

Enhancing effect of Na_2SeO_3 on the growth and physiological parameters of *Vitis vinifera* × *labrusca* 'Shuijing' under nitrogen deficiency and underlying transcriptomic mechanisms

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ABSTRACT

The differences in physiological characteristics and transcriptional response of grape growth under Na_2SeO_3 treatment with nitrogen deficiency are explored, and selenium- and nitrogen-responsive genes are screened. Grape cuttings were divided into seven groups: Control, 0.1Se + 15N, 0.2Se + 15N, 0.4Se + 15N, 0.1Se, 0.2Se, and 0.4Se. Morphological and physiological characteristics in different groups were determined, and transcriptome sequencing was analysed. Net increases in plant height, stem diameter, root volume, biomass, and contents of flavonoids, soluble sugar, and nitrogen in the leaves were higher in the 0.2Se + 15N groups than in the Se groups. 0.2Se + 15N versus Control, 0.2Se versus Control, and 0.2Se versus 0.2Se + 15N had 1196, 2238, and 1980 differentially expressed unigenes (DEGs), respectively. Analysis of these DEGs revealed that, under nitrogen supply conditions, Na_2SeO_3 treatment regulated the upregulation of some gene activities of stilbene synthase, phenylalanine ammonia lyase, 4-coumarate-COA ligase, and α -trehalose phosphate synthase; under nitrogen deficiency conditions, genes encoding auxin and gibberellin were upregulated after Na_2SeO_3 treatment; the expression of these genes plays an important role in regulating the growth of grape plants. The study elucidated the mechanism by which Na_2SeO_3 promotes plant growth under both nitrogen supply and nitrogen deficiency conditions.

Keywords: grape, Na_2SeO_3 , nitrogen, physiological parameters, transcriptome

INTRODUCTION

As a non-metallic element widely distributed in nature, selenium (Se) often exists in the form of selenite and selenate (Vallini et al., 2005). Although it is not an essential element for plants, an appropriate amount of Se is beneficial to plant growth and development (Peng et al., 2017; Trippe and Pilon-Smits, 2021). An appropriate concentration of Se can promote the growth of leaf buds and germination of seed plumule, enhance plant metabolism (Chen et al., 2020), and improve the resistance of plants to saline-alkaline (El-Badri et al., 2022) and

heavy metal stresses (Hasanuzzaman et al., 2022). An appropriate amount of Se could significantly increase the yields of *Juglans regia* L. (Wang et al., 2021a, 2021b) and winter jujube (Jing et al., 2017) and improve the fruit quality of grapes (Yang et al., 2019) and pears (Xu et al., 2019). Se is considered an essential trace element for humans. As an essential component of glutathione peroxidase, Se can eliminate free radicals in the human body and plays a role in antioxidation, delaying cell ageing, enhancing the body's resistance to diseases,

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and preventing cancer (Peng et al., 2013). Humans can obtain Se from crops; studies have reported that the application of an appropriate amount of exogenous Se can significantly increase Se content in crops (Rotruck et al., 1973) and reduce the enrichment of toxic heavy metals such as cadmium (Luo et al., 2019), chromium (Ulhasan et al., 2019), mercury (Tran et al., 2018), and arsenic (Shahid et al., 2019) in the plant body. However, a high concentration of Se can inhibit plant growth and development (Li et al., 2020) and reduce photosynthetic rate (Gao et al., 2017). Therefore, it is necessary to optimise the application amount of exogenous Se for crops.

Nitrogen is one of the essential elements for plant growth and development. It is the basic element that constitutes important structures such as nucleic acids, chlorophyll, and proteins. Additionally, it is one of the main limiting factors affecting crop growth (Luo et al., 2015). In the production of fruit trees, the utilisation rate of N fertiliser is relatively low (average of 30%) (Zhao et al., 2009). However, overapplication of N fertiliser is very common (Fan et al., 2012), which causes wastage of resources, increases production costs, deteriorates soil quality, and promotes water pollution and other environmental problems (Zhang et al., 2012). Therefore, scientific application of fertilisers and improvement of the absorption and utilisation of N fertilisers are inevitable for high-yield, high-efficiency production and sustainable development of modern agriculture.

Vitis vinifera × *labrusca* 'Shuijing' is a popular fruit globally and is widely cultivated. It has a wide range of varieties, and its fruit has a rich nutritional value and is deeply loved by consumers (Ali et al., 2011). In grape production, if the amount of N fertiliser applied is too low, it cannot meet the nitrogen absorption needs of the trees. This results in nutrient deficiency in the trees, limiting the fruit yield and quality (Wang et al., 2021a, 2021b). If the amount of N fertiliser applied is too high, it will cause an imbalance in the absorption of nutrients by the trees, which is not conducive to the improvement of fruit quality and has a negative impact on the environment (Peng et al., 2003; Zhang et al., 2013).

Transcriptome analysis is the most widely used technique to elucidate the differential gene expression of the same cell at different growth stages and growth environments through sequencing technology (Shu et al., 2013). This technique can be used to explore the mechanism of inorganic nutrient elements regulating crop growth. For example, Liu et al. (2021) used transcriptome technology to study the nitrogen response mechanism of the rice tillering stage, and Hu et al. (2022) used transcriptome technology to study the accumulation and assimilation mechanism of selenium in alfalfa leaves. Nitrogen content in oat plants is reported to increase after the application of Se fertiliser (Rayman, 2008; Lopes et al., 2017). The application of Se fertiliser and nitrogen at the same rate could increase nitrogen content in wheat plants

(Sun et al., 2017). This may be attributed to the fact that Se can affect chlorophyll synthesis and regulate the electron transfer in photosynthesis and respiration, thus improving crop photosynthetic capacity and increasing the N accumulation (Mokhele et al., 2012). Therefore, the Se application can improve the utilisation rate of N fertiliser. However, the effects of Se on the growth and development of grape plants and the appropriate concentration have not been reported. In this study, we investigated the effects of Na_2SeO_3 application on the morphological and physiological indicators of 'Shuijing' grape under various nitrogen conditions. We explore the transcription factors related to the promotion of plant growth by Na_2SeO_3 under nitrogen supply and nitrogen deficiency conditions. This provides new insights into the potential application of Na_2SeO_3 in plant nitrogen-deficient stress environments.

MATERIALS AND METHODS

Materials and sample preparation

The experiment was conducted in the Agricultural Practice Park of Kunming College (E 102°42', N 25°02', Kunming, China) from 2022 to 2023. *Vitis vinifera* × *labrusca* 'Shuijing' grape cuttings were obtained from Dongfeng Farm Management Bureau, Mile City, Yunnan, China. Pest- and disease-free cuttings with healthy growth were selected and transplanted into black nutrient pots (internal diameter = 22 cm and depth = 25 cm). Each pot was filled with 7 L of substrate (vermiculite: perlite: peat = 1: 1: 3) and contained one cutting. The seedlings were cultivated for 10 days in a glass greenhouse at 20–30°C and relative humidity of 80%–90%.

Seven treatment groups were set, repeating five plants per treatment: Control, 0.1Se + 15N ($0.1 \text{ mmol} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_3 + 15 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$), 0.2Se + 15N ($0.2 \text{ mmol} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_3 + 15 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$), 0.4Se + 15N ($0.4 \text{ mmol} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_3 + 15 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$), 0.1Se ($0.1 \text{ mmol} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_3$), 0.2Se ($0.2 \text{ mmol} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_3$), and 0.4Se ($0.4 \text{ mmol} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_3$). To the substrate in each group, 500 mL of treatment solution was poured every 3 days. The specific formulation of the treatment solution is given in Table 1. The experiment was conducted for 70 days. After the treatment, the morphological characteristics of the plants were measured and photographed. Further, healthy leaves in the middle of each plant were picked, washed with DI water thrice, dried, frozen in liquid nitrogen, and stored at −80°C for the determination of growth and physiological indicators and transcriptome sequencing. The experiment included four biological replicates.

