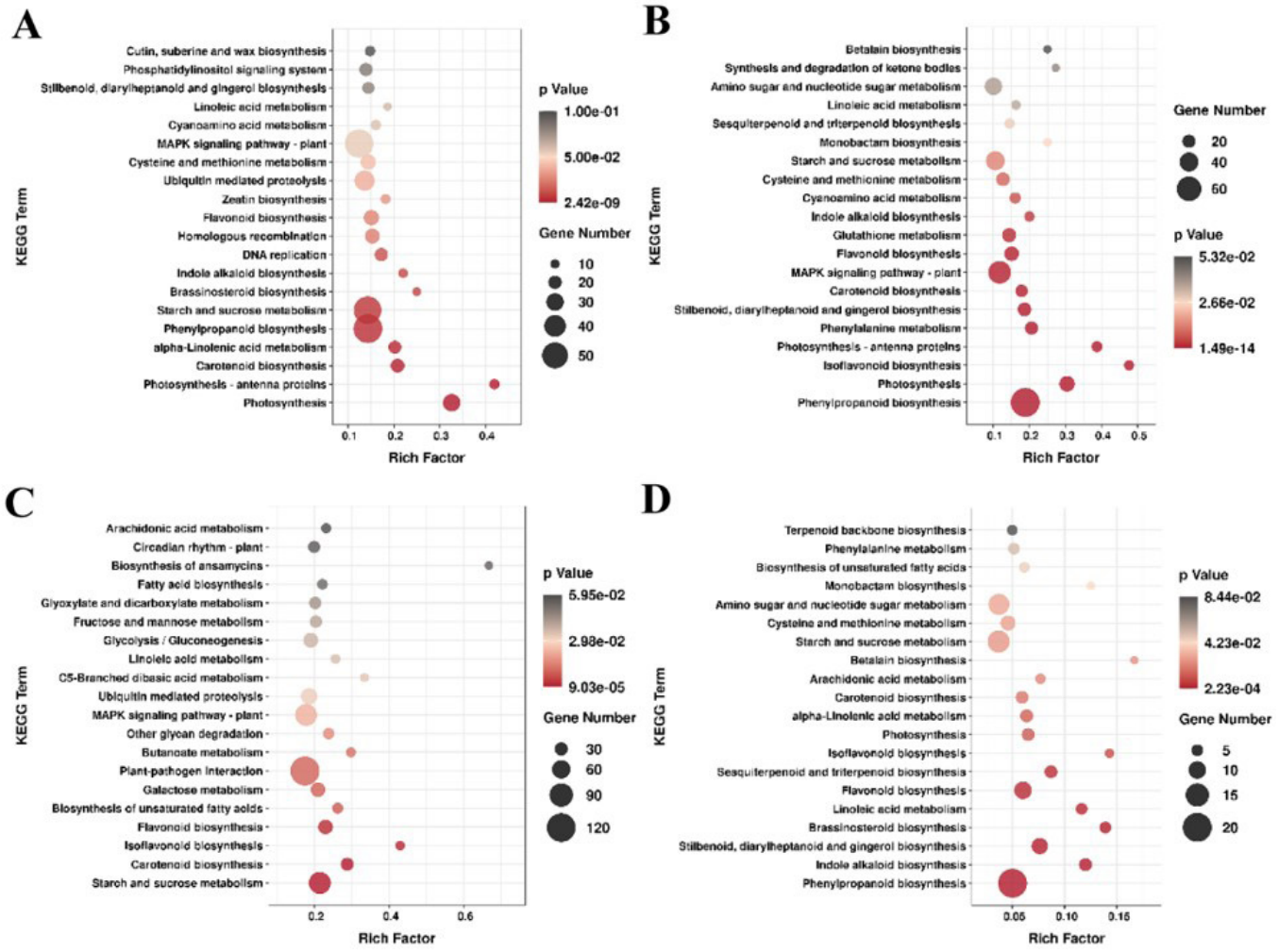

Figure 4. The KEGG enrichment of DEGs. H_2S treatment on the postharvest goji berries transcriptome in groups T2 vs CK2 (A), T4 vs CK4 (B), T6 vs CK6 (C), and three comparison groups (D). The x-axis represents the rich factor of DEGs. The y-axis represents the name of the enriched KEGG pathways.

