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Pre-harvest treatment with gibberellin (GA_3) and nitric oxide donor (SNP) enhances post-harvest firmness of grape berries

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ABSTRACT

Grapes are prone to softening, which limits their shelf life and suitability for long-distance transport. This study explored the molecular mechanisms underlying the effects of the chemical preservatives gibberellin (GA_3) and the nitric oxide donor sodium nitroprusside (SNP) on grape firmness. Enhancing grape quality, prolonging shelf life, and extending market supply were key objectives. Using transcriptomic and physicochemical analyses, the study found that treatments with 3 mmol/L GA_3 and 20 μmol/L SNP significantly increased the firmness of 'Yinhong' grapes, thereby improving overall quality. The mechanisms of action, however, differed between the two treatments. GA_3 inhibited pectin degradation and promoted cellulose accumulation, whereas SNP enhanced lignin and cellulose accumulation, by modulating cell wall metabolism. Furthermore, transcription factors such as CYPs, NAC043, and WRKY33 were identified as key regulators working in concert with target genes to influence berry firmness. These findings highlight the critical roles of GA_3 and SNP in improving grape quality and extending storage potential.

1. Introduction

Grapes (*Vitis vinifera* L.) are widely valued for their exquisite flavor and nutritional benefits (Cardone et al., 2016; Sun et al., 2020). In China, particularly in southern regions known for extensive cultivation and high yields, grapes play a vital role as both as a producer and consumer commodity (Majeed et al., 2023). However, grapes are prone to softening at room temperature, leading to a limited shelf life, and challenges during cultivation and storage. Post-harvest processes, such as transportation, and storage, often result in physical damage, including berry shedding and breakage, which compromise appearance, reduce market value and limit long-distance transportability. Enhancing grape berry firmness is thus essential to meet long-term supply demands and improve storage potential.

To address these challenges, researchers have explored chemical preservatives to improve grape texture, with nitric oxide (NO) being a prominent option due to its ability to preserve quality and delay aging (Zhong et al., 2024). NO, a bioactive compound synthesized by plants, plays critical physiological roles (Peng et al., 2023; Y. Zhu et al., 2019). Exogenous NO application reduces fruit weight loss and enhances firmness by inhibiting glycolytic enzymes and ATP synthase activity via S-nitrosylation, which decreases acetyl-CoA, ATP, ADP-glucose, and UDP-glucose activity, ultimately impeding polysaccharide biosynthesis (Sadeghi & Jabbarzadeh, 2024). Additionally, NO boosts plant defense responses, with sodium nitroprusside (SNP) often used to enhance resistance in post-harvest fruits against pathogens such as *Botrytis cinerea* in tomatoes (*Solanum lycopersicum*), and apples (*Malus domestica*) (Han et al., 2024), and Colletotrichum spp., species in mangoes (*Mangifera indica*) (Ren et al., 2020). The mechanism involves increased endogenous NO activation of reactive oxygen species metabolism, and accumulation of antifungal compounds (Coser et al., 2023). Despite its effectiveness, NO treatment remains underexplored in grape trees (Adhikary, Gill, Jawandha, Bhardwaj, & Anurag, 2021; Zheng et al., 2023), leaving its potential for enhancing grape quality uncertain.

Alongside NO, plant hormones such as abscisic acid (ABA), indole-3-acetic acid (IAA), cytokinin (CK), gibberellin (GA_3), and ethylene (M. Zhu et al., 2024) play vital roles in regulating fruit ripening. Ethylene, for instance, orchestrates various biological processes, including organ

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shedding, seed germination, flowering and ripening (Li et al., 2019) target genes and transcription factors (TFs) (Zhang et al., 2017). Gibberellin, another key hormone, influences developmental processes such as stem elongation (Qin et al., 2022), leaf expansion, seed germination, and fruit ripening. Gibberellin has been shown to delay ripening and enhance fruit firmness in crops such as tomatoes, plums (*Prunus domestica*), and peaches (*Prunus persica*) during pre- and post-harvest (Li et al., 2019; Wu et al., 2024). In grapes, GA_3 influences seed dormancy (Brumos, 2021), reduces ascorbic acid content in leaves, and impacts fruit size and vitality (W. Wang et al., 2020). Despite its widespread use, research on GA_3's effects on grape firmness remains limited.

This study aimed to investigate the effects of SNP and GA_3 treatments on grape firmness and quality, focusing on their underlying molecular mechanisms. The self-developed grape variety, 'Yinhong' was enclosed in bags 60 days after flowering and treated with varying concentrations of SNP and GA_3. Changes in grape firmness and other quality parameters were analyzed to identify optimal treatments for extending the shelf life of soft-fleshed grapes. These findings provide valuable insights for improving grape storage potential and meeting market demands.

2. Materials and methods

2.1. Plant materials

This study conducted from May to July 2023 at Dicuiyuan Vineyard in Cixi, Zhejiang, China (30° 16′6.59" N, 121 ° 25'2.18" E). The table grape variety 'Yinhong' was used, cultivated in cohesive soil, with the following characteristics: pH 6.4, soil depth 102 cm, total organic matter content 25 g/kg, alkaline nitrogen 202 mg/kg, effective phosphorus 35 mg/kg, effective potassium, 114 mg/kg, soil bulk density 112.3 cm^3, and groundwater level between 55 and 60 cm.

Forty uniform grapevines planted in 2010 and grown in rainproof greenhouses, were randomly selected and divided into 12 treatment groups: Control (TK), 2.5 μmol/L SNP, 5 μmol/L SNP, 10 μmol/L SNP, 20 μmol/L SNP, 40 μmol/L SNP, 1.5 mmol/L GA_3, 3 mmol/L GA_3, 6 mmol/L GA_3, 12 mmol/L GA_3, 24 mmol/L GA_3, and combined 5 μmol/L SNP/ 3 mmol/L GA_3 treatments (LH). Each grape bunch was sprayed during the fruit swelling period, approximately 60 days after flowering (DAF). A minimum of nine biological replicates were used for each treatment throughout the experiment.

No additional nutrients were applied to the grapevines. Standard agricultural practices, including irrigation and pest control, were maintained. Fifty berries were randomly collected during the ripening period, with half stored at −80 °C for physicochemical property analysis and RNA extraction.