Determination of growth indicators

Plant height and stem diameter were determined using a tape measure and vernier calliper, respectively; net increase of plant height = plant height at planting – plant height at the end of treatment; net increase of stem

Table 1. Experimental treatment reagent formula.

Solution	Control/mL	0.1 mmol · L ⁻¹ Na ₂ SeO ₃ + 15 mmol · L ⁻¹ NO ₃ ⁻ (0.1Se + 15N) /mL	0.2 mmol · L ⁻¹ Na ₂ SeO ₃ + 15 mmol · L ⁻¹ NO ₃ ⁻ (0.2Se + 15N) /mL	0.4 mmol · L ⁻¹ Na ₂ SeO ₃ + 15 mmol · L ⁻¹ NO ₃ ⁻ (0.4Se + 15N) /mL	0.1 mmol · L ⁻¹ Na ₂ SO ₃ (0.1Se)/mL	0.2 mmol · L ⁻¹ Na ₂ SeO ₃ (0.2Se)/mL	0.4 mmol · L ⁻¹ Na ₂ SeO ₃ (0.4Se)/mL
1.0 mol/L Ca(NO ₃) ₂	0	5	5	5	0	0	0
1.0 mol/L KNO ₃	0	5	5	5	0	0	0
0.5 mol/L K ₂ SO ₄	5	0	0	0	5	5	5
1.0 mol/L CaCl ₂	5	0	0	0	5	5	5
1.0 mol/L MgSO ₄	2	2	2	2	2	2	2
1.0 mol/L KH ₂ PO ₄	1	1	1	1	1	1	1
20.0 mmol/L FeSO ₄	1	1	1	1	1	1	1
20.0 mmol/L MnSO ₄	1	1	1	1	1	1	1
10.0 mmol/L ZnSO ₄	1	1	1	1	1	1	1
1.0 mmol/L Na ₂ MoO ₄	1	1	1	1	1	1	1
0.1 mol/L H ₃ BO ₃	1	1	1	1	1	1	1
0.01 mol/L CuSO ₄	1	1	1	1	1	1	1
0.01 mol/L Na ₂ SeO ₃	0	10	20	30	10	20	30
Deionised water	981	971	961	951	971	961	951

diameter = stem diameter at planting – stem diameter at the end of treatment; node spacing was assessed using a ruler; root volume was determined using the drainage method; leaf thickness was determined by picking 10 leaves and considering leaf thickness as 1/10 of the measured value; biomass was determined by the whole plant weighing; root/shoot ratio was calculated as the fresh weight of the belowground part/fresh weight of the aboveground part $\times 100\%$.

Measurement of starch and soluble sugar contents

The leaves dried at 80°C and ground into fine powder. Soluble sugar content was determined according to the method by Yemm and Willis (1954). Then, 0.1 g of this powder and 6 mL of 80% (v/v) ethanol were taken in a centrifuge tube, incubated at 80°C for 30 min, and centrifuged. The supernatants were made up to 25 mL with distilled water. The precipitate was used to determine starch content according to the method by Clegg (1956). The precipitate was mixed with 2 mL deionised water and placed in a boiling water bath for 15 min. To this, 2 mL of 9.2 mol $\cdot$ L⁻¹ perchloric acid was added and stirred for 15 min. The mixture was centrifuged, the supernatant was collected, and the volume was made up to 50 mL with water. This solution was used to determine starch content using anthrone reagent.

Measurement of total nitrogen and soluble protein contents

The fresh leaves were cleaned, dried at 85°C, and ground into fine powder. Further, 0.1 g of the powder was digested with K₂Cr₂O₇–H₂SO₄. Total nitrogen content was determined by the Kjeldahl method using a Kjeldahl nitrogen analyser (NKD6280, Shanghai Wanghai Environmental Science and Technology Co., Ltd., CHN). To 0.2 g of the powder, 10 mL of 50 mmol $\cdot$ L⁻¹ sodium phosphate (pH = 7.8) containing 2 mmol $\cdot$ L⁻¹ EDTA and 80 mmol $\cdot$ L⁻¹ L-ascorbic acid was added. The mixture was centrifuged. The supernatant was collected and used to determine soluble protein content using Bradford G-250 reagent (Bradford, 1976).

Measurement of flavonoid content

The contents of flavonoids were measured using a method reported previously (Liu and Zhu, 2007). In brief, 0.5 g of fresh leaves were cut into filaments and immersed in a mixture of 10 mL of 70% ethanol and 0.1 g of CaCO₃ for 24 h. Further, 0.5 mL of the extracted solution was taken in a test tube, to which 1.5 mL of 70% ethanol and 0.3 mL of 5% NaNO₂ were added and incubated for 6 min. Then, 0.3 mL of 10% Al(NO₃)₃ solution was added and incubated for 6 min. Finally, 2 mL of 4% NaOH was added and incubated for 10 min. The absorbance was determined at 510 nm using a spectrophotometer. The flavonoid content was calculated based on the standard curve.

RNA sequencing and bioinformatic analysis

After 70 days of treatment as indicated in Section 2.1, through the comprehensive analysis of morphological and physiological indicators, grape leaves from the Control, 0.2Se + 15N, and 2Se groups were finally selected as the test materials, with three replicates for each treatment. Total RNA was extracted from the grape leaves using the TRIzol method. The concentration and purity of the total RNA were examined using Nanodrop 2000 (ThermoFisher Scientific, US). The integrity of RNA and DNA or protein contamination was examined using agarose gel electrophoresis. The RIN was determined using Agilent 5300 (Agilent Technologies, US). After passing the test, the library construction and non-parametric transcriptome sequencing (Illumina Novaseq 6000, Illumina Corporation, US) were commissioned to Shanghai Major Biomedical Technology Co., Ltd., CHN. The raw reads obtained from sequencing were subjected to quality control by removing reads with adapters, N ratio >10% (indicating that base information could not be determined), and all A bases and low-quality reads (bases with a quality value of $Q_{\text{phred}} \leq 20$ accounting for more than 50% of the entire reads) to obtain high-quality clean reads for subsequent analysis (Liu et al., 2019).

Using Hisat2 software to perform sequence alignment between clean reads and the grape reference genome (http://plants.ensembl.org/Vitis_vinifera/Info/Index), obtaining positional information on the reference genome and unique sequence feature information of the sequencing sample. The reads per fragments per kilobase of transcript per million mapped reads (FPKM values) were calculated. Then, the gene and transcriptional expression levels were detected using the RSEM tool, and the *p*-adjust and fold change (FC) values were calculated using the Nbinom Test function. *p*-adjust ≤ 0.05 and $|\log_2 \text{FC}| > 1$ were used as the screening criterion to obtain differentially expressed genes (DEGs) under different treatments. Finally, differentially expressed genes based on the GO Database (<http://geneontology.org/>) and KEGG Database (<http://www.genome.jp/kegg/>) perform gene function annotation. Perform GO enrichment analysis on DEGs using Goatools software (<https://github.com/tanghaibao/GOatools>) and use Fisher's exact test; using KOBAS software (<https://bioinformaticshome.com/tools/rna-seq/descriptions/KOBAS.html>) for KEGG enrichment analysis of DEGs, the calculation principle was the same as GO functional analysis. To control the false positive rate, the Bonferroni method was used for multiple tests, with a *p*-adjust value ≤ 0.05 as the threshold. GO and KEGG pathways that meet this condition were defined as pathways significantly enriched in differentially expressed genes.