FLS, ANS, CYP75B1, HCT, CCOMT, 4CL, CCR, CAD, and *PAL* genes in flavonoid biosynthesis and phenylpropane biosynthesis pathways were positively correlated. On the other hand, H_2S treatment was negatively associated with the expression of *bglX, malZ, bamyL, GN1_2_3* and *otsB* genes in the starch sucrose metabolic pathway.

4 Discussion

The complexity of biosynthesis of flavonoids in plants reveals not only the involvement of the biosynthetic pathway of phenylpropanoid but also associate several other secondary metabolic pathways of significance, such as the flavonoid metabolic pathway, terpene biosynthetic pathway, phytohormone signaling pathway, plant circadian pathway, starch and sucrose metabolic pathway (Hu et al., 2020). Phenylpropane biosynthesis converts L-phenylalanine into a variety of aromatic compounds, namely phenols, including styrene, coumarins, flavonoids, anthocyanins, stilbenes, hydroxycinnamic acid and macromolecular lignin (Yan et al., 2021). This array of aromatic compounds plays an important physiological function in plant growth, development and plant-environment interactions. Flavonoids and anthocyanins act as disease-resistant, antioxidant or UV-absorbing compounds, protecting plants from biotic and abiotic stresses (Liu et al., 2021). PAL, 4CL, ANS and FLS play important role in the biosynthesis of flavonoids (Wang et al., 2019). Several key enzymes in the flavonoid biosynthesis pathway, such as PAL, 4CL, ANS and FLS were significantly up-regulated in postharvest goji berries treated with H_2S. H_2S activated the key enzymes involved in the biosynthesis of flavonoids in goji berries, thereby increasing the flavonoid and anthocyanin content of goji berries, improving the antioxidant activity of goji berries and delaying postharvest senescence and decay. Ni et al. (2016), Lv et al. (2022) study found that H_2S treatment increased the ascorbic acid, flavonoids and total phenolic content of postharvest grapes and jujubes, and improved antioxidant activity. Dawood et al. (2021) study was shown that H_2S enhanced artichoke seedlings phenylalanine deaminase content and increased the content of non-enzymatic antioxidants, indicating a protective effect of H_2S against oxidative damage. Our previous study reported that H_2S treatment improved