2.2. Determination of soluble solids, berry weight, and titratable acidity

Soluble solids were measured using a portable refractometer (PAL^{-1}; ATAGO, Tokyo, Japan) according to the manufacturer's instructions. Ten grapes were randomly selected, weighed three times, and the average value was recorded as the individual berry weight. Read the diameter of the fruit as the fruit size in millimeters by sliding the vernier caliper. To determine titratable acidity, 25 g of grape flesh was placed into a conical flask, an equal volume of water was added, and the mixture was ground and homogenized. Next, 100 mL of water was added to 50 g of the homogenate, which was heated in a water bath at 80 °C for 30 min with constant shaking. After heating, the mixture was cooled, diluted to 250 mL, thoroughly shaken, and filtered. For titration, 50 mL of the filtered sample was transferred to a flask, and a phenolphthalein indicator was added. The sample, was then titrated with standard NaOH solution until a stable light red color appeared, which did not fade within 30 s (the endpoint). The volume of NaOH used was recorded, and the titratable acidity content was calculated.

2.3. Determination of grape firmness

Grapes were peeled and flesh firmness was measured using a GY-4 fruit tester (Edburg, China). The probe diameter was 2 mm (P/2 columnar probe) and the test speed was 1 mm/s. The tester was pressed down vertically at a constant speed to obtain the firmness of grape peel when punctured. Thereafter, 1 cm of the grape peel was cut off and the grape was slowly pressed vertically downward at a constant speed. The peak mean value was regarded as the grape flesh firmness and firmness was expressed in Newton (N). Measure 12 berries for each treatment and repeat three times.

2.4. Determination of cellulose, hemicellulose, and lignin contents

Pectin was extracted from the grape samples and dried to a constant weight. The pectin was then ground into powder and sieved to obtain fine powder. Fourier transform infrared spectroscopy was used to examine cellulose and hemicellulose structures in the fine powder of grape flesh and peels. The sample powder was mixed with 0.001 g KBr and then compressed under a vacuum, as previously described (He et al., 2022). The scanning range of the sample was 400–4000 cm^{-1}. Subsequently, cellulose content was determined using the anthrone–sulfuric acid colorimetric assay. Hemicellulose and lignin contents were determined using their respective detection kits (Solarbio Science and Technology Co., Ltd., Beijing, China).

2.5. Transcriptome analysis

Total RNA was extracted from grape samples for transcriptome sequencing and library construction. Thereafter, the mRNA was reverse-transcribed into complementary DNA (cDNA) using random primers. Purified cDNA fragments were used for terminal repair and ligation to the Illumina sequencing adapter. The linked products were separated by agarose gel electrophoresis, amplified using PCR, and sequenced on a BGISEQ-500 platform (Da Hua, China). High quality clean data were obtained by removing reads containing adapters, unknown "N" bases, and low quality reads. Three biological replicates were used. De novo transcriptome assembly of the reference genome was performed using Trinity software. The fragments per kilobase of exon per million fragments mapped (FPKM) value was calculated using String Tie to quantify the expression abundance and variation of a single gene. Meanwhile, R software (https://www.r-project.org/; R Foundation for Statistical Computing, Vienna, Austria) was used to analyze the differential expression of RNA between two different groups, and the false discovery rate (FDR) was used to calibrate the *P*-value. Differentially expressed genes (DEGs) were determined using $P < 0.05$ and $FC > 1$ as thresholds for identifying significant differences in gene expression. The DEGs were subjected to GO and KEGG pathway enrichment analyses. Total RNA was extracted using the Rnaprep Pure polysaccharide polyphenol plant total RNA extraction kit (HuiLing, China), and cDNA was synthesized using the NovoScript Plus All in one 1st Strand cDNA Synthesis SuperMix (gDNA Purge) reverse transcription kit (Novoprotein, China). Perform qRT-PCR using the Trans start TransStart Tip Green qPCR SuperMixS kit (Novoprotein, China) and q225 fluorescence quantitative PCR instrument (Kubo, Guangzhou).Reaction system (10 μL) Template cDNA 1 μL. 1 forward and 1 reverse primer each μL. 2 x TransStart Tip Green qPCR SuperMix 5 μL. H2O replenishment to 10 μL. Reaction procedure: 95 °C pre denaturation for 60 s; 95 °C for 20 s, 60 °C for 60 s, 45 cycles. Using *VvEF1α* for internal reference genes, set 3 replicates for each reaction. Adopting $2^{-\Delta\Delta Ct}$ method was used for data analysis, SPSS 26.0 was used for one-way ANOVA, and software was used to plot gene expression levels. The qRT-PCR primers are shown in Supplementary Table 1.

2.6. Data analysis

Results were expressed as means ± standard errors. All statistical differences and correlation analysis of the parameters between phenotypes, such as grape pulp firmness and cellulose content, and differential gene expression levels were analyzed by least significant difference (LSD) of one-way ANOVA using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA) software.A heatmap was generated using the heatmap function in R.

3. Results and analysis

3.1. NO and GA_3 alter grape firmness and physiological parameters

The effects of GA_3 and SNP at various concentrations on the quality of 'Yinhong' grapes were evaluated by measuring parameters such as soluble solids, titratable acidity, weight, and firmness. The results are summarized in Figs. 1, 2, and Table 1.

The firmness of the fruit flesh showed an initial increase followed by a decrease with the increase of SNP concentration (Fig. 2A), peaking at 20 μmol/L and then increased again at 40 μmol/L. Compared with TK, the peel firmness of the SNP treated group decreased, but did not reach a significant difference (Fig. 2B). This may be due to changes in the degree of cell wall cross-linking or the effect on intercellular adhesion after SNP treatment, but the peel firmness increased at 40 μmol/L. SNP treatments altered grape berry color without significantly affecting size or weight. Soluble solid content showed a slight increase with higher SNP concentrations but remained slightly lower than the TK group. Titratable acidity decreased with increasing SNP concentration (Fig. 2).

GA_3 treatments significantly enhanced both flesh and peel firmness, with the highest firmness observed at 3 mmol/L GA_3. Additionally, GA_3 increased berry weight and size, delayed ripening by approximately one week, and improved soluble solid content, reaching a maximum of 18.30°Brix at 3 mmol/L GA_3. However, soluble solids showed no consistent trend across concentrations. The increased firmness of both flesh and peel under GA_3 treatment may be linked to elevated cellulose content (Fig. 2A, B, G).