Data analysis

Experimental data were organised using Microsoft Excel 2019 software. SPSS 27 software (International

Business Machines Corporation, US) was used for one-way ANOVA and Duncan's test to compare growth and physiological indicators. The results were expressed as the mean $\pm$ standard deviation of four replicates. $p < 0.05$ was considered significant. Graphs were plotted using GraphPad Prism 9 software (GraphPad software, LLC., US) and Origin 2021 software (OriginLab Corporation, US).

RESULTS AND ANALYSIS

Effects of Na_2SeO_3 treatment on the growth and physiological characteristics of grape under various nitrogen conditions

Significant differences were observed in the growth status of grape plants in various groups after 70 days of treatment (Figure 1A). The 0.2Se + 15N group exhibited the most vigorous growth, and the Control exhibited the weakest growth of above- and belowground parts. Compared with the Control, the 0.1Se + 15N, 0.2Se + 15N, 0.4Se + 15N, 0.1Se, 0.2Se, and 0.4Se groups exhibited an increase in the net growth of grape plants by 114.32%, 145.02%, 73.00%, 78.88%, 93.15%, and 26.19%, respectively; net growth of stem diameter by 21.54%, 38.31%, -23.87%, -34.37%, 6.93%, and -18.96%, respectively; internodal spacing by 27.55%, 30.41%, 11.27%, 36.88%, 44.97%, and 6.46%, respectively; root volume by 82.46%, 144.52%, 72.44%, 46.62%, 82.59%, and 56.63%, respectively; leaf thickness by -5.90%, 5.62%, 1.76%, 9.84%, 13.39%, and 5.39%, respectively; biomass by 84.83%, 112.51%, 52.03%, 55.76%, 76.89%, and 55.94%, respectively; and root/shoot ratio by 25.14%, 36.67%, 26.90%, 30.58%, 48.62%, and 22.10%, respectively (Figure 1B). Additionally, they exhibited an increase in flavonoid content by 12.36%, 21.98%, 15.16%, -5.27%, -9.56%, and -14.78%, respectively (Figure 1C); starch content by 14.58%, 22.12%, 8.60%, 41.18%, 61.86%, and 51.52%, respectively; soluble sugar content by 24.74%, 59.93%, 37.83%, 27.40%, 11.55%, and 7.89%, respectively; total nitrogen content by 79.98%, 73.17%, 69.97%, 33.61%, 49.06%, and 42.04%, respectively; and soluble protein content by 38.86%, 89.22%, 10.78%, 5.10%, 51.85%, and 6.09%, respectively (Figure 1D), compared with the Control. Overall, Na_2SeO_3 treatment considerably increased root volume, biomass accumulation, and root/shoot ratio and promoted plant height and nitrogen uptake under various nitrogen conditions.

Transcriptome analysis of grape leaves after Na_2SeO_3 treatment under various nitrogen conditions

The Control, 0.2Se + 15N, and 0.2Se groups were selected for transcriptome analysis based on the results described in Section 3.1. Transcriptome sequencing was performed on 9 samples using the Illumina platform, and 54.99 Gb clean bases and 0.38 Gb raw reads were obtained. The clean reads, error rate, GC content for

each sample, and percentage of Q20 and Q30 bases were 0.37 Gb, <0.0258%, 45.69%–46.82%, >97.73%, and >93.32%, respectively (Table 2). This indicated that the accuracy of the sequencing data was high, which met the requirements of quality control and facilitated data analysis at the later stage. Using Trinity to assemble high-quality sequences from scratch, 147924 transcripts were obtained, with an N50 length of 2268 bp, and the C value was 89.7%, with an S value of 64.2% and a D value of 25.5%. A total of 89421 unigenes were obtained, with an N50 length of 1954 bp, and the C value was 73.5%, with an S value of 70.2% and a D value of 3.3% (Table 3). The clean reads of each sample were compared with the reference sequences obtained from the Trinity assembly to obtain the mapping results of each sample, which was also the basis for the quantification of the genes and transcripts of each sample. The mapped ratio of each sample was >87.96% (Table 4). Overall, the quality of the samples was good and suitable for subsequent bioinformatic analysis.

The correlation coefficients of the transcriptome sequencing samples between different replicates of the Control, 0.2Se, and 0.2Se + 15N groups were >0.94, 0.99, and 0.97, respectively (Figure 2A), indicating good reproducibility within the groups. These groups contained 18077, 17922 and 17555 unigenes, respectively, with 16861 shared unigenes (Figure 2B), that the expression estimated for transcripts assembled from all samples simultaneously. Based on $\text{FDR} < 0.05$ and $|\log_2\text{FC}| \geq 1$, the DEGs in various groups were identified. Visualisation of results using a volcano plot indicated that 0.2Se + 15N versus Control, 0.2Se versus Control, and 0.2Se versus 0.2Se + 15N had 1196 (395 up- and 801 downregulated), 2238 (520 up- and 1718 downregulated), and 1980 (678 up- and 1302 downregulated) DEGs, respectively (Figures 2D–2F), and 0.2Se + 15N versus Control, 0.2Se versus Control, and 0.2Se versus 0.2Se + 15N had 294, 753, and 294 DEGs, respectively. These comparison groups had 179 co-expressed unigenes (Figure 2C). Overall, Na_2SeO_3 treatment considerably promoted the production of DEGs and grape growth under various nitrogen conditions.

GO pathway enrichment analysis of DEGs in grape after Na_2SeO_3 treatment under various nitrogen conditions

To clarify the biological functions associated with the DEGs after Na_2SeO_3 treatment under various nitrogen conditions, GO pathway enrichment analysis was conducted on the top 40 DEGs with $\text{FDR} < 0.05$ in 0.2Se + 15N versus Control, 0.2Se versus Control, and 0.2Se + 15N versus 0.2Se (Figure 3). DEGs present in all three comparisons were involved in catalytic activity (GO:0003824), oxidoreductase activity (GO:0016491), transferase activity, transferring acyl groups (GO:0016746), transferase activity, transferring acyl groups other than amino-acyl groups (GO:0016747), tetrapyrrole binding (GO:0046906), heme binding