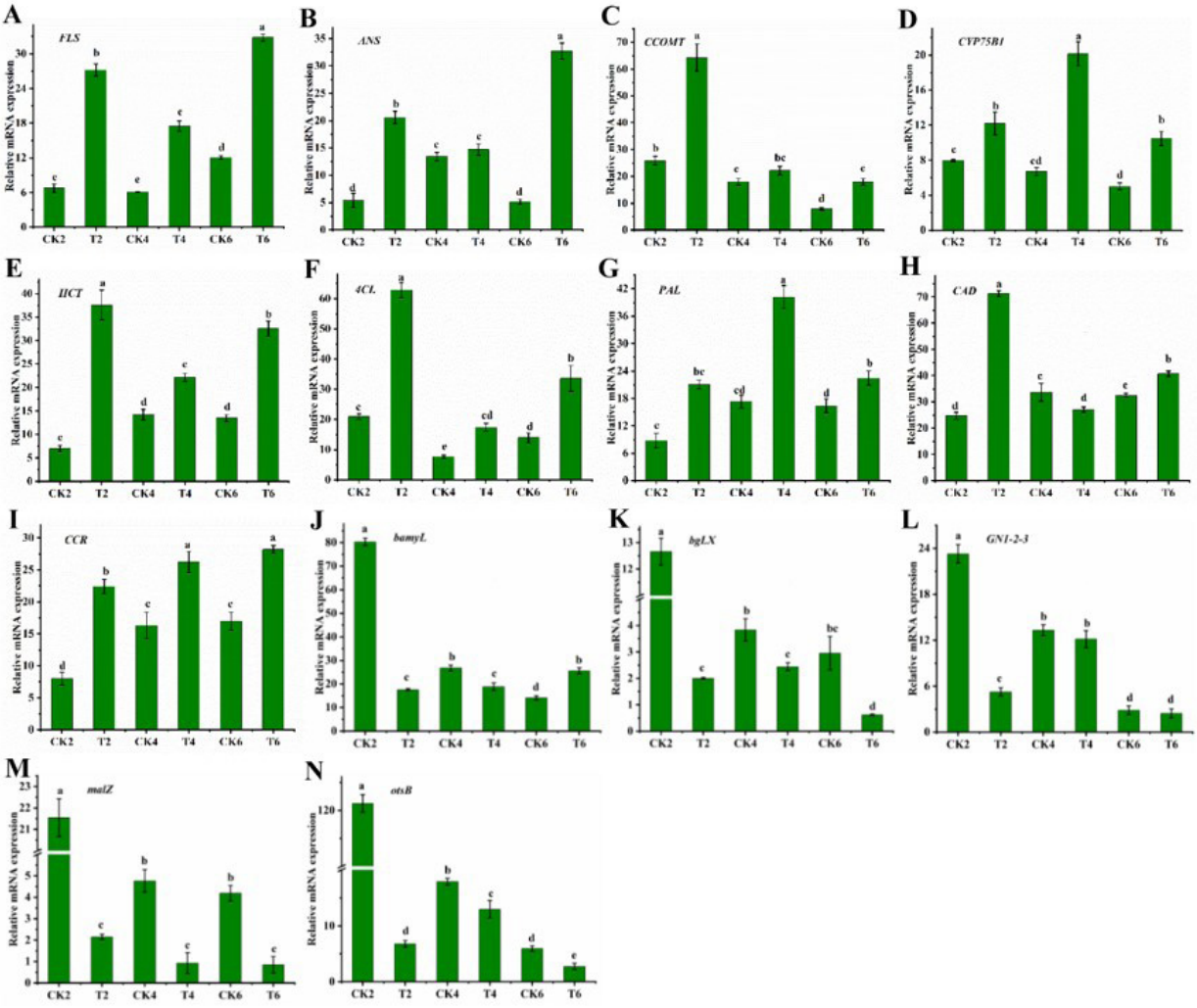

Figure 5. Relative mRNA expression of selected genes in post-harvested goji berries (A-N). Data are presented as means ± SD (n = 3). Different lowercase letters represent significant differences at $P < 0.05$ level.