Drying grape berries was challenging due to their high pectin and moisture content, so grape powder was used for analysis. Fourier transform infrared spectroscopy (FTIR) revealed no significant changes in cellulose and hemicellulose structures after GA_3 and SNP treatments (Fig. 2K). Similar cellulose-related absorption peaks appeared at 3400, 2900, 1430, 1370, and 890 cm^{-1}. Key peaks included O—H (3300–3500 cm^{-1}), C—H (2900 cm^{-1}), C—O—H bending (1430 and 1370 cm^{-1}), and the B-(1 → 4)-glycosidic bond (890 cm^{-1}). Hemicellulose was characterized by xylan peaks (1161–988 cm^{-1}) with a pyran ring structure and arabinose side chains. GA_3 and SNP treatments showed similar effects on cellulose and hemicellulose structure but increased cellulose and hemicellulose content. Cellulose content increased most with 3 mmol/L GA_3, followed by 24 mmol/L > 6 mmol/L > 1.5 mmol/L > 12 mmol/L > 0 mmol/L. SNP treatments caused minor cellulose variations. Hemicellulose content in grape flesh rose under all treatments, with significant differences in grape peel. Lignin content in grape flesh showed no significant change, while grape peel lignin increased slightly across treatments.

3.2. NO and GA_3 induce transcriptional changes in relations to grape firmness

To identify genes involved in regulating grape firmness after GA_3 and SNP application, transcriptomic analysis was performed on 'Yinhong' grapes showing significant firmness changes, and 14 genes were randomly selected to validate the transcriptome data, confirming the validity of the transcriptome data (Supplement Fig. 1). A total of 5126, 272, and 5669 differentially expressed genes (DEGs) were identified in GA_3 vs. TK, SNP vs. TK, and LH vs. TK comparisons, respectively. Among these, 103 DEGs were common across all treatments, while 43 were shared between LH and SNP, 32 between GA_3 and SNP, and 3770 between LH and GA_3. SNP treatment resulted in fewer DEGs than GA_3, suggesting GA_3 had a more substantial impact. These findings indicate that both GA_3 and SNP significantly alter the transcriptional profile of 'Yinhong' grapes (Supplement Fig. 2).

Volcano plots of DEGs (Fig. 3) and GO/KEGG enrichment analyses revealed their involvement in diverse biological processes, molecular functions, and cellular components. GA_3 treatment mainly enriched DEGs in cytoplasmic processes and biological responses to temperature, abiotic stress, and hydrogen peroxide. It also impacted pathways such as amino acid biosynthesis, carbon metabolism, plant circadian rhythms, and photosynthesis-related carbon sequestration, suggesting GA_3's role in environmental adaptability and energy conversion mechanisms.

SNP treatment primarily enriched DEGs linked to cell wall metabolism, including hemicellulose, xyloglucan, and polysaccharide metabolism. KEGG analysis highlighted pathways such as phenylalanine, tyrosine, and tryptophan biosynthesis, phenylpropanoid biosynthesis, MAPK signaling, and hormone signal transduction, indicating SNP's influence on cell wall integrity and hormonal regulation.

In summary, GA_3 had an impact on gene expression related to photosynthesis and stress responses, whereas SNP mainly modulated cell wall metabolism and hormone signaling pathways, both of which contributed to grape firmness. These findings provide insights into how GA_3 and SNP enhance fruit quality and suggest molecular targets for improving grape firmness.

3.3. Analysis of DEGs

3.3.1. Phenylpropane metabolic pathway

A thorough analysis of the transcriptome data revealed that 66 differentially expressed genes (DEGs) were associated with phenylalanine synthesis (see Fig. 4A and Supplementary Table 2). The DEGs identified were classified into eight categories, with each DEG exhibiting a unique expression pattern that reflected their various mechanisms of action in regulating fruit firmness.

The expression levels of group C1 genes, which included peroxidase (POD), laccase (LAC), and cinnamoyl-CoA reductase (CCR) genes, decreased significantly after GA_3 treatment, whereas the expression levels of 4-Coumarate: Coenzyme A Ligase (4CL) and Phenylalanine ammonia-lyase (PAL) genes in group C4 increased significantly after GA_3 treatment. Although the effect of combined SNP/GA_3 treatment was

Fig. 1. Grape clusters treated with different concentrations of SNP and GA_3.
Note: Each treatment was labelled under the respective grape clusters.