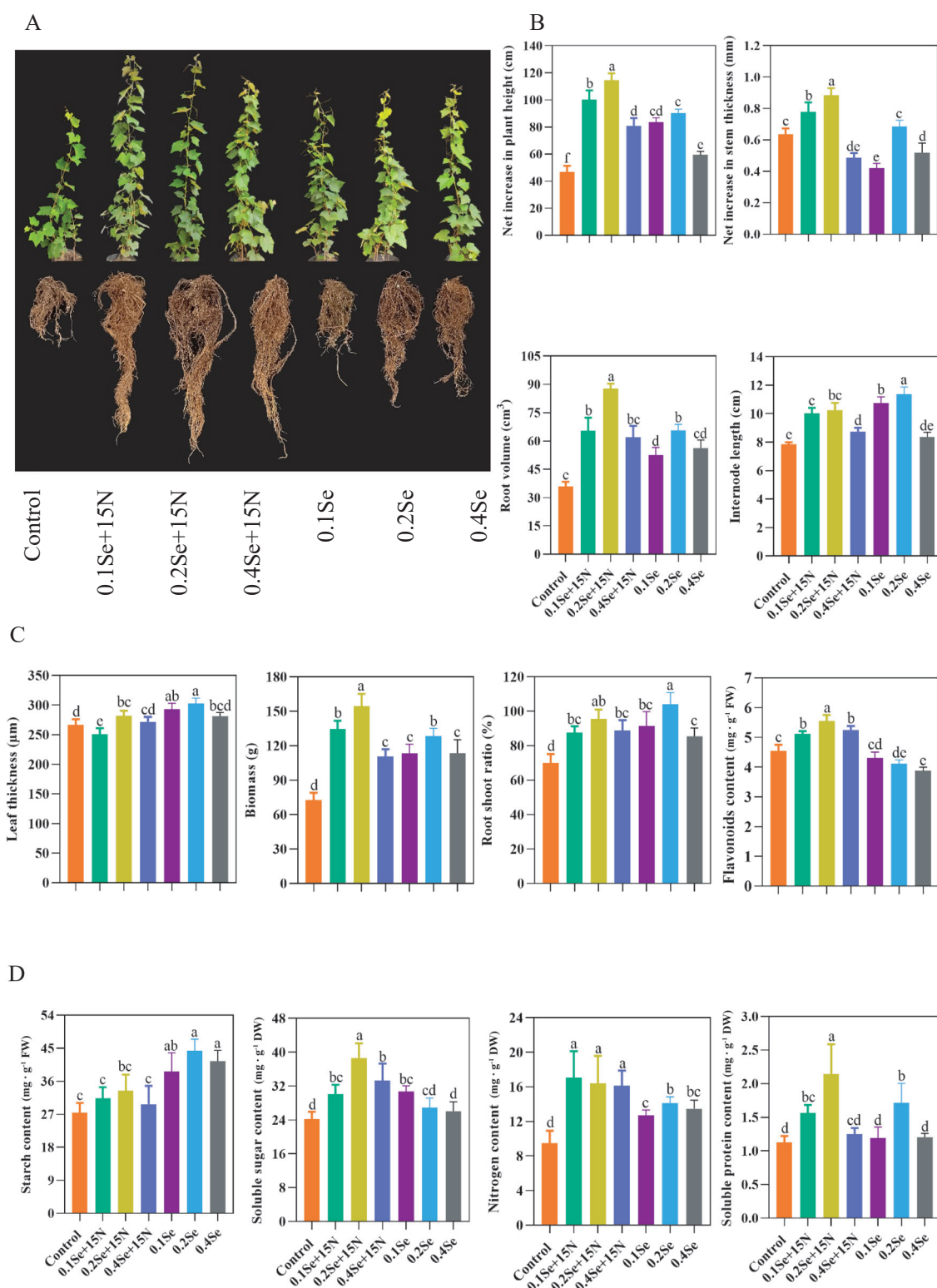


Figure 1. Effects of Na_2SeO_3 treatment on the growth and physiological characteristics of grape under various nitrogen conditions. (A) The growth status of grape plants; (B) the net growth of plant height, the net growth of stem thickness, root volume and internode length of grape; (C) leaf thickness, biomass, root shoot ratio and flavonoids content of grape; (D) starch content, soluble sugar content, nitrogen content and soluble protein content of grape. The results were shown as mean $\pm$ standard deviation ($n = 4$), and one-way ANOVA was used to compare the significant differences among the treatments. Different lowercase letters on the bar graph indicate significant differences at the $p < 0.05$ level. Control group is Control; 0.1 mmol $\cdot$ L $^{-1}$ + 15 mmol $\cdot$ L $^{-1}$ N is 0.1Se + 15 N; 0.2 mmol $\cdot$ L $^{-1}$ + 15 mmol $\cdot$ L $^{-1}$ N is 0.2Se + 15 N; 0.4 mmol $\cdot$ L $^{-1}$ + 15 mmol $\cdot$ L $^{-1}$ N is 0.4Se + 15 N; 0.1 mmol $\cdot$ L $^{-1}$ Na_2SeO_3 is 0.1Se; 0.2 mmol $\cdot$ L $^{-1}$ Na_2SeO_3 is 0.2Se; 0.4 mmol $\cdot$ L $^{-1}$ Na_2SeO_3 is 0.4Se.

Table 2. Grape transcriptome sequencing quality data treated with Na₂SeO₃ and nitrogen.

Sample	Raw reads	Clean reads	Clean bases	Error rate (%)	Q20 (%)	Q30 (%)	GC content (%)
Control-1	44342452	43068728	6392908153	0.0252	97.92	93.97	46.14
Control-2	51674990	50251708	7420078791	0.0255	97.81	93.73	46.66
Control-3	58656668	56709132	8314120252	0.0251	97.96	94.14	46.80
0.2Se + 15N-1	61956434	60368950	8886922304	0.0252	97.94	94.10	46.57
0.2Se + 15N-2	53393402	51882436	7638583022	0.0256	97.77	93.68	46.64
0.2Se + 15N-3	51910586	50527188	7411722373	0.0249	98.04	94.32	46.82
0.2Se-1	43739408	42003504	6241771742	0.0258	97.69	93.44	46.06
0.2Se-2	45886300	44202816	6568402293	0.0256	97.80	93.66	45.69
0.2Se-3	46115382	44372272	6564671570	0.0257	97.73	93.54	45.94

Clean reads: The total number of entries in the sequencing data after quality control; Clean bases: Total amount of sequencing data after quality control; Error rate (%): The average error rate of sequencing bases corresponding to quality control data; Q20 and Q30 (%): respectively refer to the percentage of bases with sequencing quality above 99% and 99.9% in total bases; GC content (%): The percentage of the total number of G and C bases corresponding to the quality control data to the total number of bases.

Table 3. Assembly quality for transcript and unigene of grape.

Type	Unigene	Transcript
Total number	89,421	147,924
N50 length (bp)	1954	2268
BUSCO	C: 73.5%[S: 70.2%; D: 3.3%]	C: 89.7%[S: 64.2%; D: 25.5%]

Total number, the number of sequence entries of the assembled unigene/transcript. N50 length, sort the assembled unigene/transcript in descending order of length, and accumulate the length of the transcript to half of the total length, corresponding to the length of the transcript. BUSCO, using BUSCO to evaluate assembly integrity score, the higher the score, the better the complete (C). Complete (C) represents the proportion of sequences that reach the desired length in the assembled sequence compared to the total BUSCO sequence, consisting of two parts. S represents a sequence that can align with one gene in the database, and D represents a sequence that can align with multiple genes in the database.

(GO:0020037), extracellular region (GO:0005576), cell wall (GO:0005618), external encapsulating structure (GO:0030312), oxidation-reduction process (GO:0055114), response to abiotic stimulus (GO:0009628), and cell wall organisation or biogenesis (GO:0071554). DEGs only present in 0.2Se + 15N versus Control were involved in DNA binding (GO:0003677), DNA-binding transcription factor activity (GO:0003700), lyase activity (GO:0016829), plasmodesma (GO:0009506), polymeric cytoskeletal fibre (GO:0099513), supramolecular polymer (GO:0099081), response to stimulus (GO:0050896), transmembrane transport (GO:0055085), lipid biosynthetic process (GO:0008610), small molecule biosynthetic process (GO:0044283), microtubule-based process (GO:0007017), response to oxygen-containing compound (GO:1901700), response to external stimulus (GO:0009605), ion transmembrane transport (GO:0034220), mitotic cell cycle process (GO:1903047), organic acid biosynthetic process (GO:0016053), carboxylic acid biosynthetic process (GO:0046394), regulation of cell cycle

Table 4. Grape comparison and statistics of sequencing data and assembly results treated with Na₂SeO₃ and nitrogen.

Sample	Clean reads	Mapped reads	Mapped ratio (%)
Control-1	43068728	38170787	88.63
Control-2	50251708	44199319	87.96
Control-3	56709132	50304056	88.71
0.2Se + 15N-1	60368950	53347721	88.74
0.2Se + 15N-2	51882436	45851321	88.27
0.2Se + 15N-3	50527188	44810011	88.34
0.2Se-1	42003504	37275528	88.37
0.2Se-2	44202816	39017275	88.38
0.2Se-3	44372272	39199620	88.68

Clean reads: the number of filtered sequencing data entries; mapped reads: the number of clean reads that can be compared to the assembled transcript; mapped ratio: the percentage of clean reads that can be located on the assembled transcript.