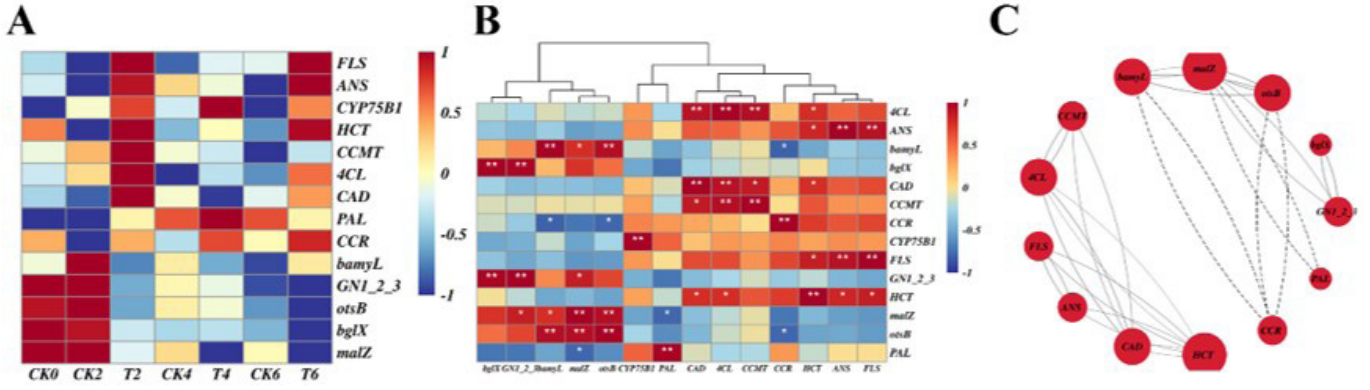

Figure 6. Relative mRNA expression of selected genes and expression heatmap in post-harvested goji fruits. Gene expression heatmap (A), correlation heatmap (B), correlation network (C). Data are presented as means ± SD (n = 3).

the activity of the antioxidant system in goji berries (Wang et al., 2023). Altogether, H_2S has been shown to increase the antioxidant activity of fruits in postharvest storage of a variety of fruits and vegetables (Chen et al., 2021; Deshi et al., 2020; Siddiqui et al., 2021; Zhong et al., 2021), which is consistent with our findings.

Postharvest softening of fruit mainly involves changes in the structural components of the cell wall, energy and starch metabolism, hormone secretion and regulation and pathogenic bacterial infestation. Fruit softening is closely related to starch metabolism and the metabolism of cell wall substances such

as pectin. Starch metabolism is a major factor in postharvest softening of fruit. The dynamic balance between starch and soluble sugars gives the cells a certain morphology (Lee & Jeon, 2020). During postharvest storage, the cell wall material is degraded by hydrolytic enzymes such as polygalacturonase, cellulase, β-glucosidase, a-amylase and β-amylase, resulting in the loosening of the fruit cytoskeleton structure and the escape of cell juice, leading to fruit softening and autolysis. β-glucosidase belongs to the cellulase group and has an important influence on the degradation and stabilization of the fruit cell wall. β-glucosidase is involved in the glycation of cellulose in the cell wall, which eventually hydrolyses the cellulose into glucose, resulting in the disintegration of the cell wall structure and the softening of the fruit and the onset of rot. H_2S treatment delayed the pectin degradation of Chilean strawberry during storage, significantly reduced the expression of pectin dissolving enzymes such as *FcPG1* and *FcPL1* genes, and reduced the expression of *FcXTH1* (xylan endoglycosidase/hydrolase 1) genes (Molinett et al., 2021). The cotreatment of H_2S and NO maintains high CHI (chitinase) and GNS (β- 1,3-glucanase) and reduce PME, PG and EGase (endonuclease- β- 1,4-glucanase) activity, reducing the decay rate of berry (Elam et al., 2022). H_2S treatment of sweet cherry can reduce PG, PL β- GUL and β- The activity of GAL controls the development of surface pitting by stabilizing cell wall structure and regulating cell wall catabolism (Zhi et al., 2018). H_2S delays ripening and senescence in kiwifruit by regulating cell wall degrading enzyme genes (Lin et al., 2020). Our study reported that starch and cell wall material metabolizing enzymes were inhibited during storage of postharvest goji berries treated with H_2S, and the expression of β-amylase, glucan endo-1,3-β-glucosidase, alglucose-6-phosphate phosphatase, β-glucosidase and α-glucosidase genes was significantly down-regulated.

5 Conclusion

Our data speculated that the expression of PAL, 4CL, FLS, and ANS key genes for flavonoid biosynthesis was up-regulated after H_2S treatment of postharvest goji berries. While the expression of genes related to sucrose and starch metabolism was down-regulated, suggesting that H_2S treatment may be associated with anti-oxidant system activation, increasing the antioxidant activity of goji berries and slow down carbon metabolism to delay the softening and senescence of goji fruit after harvesting. These results provide insights into the effect of H_2S on postharvest goji berries at the transcriptional level and lay the basis for application of H_2S as gas regulator for preservation of goji berry.

Conflict of interest

The authors declare no conflict of interest.

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