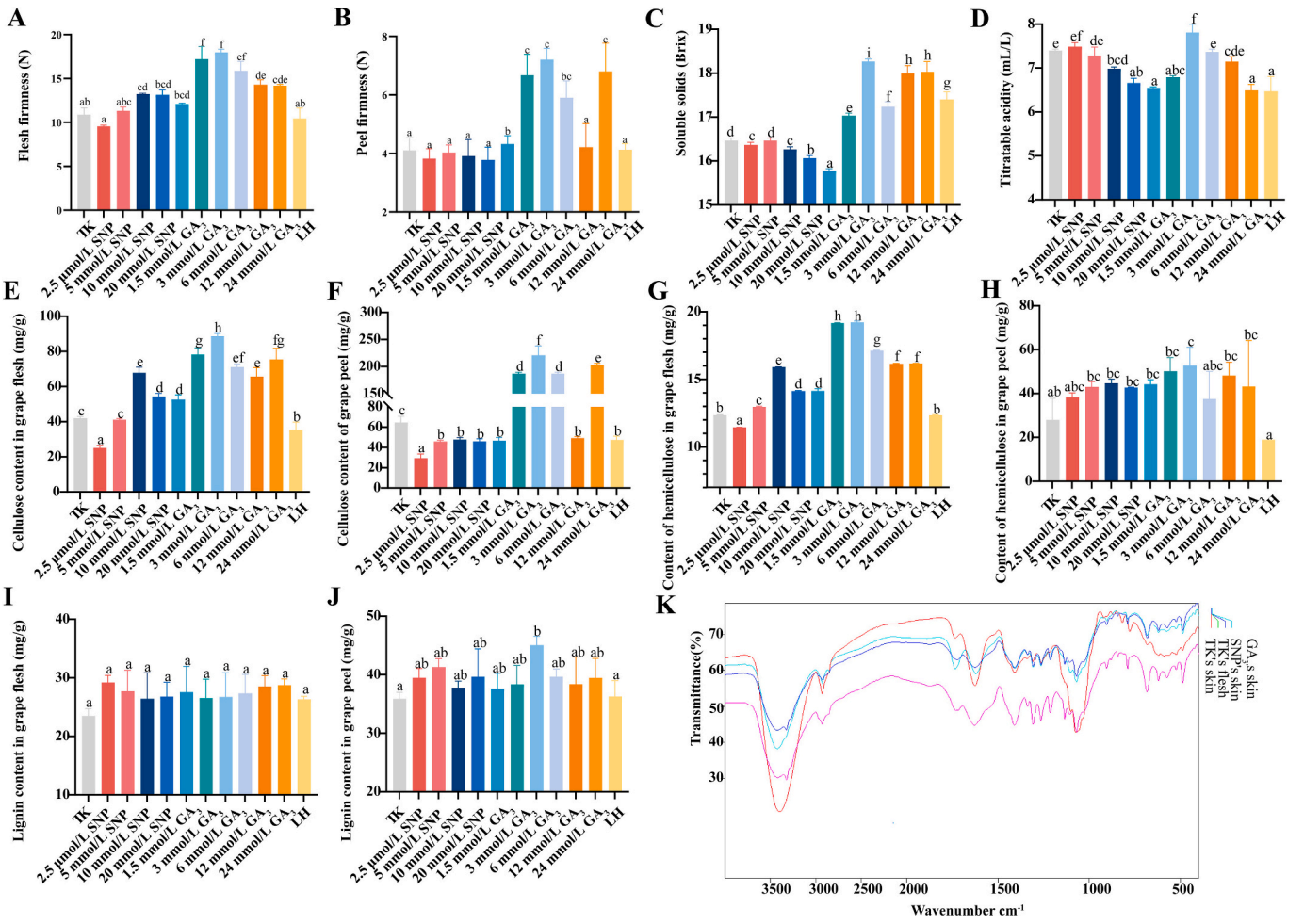

Fig. 2. The intrinsic quality of grape fruits treated with different chemical preservatives. A. flesh firmness, B. peel firmness, C. Soluble solid concentration, D. titratable acid concentration, E. flesh cellulose content, F. peel cellulose content, G. flesh hemicellulose content, H. peel hemicellulose content, I. flesh lignin content, J. peel lignin content, K. Infrared spectra of powders treated with different chemical preservatives, Note: abcdefgh represents significance analysis.

Table 1
Basic appearance quality of grapes treated with different chemical preservatives.

Groups	Fruit color	Fruit weight (g)	Fruit size (diameter/mm)
TK	Dark red/yellow green	10.63 ± 0.11	23.4 ± 0.01
LH	Dark red/yellow green	10.93 ± 0.04	25.1 ± 0.01
2.5 μmol/L SNP	Dark red/yellow green	11.77 ± 0.10	24.3 ± 0.31
5 μmol/L SNP	Dark red/yellow green	12.11 ± 0.10	23.11 ± 0.07
10 μmol/L SNP	Dark red	12.35 ± 0.07	25.17 ± 0.02
20 μmol/L SNP	Dark red/yellow green	12.04 ± 0.06	24.13 ± 0.06
40 μmol/L SNP	Dark red/yellow green	11.63 ± 0.03	23.53 ± 0.01
1.5 mmol/L GA_3	Pink	12.14 ± 0.06	28.9 ± 0.02
3 mmol/L GA_3	Pink	13.96 ± 0.05	29.4 ± 0.07
6 mmol/L GA_3	Pink	12.25 ± 0.05	26.9 ± 0.01
12 mmol/L GA_3	Pink	13.25 ± 0.04	32.2 ± 0.01
24 mmol/L GA_3	Pink	11.99 ± 0.05	29.1 ± 0.01

slightly lower than that of GA_3 treatment only, GA_3 treatment exerted a positive regulatory effect on the expression of these genes. The inhibitory effect of GA_3 treatment on the expression of group C1 genes, especially *POD* and *LAC*, could reduce the activity of cell wall degrading enzymes and delay the fruit softening process. The increase in the expression levels of group C4 genes, especially *PAL* genes, indicates that GA_3 treatment activates the phenylpropanoid metabolic pathway, promotes lignin synthesis, and enhances fruit firmness. GA_3 treatment can effectively regulate grape firmness by inhibiting the expression of cell wall-degrading enzyme-related genes and promoting the expression of lignin synthesis-related genes. The expression levels of group C3 genes including POD genes (*Vitvi11g01258*, *Vitvi16g00139*, and *Vitvi17g00148*) decreased significantly under SNP treatment, indicating that lignin synthesis was activated, thereby enhancing grape firmness. As a key component of cell walls, lignin enhances lignin synthesis, which in turn, improves the mechanical strength of fruit cell walls and reduces fruit softening. The expression levels of group C2 genes showed a trend similar to that exhibited group C4 genes under combined SNP/GA_3 treatment; however, the expression levels of certain genes increased significantly. Slight differences were observed in gene expression under the different treatments. The expression levels of genes in groups C5 and C8, namely PAL genes were higher than those of the control group under combined SNP/GA_3 treatment, suggesting that the combined treatment effectively activated the phenylpropanoid metabolic pathway through synergistic effects, thereby promoting lignin synthesis and enhancing grape firmness.