(GO:0051726), inorganic ion transmembrane transport (GO:0098660). DEGs only present in 0.2Se versus Control were involved in monooxygenase activity (GO:0004497), Golgi apparatus (GO:0005794), protein phosphorylation (GO:0006468), external encapsulating structure organisation (GO:0045229), and cell wall organisation (GO:0071555). DEGs only present in 0.2Se versus 0.2Se + 15N were involved in apoplast (GO:0048046), organic substance catabolic process (GO:1901575), on transport (GO:0006811), regulation of biological quality (GO:0065008), homeostatic process (GO:0042592), cation transport (GO:0006812), metal ion transport (GO:0030001), response to inorganic substance (GO:0010035), and inorganic ion homeostasis (GO:0098771). Overall, the high-frequency occurrence of GO-enriched DEGs after Na₂SeO₃ treatment may be a key factor in promoting grape growth under various nitrogen conditions.

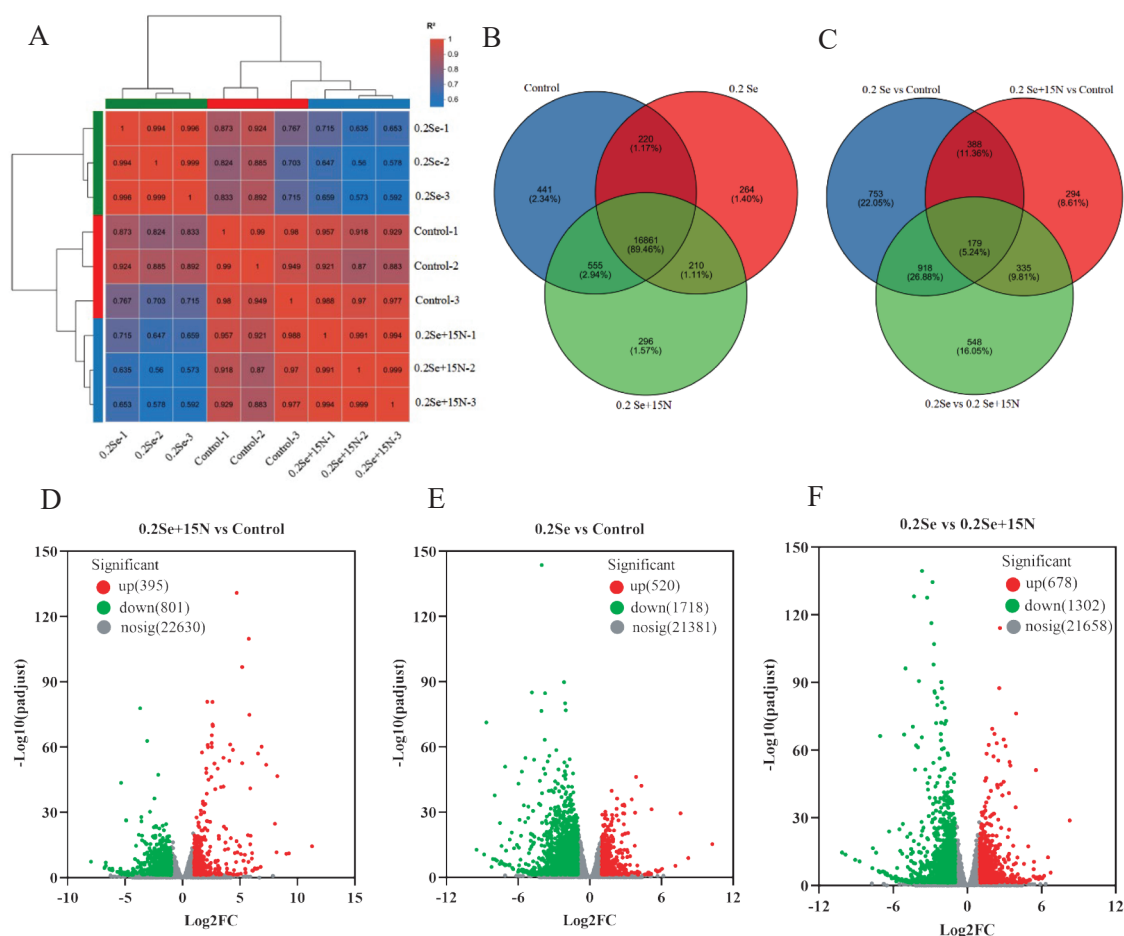


Figure 2. Transcriptome analysis of grape leaves after Na_2SeO_3 treatment under various nitrogen conditions. (A) Correlation between samples; (B) unigene distribution and group overlap; (C) Venn diagram displaying the number of DEGs in each comparison group and group overlap; (D–F) The DEGs in 0.2Se + 15N versus Control, 0.2Se versus Control, and 0.2Se + 15N vs 0.2Se are plotted in a volcano plot. Control group is Control; 0.1 $\text{mmol} \cdot \text{L}^{-1}$ + 15 $\text{mmol} \cdot \text{L}^{-1}$ N is 0.1Se + 15 N; 0.2 $\text{mmol} \cdot \text{L}^{-1}$ + 15 $\text{mmol} \cdot \text{L}^{-1}$ N is 0.2Se + 15 N; 0.4 $\text{mmol} \cdot \text{L}^{-1}$ + 15 $\text{mmol} \cdot \text{L}^{-1}$ N is 0.4Se + 15 N; 0.1 $\text{mmol} \cdot \text{L}^{-1}$ Na_2SeO_3 is 0.1Se; 0.2 $\text{mmol} \cdot \text{L}^{-1}$ Na_2SeO_3 is 0.2Se; 0.4 $\text{mmol} \cdot \text{L}^{-1}$ Na_2SeO_3 is 0.4Se.

KEGG pathway enrichment analysis of DEGs in grape after Na_2SeO_3 treatment under various nitrogen conditions

To further reveal the growth- and physiology-related functions of DEGs affected by Na_2SeO_3 treatment under various nitrogen conditions, based on the results of GO pathway enrichment analysis, the entries with $P_{\text{adjust}} < 0.05$ and a higher number of enriched genes were further selected for KEGG pathway enrichment analysis (Figure 4). DEGs present in all three comparisons were involved in flavonoid biosynthesis (map00941), phenylpropanoid biosynthesis (map00940), starch and sucrose metabolism (map00500), plant hormone signal transduction (map04075), MAPK signalling pathway—plant (map04016), and plant-pathogen interaction (map04626). DEGs only present in 0.2Se + 15N versus Control were involved in stilbenoid, diarylheptanoid, and gingerol biosynthesis (map00945) and glycine, serine, and threonine metabolism (map00260). DEGs only present in 0.2Se versus Control were involved in tyrosine metabolism (map00350), phagosome (map04145), and

endocytosis (map04144). DEGs only present in 0.2Se versus 0.2Se + 15N were involved in protein processing in the endoplasmic reticulum (map04141), galactose metabolism (map00052), and oxidative phosphorylation (map00190). These results indicate that flavonoid biosynthesis (map00941), phenylpropanoid biosynthesis (map00940), starch and sucrose metabolism (map00500), and plant hormone signal transduction (map04075), which are relatively abundant and present in all three comparison groups, play important roles in promoting grape plant growth.