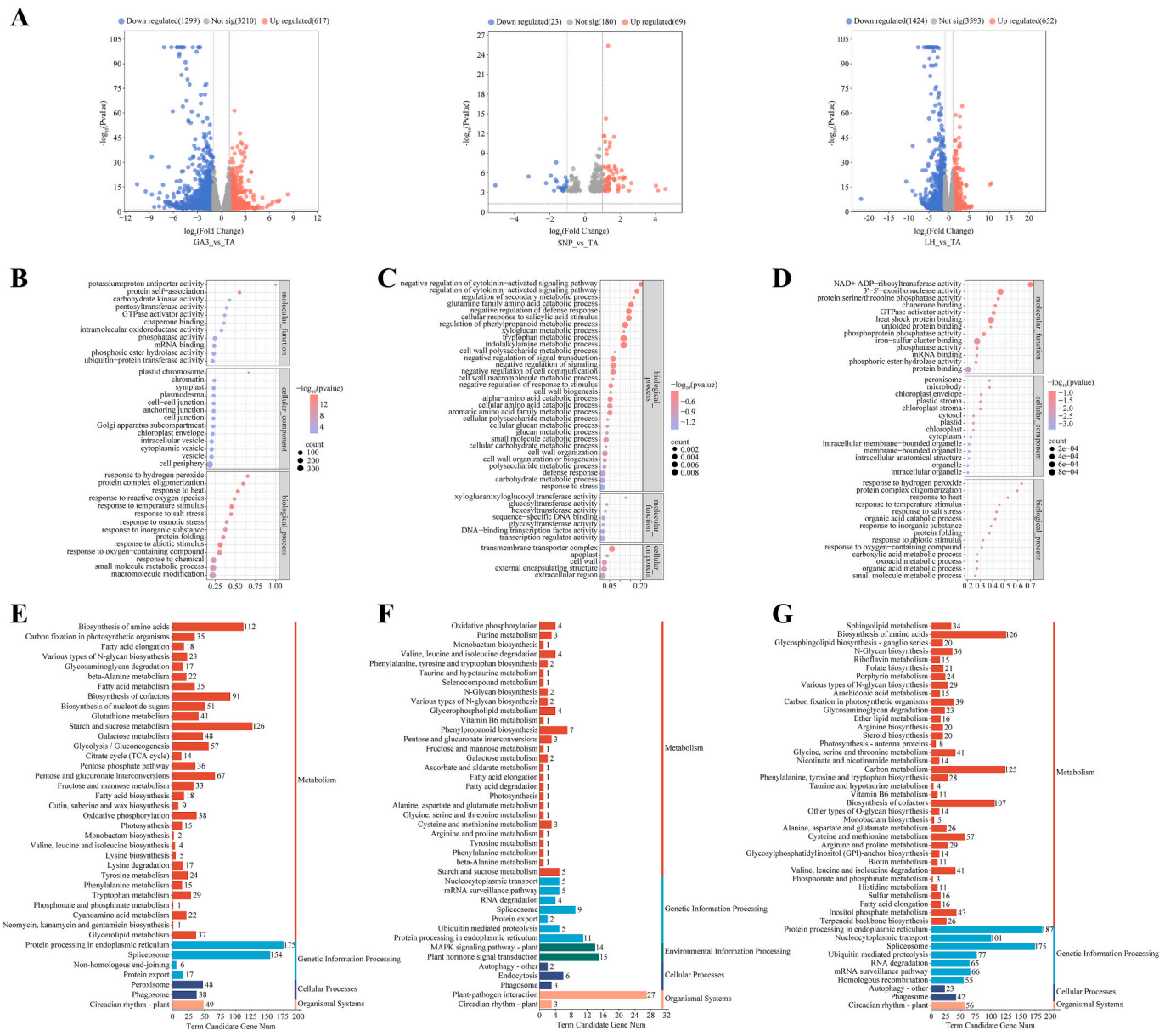

Fig. 3. Presents the transcriptome analysis results for different treatments. A shows volcano plots of differential gene expression for the control group (TA), 3 mmol/L GA_3 treatment (GA), 20 μmol/L SNP treatment (SNP), and the combined treatment of 3 mmol/L GA_3 and 20 μmol/L SNP (combined). B–G present the classification results of GO enrichment analysis classification, highlighting the biological processes affected by the treatments. G–H shows the classification results of KEGG enrichment analysis classification, providing insights into the metabolic pathways altered by the treatments.

In addition, the number of genes in group C6 was relatively low and gene expression was significantly upregulated under all treatments, suggesting that the genes are involved in regulating grape firmness. Variations in the expression of *CCR*, *PAL*, and *POD* genes under the different treatment conditions demonstrate their key roles in regulating grape firmness. For example, the expression level of *PAL* (*Vitvi06g04091*) increased under GA_3 and combined SNP/GA_3 treatments; however, its expression was inhibited under SNP treatment. The results indicate that GA_3 and SNP exert contrasting effects on *PAL* genes in 'Yinhong' grapes. SNP treatment significantly increased the expression levels of some *POD* and *LAC* genes, which could be due to the activation of gene transcription associated with phenylpropanoid synthesis by SNP, thereby promoting lignin synthesis and enhancing grape firmness.

3.3.2. *Pectin metabolism*

Pectin has been extensively studied in relation to grape firmness and softening. A total of 76 DEGs, which are closely associated with pectin metabolism and grape firmness were identified (Fig. 4B, Supplementary Table 3). Specifically, the expression levels of genes involved in pectin hydrolysis decreased significantly after treatment with GA_3. The expression levels of key enzymes, such as (PL; *Vitvi09g0150* and *Vitvi09g01510*) and pectinesterase (PE; Vitvi04g02246 and Vitvi09g00018) were downregulated, which is consistent with the findings of previous studies on the inhibitory effect of GA_3 on pectin metabolism. However, the expression levels of genes in groups C1 and C2 increased significantly after combined SNP/GA_3 treatment, with the expression levels of genes in group C1 being the highest. The expression levels of polygalacturonase (PG), PE, and PL genes increased significantly, which could accelerate the degradation of pectin and promote grape softening. Similarly, the SNP treatment group showed increased expression levels of these genes. The results of this study confirmed that the chemical preservative, GA_3 effectively controls the grape firmness and prolongs their shelf life by inhibiting the expression of genes related to pectin decomposition. In contrast, combined SNP/GA_3 treatment upregulated