Key DEGs in grape after Na_2SeO_3 treatment under various nitrogen conditions

DEGs involved in flavonoid and phenylpropane biosynthesis

Under various nitrogen conditions, the DEGs involved in biosynthesis of flavonoids and phenylpropanes were significantly enriched after Na_2SeO_3 treatment (Figure 4). Control versus 0.2Se + 15N had 30 DEGs

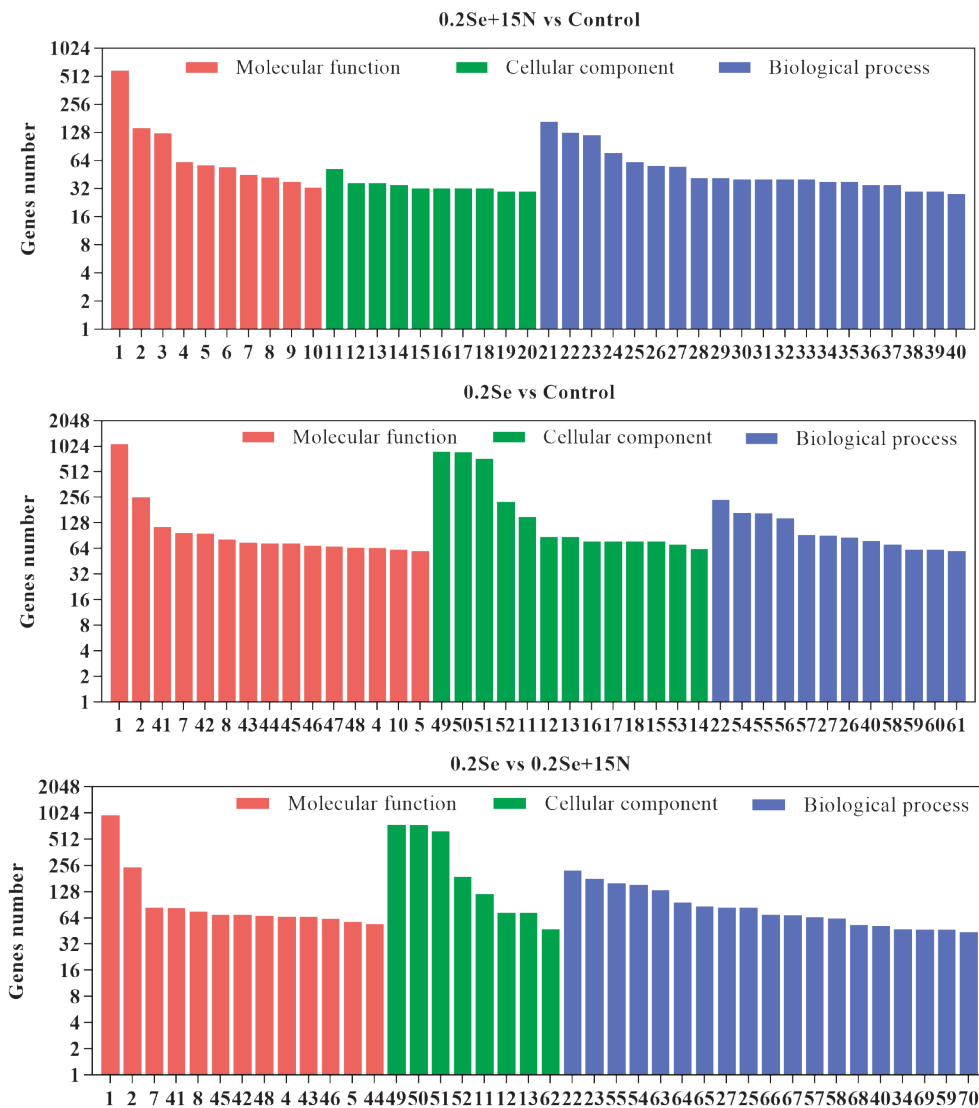


Figure 3. GO pathway enrichment analysis of DEGs in grape after Na_2SeO_3 treatment under various nitrogen conditions. The numbers listed on the horizontal axis represent the top 40 GO-enriched entries in different comparison groups ($P_{\text{adjust}} < 0.05$). Control group is Control; $0.2 \text{ mmol} \cdot \text{L}^{-1} + 15 \text{ mmol} \cdot \text{L}^{-1} \text{ N}$ is 0.2 Se + 15 N; $0.2 \text{ mmol} \cdot \text{L}^{-1} \text{ Na}_2\text{SeO}_3$ is 0.2 Se. 1. Catalytic activity; 2. oxidoreductase activity; 3. DNA binding; 4. transferase activity, transferring acyl groups; 5. transferase activity, transferring acyl groups other than amino-acyl groups; 6. DNA-binding transcription factor activity; 7. tetrapyrrole binding; 8. heme binding; 9. lyase activity; 10. cytoskeletal protein binding; 11. extracellular region; 12. cell wall; 13. external encapsulating structure; 14. supramolecular complex; 15. plasmodesma; 16. anchoring junction; 17. cell-cell junction; 18. cell junction; 19. polymeric cytoskeletal fibre; 20. supramolecular polymer; 21. response to stimulus; 22. oxidation-reduction process; 23. response to stress; 24. transmembrane transport; 25. response to chemical; 26. cell cycle process; 27. response to abiotic stimulus; 28. lipid biosynthetic process; 29. small molecule biosynthetic process; 30. microtubule-based process; 31. response to oxygen-containing compound; 32. response to external stimulus; 33. ion transmembrane transport; 34. response to oxidative stress; 35. mitotic cell cycle process; 36. organic acid biosynthetic process; 37. carboxylic acid biosynthetic process; 38. regulation of cell cycle; 39. inorganic ion transmembrane transport; 40. cell wall organisation or biogenesis; 41. transferase activity, transferring glycosyl groups; 42. transferase activity, transferring hexosyl groups; 43. hydrolase activity, acting on glycosyl bonds; 44. UDP-glycosyltransferase activity; 45. oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; 46. hydrolase activity, hydrolysing O-glycosyl compounds; 47. monooxygenase activity; 48. iron ion binding; 49. intrinsic component of membrane; 50. integral component of membrane; 51. membrane; 52. plasma membrane; 53. Golgi apparatus; 54. carbohydrate metabolic process; 55. catabolic process; 56. protein phosphorylation; 57. polysaccharide metabolic process; 58. cellular carbohydrate metabolic process; 59. cellular polysaccharide metabolic process; 60. external encapsulating structure organisation; 61. cell wall organisation; 62. apoplast; 63. organic substance catabolic process; 64. ion transport; 65. regulation of biological quality; 66. homeostatic process; 67. cation transport; 68. metal ion transport; 69. response to inorganic substance; 70. inorganic ion homeostasis.