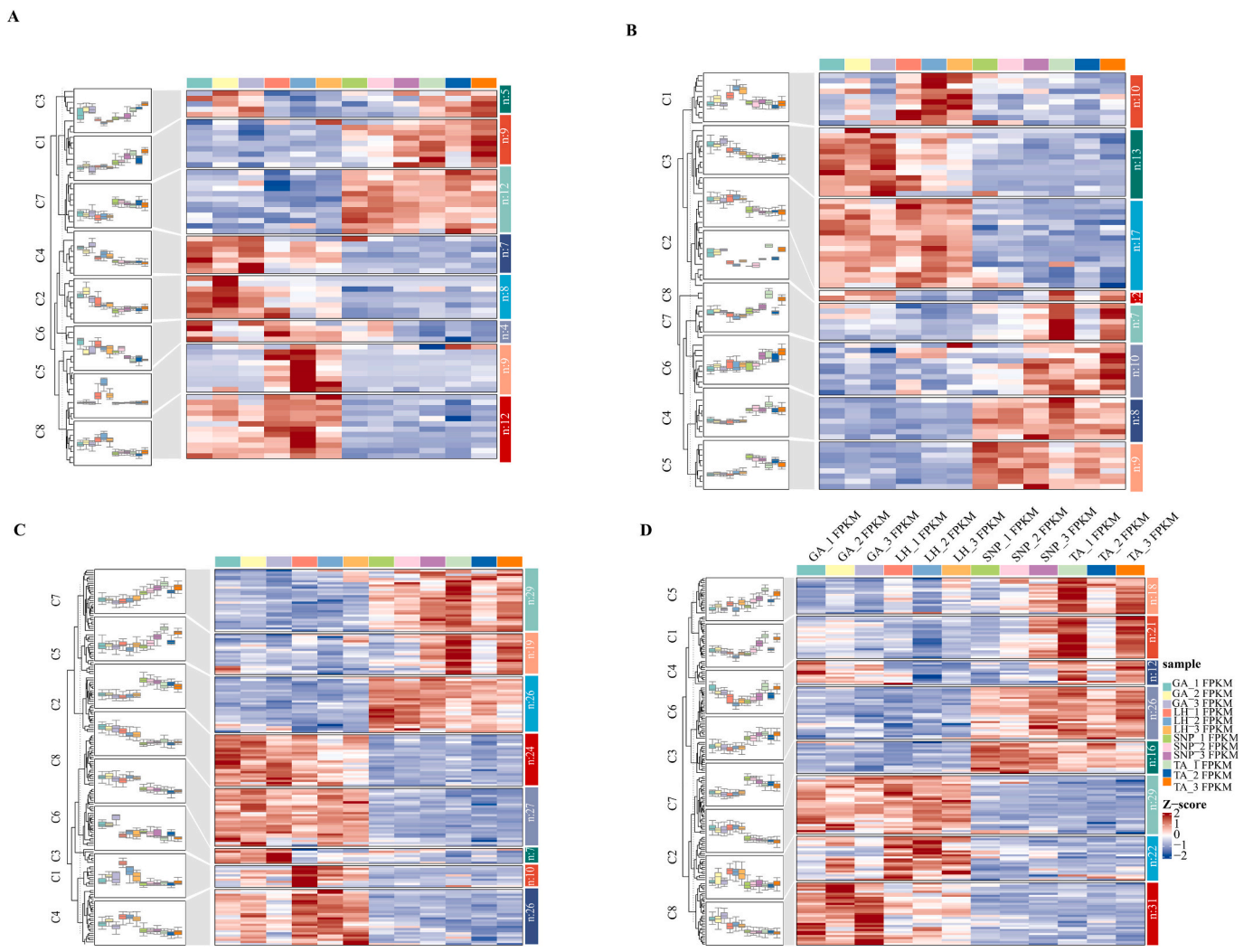

Fig. 4. K-means clustering of differentially expressed genes (DEGs) in various metabolic pathways. (A) Phenylpropanoid metabolism, (B) Pectin metabolism, and (C) Cellulose metabolism, hemicellulose, and polysaccharide metabolism. A heatmap displays the expression levels of DEGs within each cluster, with color intensity indicating the magnitude of expression changes. Line graphs represent the gene expression trends for each cluster across the metabolic pathways, showing upregulation and downregulation patterns.

the expression of pectin-degrading enzymes(Meneses et al., 2020).

3.3.3. *Cellulose and hemicellulose metabolism*

GO and KEGG pathway enrichment analyses of DEGs revealed that 168 DEGs were associated with cellulose metabolism (Fig. 4C, Supplementary Table 4). The expression levels of genes in C7, C5, and C2 groups decreased significantly, whereas those of genes in C8 and C6 groups increased significantly under GA_3 treatment. Notably, under GA_3 treatment, the expression levels of genes encoding cellulose synthase A (CesA) increased significantly, such as sucrose synthase (SUS; *Vitvi18g04660*) and CesA (*Vitvi19g00700* and *Vitvi08g01897*), which could promote cellulose synthesis and enhance grape firmness. Specifically, the expression level of SUS (*Vitvi18g04660*) increased by 50 % and the expression level of CesA (*Vitvi19g00700*) increased by 30 %. However, the expression levels of genes encoding key enzymes involved in cellulose degradation, such as endoglucanase (*EG*; *Vitvi11g00487* and *Vitvi12g00440*) and β-glucosidase (*β-BGL*; *Vitvi06g00593*), decreased significantly during grape ripening. The results suggest that GA_3 regulates cellulose metabolism by inhibiting the activity of enzymes involved in cellulose degradation, thereby preventing premature grape softening.

The expression levels of β-glucosidase and endoglucanase increased significantly during grape maturation under SNP treatment, indicating that SNP promotes cellulose metabolism by upregulating the expression of related genes. The expression levels of *CesA* (*Vitvi13g00246*, *Vitvi11g00726*, and *Vitvi18g04497*) and *SUS* (*Vitvi18g04669* and *Vitvi18g03016*) increased significantly under combined SNP/GA_3 treatment. Conversely, the expression levels of glucan endo-1,3-beta-glucosidase 4 (*Vitvi19g00557*, *Vitvi01g00468*, *Vitvi02g00248*, *Vitvi10g00085, Vitvi11g00487*, and *Vitvi12g00440*) decreased significantly under combined SNP/GA_3 treatment. Overall, the results indicate that the chemical preservatives, GA_3 and SNP induce the expression of genes related to cellulose synthesis and inhibit the expression of genes related to cellulose degradation, in turn, leading to the accumulation of cellulose and enhancing firmness.

Regarding hemicellulose metabolism, a total of 175 DEGs were identified in this study (Fig. 4D, Supplementary Table 5). The expression levels of fruit softening-related genes in groups C1 and C5, which included xyloglucan: xyloglucosyl transferase (*Vitvi11g01268* and *Vitvi11g01675*) and endoglucanase (*Vitvi02g00125*), decreased significantly after treatment with SNP and GA_3, which enhanced grape firmness. The expression levels of genes in group C4 decreased under combined SNP/GA_3 and SNP treatments, whereas those of genes in groups C6 and C3, such as β-galactosidase (*Vitvi09g00191* and *Vitvi13g01417*), increased after SNP treatment. Notably, the expression levels of genes in groups C7, C8, and C2 increased significantly under GA_3 and combined SNP/GA_3 treatments, although no significant

difference was observed in the gene expression levels under SNP treatment. Specifically, variations in the expression levels of genes in group C2, particularly β-galactosidase (*Vitvi01g00552* and *Vitvi07g01366*), were most significant after combined SNP/GA_3 treatment. These results suggest that β-galactosidase affects grape firmness by promoting the expansion and degradation of cell walls, metabolic recovery of galactose and glycoproteins, and conversion of signaling molecules during ripening.

Cellulose and hemicellulose are the major components of plant cell walls(Xu et al., 2023), and their metabolic regulation is crucial for maintaining the grape firmness. The chemical preservatives GA_3 and SNP had a substantial impact on cellulose metabolism by regulating the expression of genes related to cellulose synthesis and degradation. GA_3 treatment promoted cellulose accumulation by upregulating the expression of genes encoding cellulose synthase, while inhibited cellulase activity, thereby delaying grape softening and enhancing grape firmness. SNP treatment had the opposite effect by upregulating the expression of cellulase, accelerating cellulose degradation, and reducing grape firmness. The changes in β-galactosidase expression in hemicellulose metabolism reveal its dynamic regulatory role in cell wall degradation by enhancing the degradation of structural polysaccharides in the cell wall, in turn, releasing free monosaccharides, regulating cell growth and development, and reducing grape firmness. The regulation of gene expression associated with polysaccharide metabolism by chemical preservatives influences grape firmness by promoting the accumulation and transformation of polysaccharides.