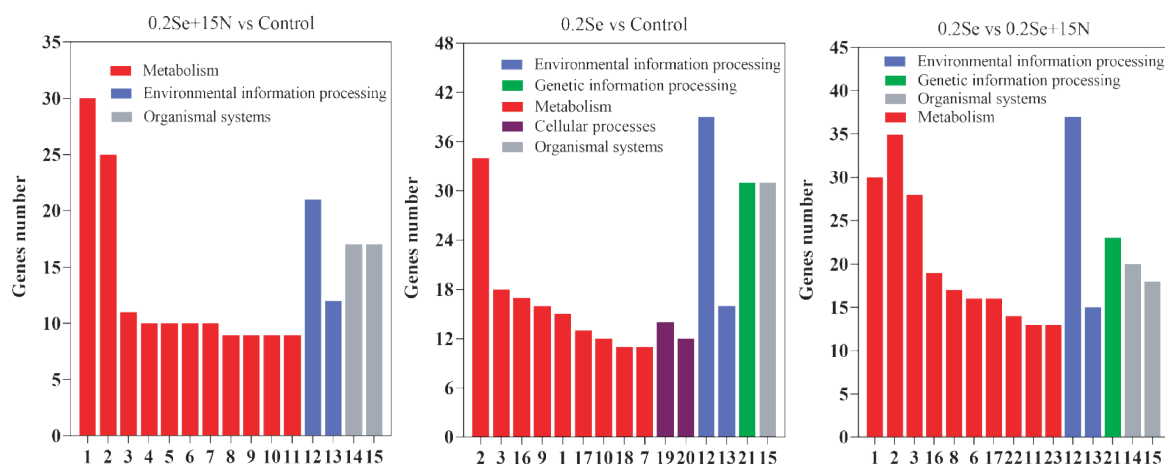


Figure 4. KEGG pathway enrichment analysis of DEGs in grape after Na_2SeO_3 treatment under various nitrogen conditions. The numbers listed on the horizontal axis represent the top 15 KEGG enrichment items in different treatment comparisons ($P_{\text{adjust}} < 0.05$). Control group is Control; $0.2 \text{ mmol} \cdot \text{L}^{-1} + 15 \text{ mmol} \cdot \text{L}^{-1} \text{ N}$ is 0.2 Se + 15 N; $0.2 \text{ mmol} \cdot \text{L}^{-1} \text{ Na}_2\text{SeO}_3$ is 0.2 Se. 1. Flavonoid biosynthesis; 2. phenylpropanoid biosynthesis; 3. starch and sucrose metabolism; 4. stilbenoid, diarylheptanoid, and gingerol biosynthesis; 5. glycine, serine, and threonine metabolism; 6. glycerolipid metabolism; 7. cysteine and methionine metabolism; 8. glutathione metabolism; 9. pentose and glucuronate interconversions; 10. monoterpene biosynthesis; 11. glycolysis/gluconeogenesis; 12. plant hormone signal transduction; 13. MAPK signalling pathway–plant; 14. circadian rhythm–plant; 15. plant-pathogen interaction; 16. glycerophospholipid metabolism; 17. amino sugar and nucleotide sugar metabolism; 18. tyrosine metabolism; 19. phagosome; 20. endocytosis; 21. protein processing in endoplasmic reticulum; 22. galactose metabolism; 23. oxidative phosphorylation.

related to flavonoid biosynthesis pathway, wherein 12 DEGs encoding CHS, ST, PGT1, and E2.3.1.133 were significantly upregulated ($\text{FDR} < 0.05$). Control versus 0.2Se had nine DEGs related to flavonoid biosynthesis pathway, and all of them were significantly downregulated ($\text{FDR} < 0.05$); 0.2Se + 15N versus 0.2Se had 17 DEGs related to flavonoid biosynthesis pathway, wherein 11 DEGs encoding CYP75A, CHS, ST, E2.1.1.104, DFR, F3H, and LAR were significantly upregulated ($\text{FDR} < 0.05$) (Table 5, Figure 5). Control versus 0.2Se + 15N had nine DEGs related to phenylpropane biosynthesis pathway, wherein six DEGs encoding phenylalanine ammonia lyase (PAL), E1.11.1.7, E2.3.1.133, and 4-coumarate-CoA ligase (4CL) were significantly upregulated ($\text{FDR} < 0.05$). Control versus 0.2Se had 22 DEGs related to phenylpropane biosynthesis pathway, wherein two DEGs encoding E3.2.1.21 and E1.11.1.7 were significantly upregulated ($\text{FDR} < 0.05$); 0.2Se versus 0.2Se + 15N had 35 DEGs related to phenylpropane biosynthesis pathway, wherein DEGs encoding E3.2.1.21, E1.11.1.7, CYP73A, and bglX were significantly upregulated ($\text{FDR} < 0.05$) (Table 6, Figure 5). Overall, the synergistic effect of Na_2SeO_3 and nitrogen was beneficial for the biosynthesis of flavonoids and phenylpropanes in grape leaves.

DEGs involved in plant hormone signal transduction

Under various nitrogen conditions, the DEGs involved in plant hormone signal transduction in grape leaves

were significantly enriched after Na_2SeO_3 treatment (Figure 6). Control versus 0.2Se + 15N had nine such DEGs, wherein four DEGs encoding SAUR, PR1, IRAK4, and AHP were significantly upregulated ($\text{FDR} < 0.05$). Control versus 0.2Se had 39 such DEGs, wherein 20 DEGs encoding ARR-A, SAUR, GH3, PR1, IAA, GID1, IRAK4, AHP, TGA, and PP2C were significantly upregulated ($\text{FDR} < 0.05$); 0.2Se + 15N versus 0.2Se had 20 such DEGs, wherein 11 DEGs encoding ARR-A, SAUR, GH3, PR1, IAA, GID1, TGA, and PP2C were upregulated significantly ($\text{FDR} < 0.05$) (Table 7, Figure 6). Overall, Na_2SeO_3 treatment significantly upregulated more DEGs related to auxin and gibberellin (GA) than significantly downregulated, thereby promoting grape growth.

DEGs involved in starch and sucrose metabolisms

KEGG pathway enrichment analysis revealed that the DEGs involved in starch and sucrose metabolisms were enriched after Na_2SeO_3 treatment (Figure 4). Various unigenes, including E3.2.1.21, WAXY, TPS, bglX, E3.2.1.4, E2.7.1.4, E2.4.1.13, GN4, malZ, AMY, INV, and HK, were involved in starch and sucrose metabolism pathways (Figure 7). Control versus 0.2Se + 15N had four DEGs related to starch and sucrose metabolisms, wherein two DEGs encoding TPS were significantly upregulated ($\text{FDR} < 0.05$). Control versus 0.2Se had 13 DEGs related to starch and sucrose metabolisms, wherein only one DEG encoding E3.2.1.21 was significantly upregulated ($\text{FDR} < 0.05$);