3.3.4. *Weighted gene co-expression network analysis*

Weighted gene co-expression network analysis (WGCNA) was conducted, revealing 22 different gene expression modules. These modules were enriched for pathways such as phenylalanine biosynthesis, polysaccharide metabolism, and MAPK signaling transduction (Supplementary Fig. 2, Supplementary Table 6). For example, the MEyellow, Meblue, and Meyelowgreen modules had 73, 81, and 46 genes,

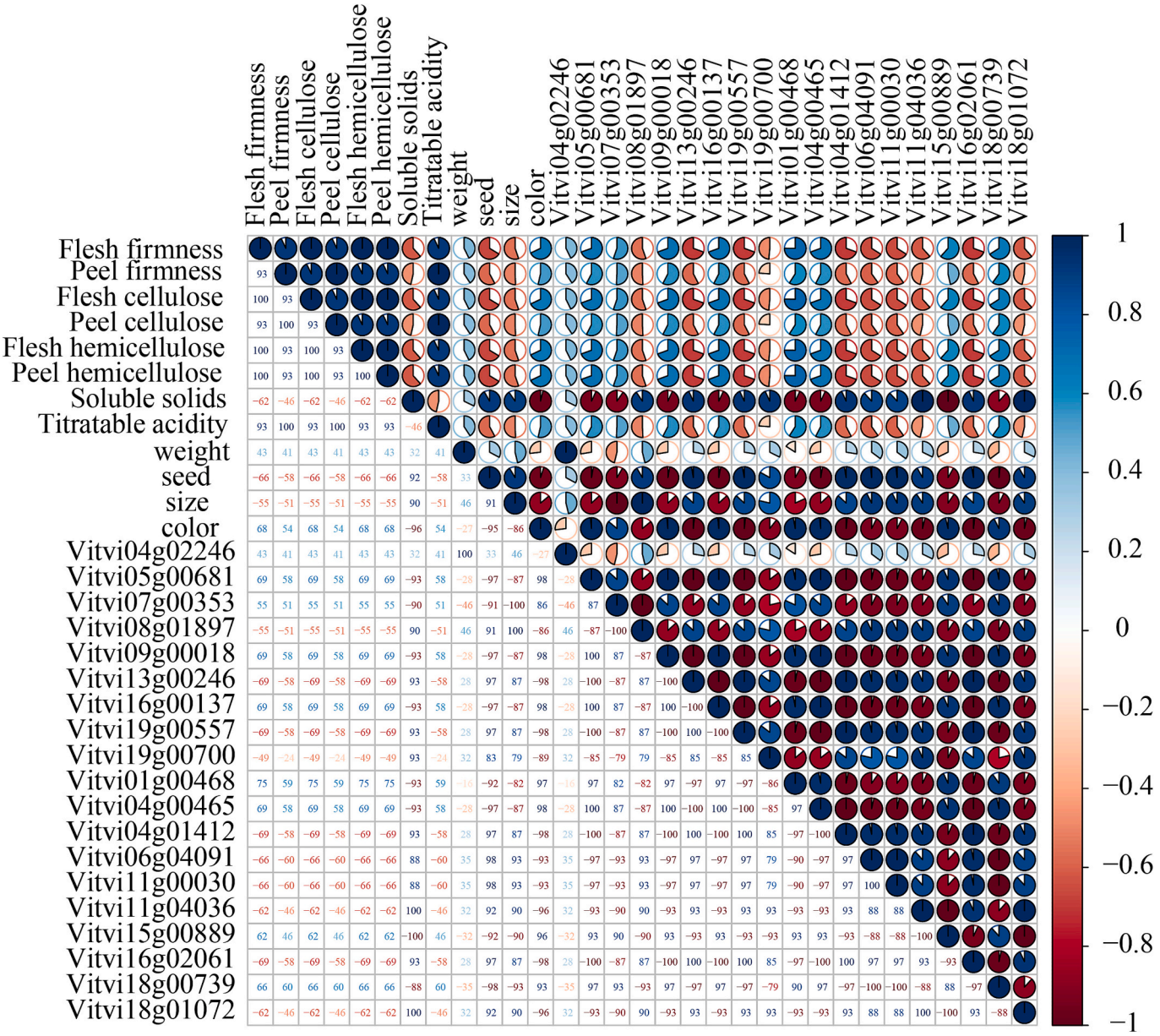

Fig. 5. Presents the Pearson correlation analysis between key genes and various physiological parameters, including fruit hardness, cellulose content, hemicellulose content, soluble solids, and titratable acids. The correlation coefficients are expressed as percentages, ranging from −1 to +1. A coefficient close to +1 indicates a strong positive correlation, a coefficient close to −1 indicates a strong negative correlation, and a coefficient close to 0 suggests no significant correlation.

respectively, which exhibited the highest correlation with grape firmness. Further analysis was conducted using the JASPAR database to identify potential target genes for differentially expressed TFs in all modules (Supplementary Table 7). Five genes, namely *CYP73A5* (*Vitvi16g02061*), *CYP84A1* (*Vitvi04g01412*), *CYP83B1* (*Vitvi18g01072*), *NAC043* (*Vitvi15g00889*), and *WRKY33* (*Vitvi18g00739*) were strongly correlated with TFs and were likely to be the candidate genes involved in cell wall metabolism.

3.4. Correlation analysis

To elucidate the molecular mechanisms underlying changes in grape firmness under different treatment conditions, Pearson's correlation analysis was conducted on physiological and transcriptomic data (Fig. 5). The results revealed that cellulose and hemicellulose contents were significantly positively correlated with grape flesh and peel firmness, suggesting their combined effect on grape firmness. Conversely, cellulose and hemicellulose contents were negatively correlated with soluble solid content, implying that cellulose content increased with a decrease in sugar content.

A significant correlation was observed between polygalacturonase, cellulase, sucrose synthase, and the physiological indicators assessed, particularly with soluble solids, suggesting that these enzymes may play a role in regulating the grape ripening process. It is noteworthy that the correlation coefficients between *Viti04g02246* and grape firmness are 0.60 and 0.51, respectively, suggesting that *Viti04g02246* may play a significant role in regulating grape firmness. Positive correlations were observed between *CesA* (*Vitvi04g00465* and *Viti19g00700*) and grape firmness ($r = 0.69$ and $r = 0.49$, respectively), suggesting that increased *CesA* expression altered cell wall components associated with firmness, ultimately modifying grape firmness. Furthermore, five TFs exhibited distinct correlations, where three CPY TFs were significantly negatively correlated with grape firmness ($r = -0.69$), whereas WRKY and NAC043 were positively correlated with grape firmness ($r = 0.62, 0.66$). These correlations suggest a complex interplay between these TFs and grape firmness, which is possibly achieved through their roles in regulating cell wall metabolism and grape maturation.

4. Discussion

The use of chemical preservatives has been widely shown to extend food shelf life, reduce post-harvest losses, and enhance the market value of fruits during long-distance transportation (Li et al., 2019; Yan, Gagalova, Gerbrandt, & Castellarin, 2024). While much of the research focuses on the efficacy of GA_3 and SNP in post-harvest fruit preservation and disease prevention (García-Rojas et al., 2018), limited studies explore their effects at various fruit development stages or the molecular mechanisms underlying firmness regulation. Grape firmness, a critical quality attribute, plays a vital role in extending storage potential, resisting decay and mechanical damage, and improving market appeal.

This study addressed this gap by analyzing the effects of exogenous GA_3 and SNP on grape firmness. The results demonstrated that both GA_3 and SNP treatments significantly improved firmness. Low SNP concentrations had minimal effects, while higher concentrations strongly enhanced firmness. GA_3 treatment not only improved firmness but also positively impacted grape attributes like color, brightness, and weight (Dong et al., 2023; Li et al., 2024). However, GA_3 increased titratable acidity, potentially raising fruit astringency. Combined GA_3 and SNP treatments slightly reduced firmness compared to individual treatments, though their overall effect remained consistent. These findings align with previous research on grapes and apples.

Transcriptomic and correlation analyses revealed that SNP induced the expression of genes linked to phenylpropanoid synthesis, including PAL1, PAL2, 4CL, and POD, promoting lignin synthesis and firmness. SNP also upregulated CesA expression, while altering β-galactosidase expression to influence cell wall dynamics. GA_3 inhibited pectin degradation by suppressing genes like *Vitvi07g00351* and *Vitvi13g01123*, reducing pectinase activity and delaying softening. It also enhanced CesA expression and inhibited cellulose degradation-related genes (*Vitvi12g00440*), further improving firmness. Interestingly, combined GA_3 and SNP treatments resulted in lower cellulose and hemicellulose levels compared to single treatments, suggesting complex interactions in cell wall metabolism. Key transcription factors (TFs), such as CYP74A and CYP73A5, were implicated in jasmonic acid biosynthesis, cell wall remodeling, and softening. Other TFs like CYP84A1, linked to IAA biosynthesis (Y. Wang et al., 2021), and NAC (Liu et al., 2022) and WRKY families, associated with stress response and cell wall remodeling, positively correlated with firmness (W. Li et al., 2020; Rao et al., 2022).

In summary, GA_3 and SNP treatments enhance grape firmness by modulating genes related to cell wall metabolism. These findings provide valuable insights into the role of chemical preservatives in improving grape quality and extending shelf life, emphasizing the significance of GA_3 and SNP in optimizing post-harvest fruit handling.

5. Conclusion

This study conducted a comprehensive analysis of transcriptomic data and physicochemical parameters associated with grape firmness, focusing on the effects of varying concentrations of the chemical preservatives GA_3 and SNP. The findings revealed that GA_3 and SNP enhanced grape firmness through distinct mechanisms. SNP primarily extended shelf life by stimulating antioxidant enzymes, modulating phenylacetone synthesis, and regulating the synthesis and degradation of cellulose and pectin, thereby altering cell wall metabolism. Conversely, GA_3 achieved the highest firmness by upregulating transcription, activating sucrose synthase and cellulose synthase expression, and inhibiting PG activity, which is associated with pectin degradation and fruit softening. This also resulted in increased soluble solid content, enhancing overall grape quality. However, combined GA_3 and SNP treatments slightly reduced firmness, likely due to synergistic interactions between the two preservatives. Based on these results, GA_3 emerges as a more effective option for commercially mitigating grape softening in 'Yinhong' grapes compared to SNP. These findings provide a solid theoretical basis for the development of improved chemical preservation strategies aimed at enhancing grape firmness and extending shelf life.

CRediT authorship contribution statement

Lingling Hu: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Qianqian Zheng:** Data curation. **Zhihui Chen:** Writing – review & editing. **Yi Qin:** Methodology. **Haoxuan Si:** Methodology, Investigation. **Jiayi Ji:** Methodology, Investigation. **Qing Li:** Methodology. **Zhongyi Yang:** Writing – review & editing, Visualization, Validation. **Yueyan Wu:** Writing – review & editing, Validation, Resources, Funding acquisition.

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 related to this article are available online. Supplementary Table 1 qRT PCR primers. Supplementary Table 2 Lignin pathway differential genes FPKM. Supplementary Table 3 Pectin pathway differential genes FPKM. Supplementary Table 4 Cellulose pathway differential genes FPKM. Supplementary Table 5 Hemicellulose pathway differential genes FPKM. Supplementary Table 6 Weighted Gene Co expression Network Analysis. Supplementary Table 7 Potential target genes for predicting differentially expressed transcription factors in all modules. Supplementary Figure 1 qRT PCR Validation of Differentially Expressed Genes Six genes identified as significantly differentially expressed in RNA Seq analysis were randomly selected for validation using qRT PCR. The comparison between RNA Seq and qRT PCR results is shown in the figure. Data from RNA Seq is presented as gene expression levels (FPKM), while qRT PCR data is shown as relative expression levels. Error bars represent the standard error of the mean (SEM) based on three independent biological replicates. These results confirm the reliability of the RNA Seq data through independent validation. Supplementary Figure 2 Vinn Diagram and Differential Expression Heatmap for Different Treatment Groups. The Venn diagram illustrates the overlap of differentially expressed genes (DEGs) between different treatment groups. Genes identified as significantly differentially expressed ($p < 0.05$) in each treatment group are shown, with the number of unique and shared DEGs indicated in the respective sections of the diagram. The heatmap depicts the expression patterns of significantly differentially expressed genes across all treatment groups. Gene expression values are normalized and presented as log2 fold change relative to control. Clustering of genes and samples was performed using hierarchical clustering based on Euclidean distance. The color scale represents gene expression levels, with blue indicating high expression and grey indicating low expression. The results highlight the distinct gene expression profiles associated with each treatment group. Supplementary Figure 3: Weighted Gene Co Expression Network Analysis (WGCNA) The weighted gene co expression network was constructed using the top 5000 most variable genes across all samples. Gene modules were identified using hierarchical clustering based on the TOM. Each module is represented by an eigengene, which is the first principal component of the gene expression profiles within that module. Modular trait associations were computed to identify modules significantly correlated with specific traits or clinical variables. A heatmap of the modular trait relationships is shown, where each row represents a module and each column. Representatives a trait. Supplementary material phenotype raw data. The findings and contributions presented in this study are publicly accessible. These data can be found at the following location: the National Center for Biotechnology Information (NCBI) biological project database, with the registration number PRJNA1175992 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1175992; accessed on 21 October 2024).

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