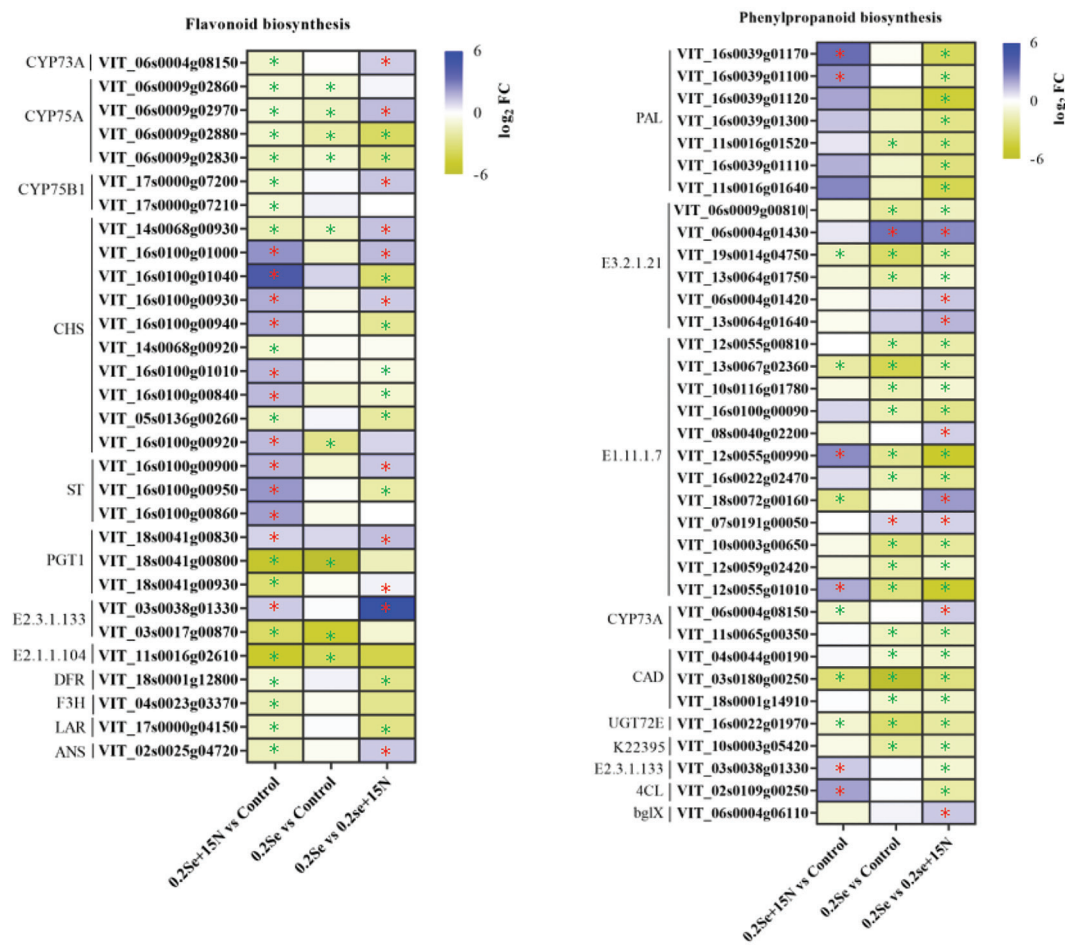


Figure 5. Key DEGs involved in flavonoid and phenylpropane biosynthesis in grape after Na₂SeO₃ treatment under various nitrogen conditions. CYP73A: nodulation receptor kinase; CYP75A: flavonoid 3',5'-hydroxylase; CYP75B1: flavonoid 3'-monooxygenase; CHS/ST: stilbene synthase; PGT1: UDP-glycosyltransferase; E2.3.1.133: stemmadenine O-acetyltransferase; E2.1.1.104: probable caffeoyl-CoA O-methyltransferase; DFR: dihydroflavonol 4-reductase; F3H: naringenin,2-oxoglutarate 3-dioxygenase; LAR: leucoanthocyanidin reductase; ANS: leucoanthocyanidin dioxygenase; E3.2.1.21: beta-glucosidase; E1.11.1.7: lignin-forming anionic peroxidase; CYP73A: trans-cinnamate 4-monooxygenase; CAD: probable mannitol dehydrogenase; UGT72E: anthocyanidin 3-O-glucosyltransferase; E2.3.1.133: stemmadenine O-acetyltransferase; 4CL: 4-coumarate-CoA ligase; bglX: xylan 1,4-beta-xylosidase. *FDR < 0.05, where red represents significant upregulation and green represents significant downregulation. PAL, phenylalanine ammonia lyase.

0.2Se + 15N versus 0.2Se had 28 DEGs related to starch and sucrose metabolisms, wherein nine DEGs encoding E3.2.1.21, WAXY, TPS, bglX, and AMY were significantly upregulated (FDR < 0.05) (Table 8, Figure 7).

DISCUSSION

Effects of Na₂SeO₃ treatment on growth and physiological characteristics of grape under various nitrogen conditions

The growth, morphology, and distribution of roots are affected by the nutrients in soil (Lynch, 2011). In this study, grape root growth was significantly better in the Se and Se + N groups than in the Control groups, and

the combination of Se and N was more effective than Se alone. Se and N were closely related to the growth and development of grape. The application of 0.2 mmol · L⁻¹ Na₂SeO₃ + 15 mmol · L⁻¹ NO₃⁻ significantly increased the root volume, plant height, and biomass of grape compared with other treatments. Grape plant growth was inhibited when Na₂SeO₃ concentration increased to 0.4 mmol · L⁻¹. These were consistent with previous studies (El-Hendawy et al., 2017; Wang et al., 2019). The Se application can significantly increase the contents of soluble solids in plants (Fan et al., 2024). In this study, Se + N application significantly increased soluble sugar content in grape leaves, with 0.1 mmol · L⁻¹ Na₂SeO₃ treatment exhibiting the most significant increase. Se + N or Se application increased nitrogen and soluble protein contents in grape leaves; however,

Table 5. Differential expression results of flavonoid biosynthesis.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control		0.2Se versus Control		0.2Se versus 0.2Se + 15N	
			Log ₂ FC	FDR	Log ₂ FC	FDR _t	Log ₂ FC	FDR _t
Trans-cinnamate 4-monooxygenase	VIT_06s0004g08150	CYP73A	-1.24	0.00489	-0.13	0.51476	1.14	0.00104
Chalcone synthase	VIT_14s0068g00930	CHS	-1.65	0.03460	-1.39	0.00004	1.28	0.01740
Dihydroflavonol reductase	VIT_18s0001g12800	DFR	-1.11	0.01526	0.36	0.02693	-1.49	0.00002
Stilbene synthase	VIT_16s0100g01000	CHS	2.44	0.00540	-1.09	0.59531	3.67	0.00024
Stilbene synthase	VIT_16s0100g00860	ST	2.15	0.00000	-0.54	0.45448	-0.27	0.10247
Flavanone 3-hydroxylase	VIT_04s0023g03370	F3H	-1.73	0.03047	-0.45	0.01205	1.29	0.06844
Glycosyltransferase	VIT_18s0041g00830	PGT1	1.01	0.04337	0.90	0.09188	1.29	0.04528
Flavonoid-3'- hydroxylase	VIT_17s0000g07200	CYP75B1	-1.20	0.03266	0.16	0.35881	1.38	0.00276
Unnamed protein product	VIT_17s0000g04150	LAR	-1.41	0.00198	0.11	0.55600	-1.54	0.00039
Stilbene synthase	VIT_16s0100g01040	CHS	4.44	0.03436	1.01	1.00000	-3.39	0.06491
Anthocyanidin synthase	VIT_02s0025g04720	ANS	-1.57	0.01520	-0.41	0.01691	1.18	0.03871
Stilbene synthase	VIT_16s0100g00930	CHS	1.88	0.00301	-0.61	0.60305	2.48	0.00000
Flavonoid 3',5'-hydroxylase	VIT_06s0009g02970	CYP75A	-1.00	0.00000	-1.43	0.00000	1.41	0.00252
Flavonoid 3',5'-hydroxylase	VIT_06s0009g02880	CYP75A	-1.22	0.00346	-1.98	0.00000	-2.73	0.38401
Anthocyanin acyltransferase	VIT_03s0017g00870	E2.3.1.133	-3.60	0.00000	-4.76	0.00000	-1.14	0.12038
Stilbene synthase	VIT_16s0100g00940	CHS	1.82	0.00030	-0.44	0.60887	-2.25	0.00001
Chalcone synthase isoform	VIT_14s0068g00920	CHS	-1.23	0.03394	-0.29	0.13223	0.96	0.038498
Unnamed protein product	VIT_11s0016g02610	E2.1.1.104	-4.99	0.02208	-3.75	0.07667	1.26	1.00000
Stilbene synthase	VIT_16s0100g01010	CHS	1.62	0.00326	-0.45	0.65109	-2.05	0.00075
Flavonoid 3',5'-hydroxylase	VIT_06s0009g02860	CYP75A	-1.03	0.03085	-1.03	0.01032	0.02	0.94689
Flavonoid 3'-monooxygenase	VIT_17s0000g07210	CYP75B1	-1.09	0.00124	0.33	0.28077	0.14	0.50203
UDP- glycosyltransferase	VIT_18s0041g00800	PGT1	-5.41	0.00000	-7.00	0.00000	-1.62	1.00000
Hypothetical protein	VIT_06s0009g02830	CYP75A	-1.42	0.00052	-1.07	0.00000	-1.37	0.04739
UDP- glycosyltransferase	VIT_18s0041g00930	PGT1	-3.30	0.03199	-0.35	0.58657	-1.09	0.00002
Unnamed protein product	VIT_03s0038g01330	E2.3.1.133	1.20	0.0000	0.10	0.66635	7.94	0.00000
Stilbene synthase	VIT_16s0100g00920	CHS	1.57	0.03144	-2.68	0.03760	0.92	0.07035
Stilbene synthase	VIT_16s0100g00900	ST	1.68	0.02265	-1.04	0.37134	2.71	0.00043
Hypothetical protein	VIT_16s0100g00950	ST	2.31	0.04592	-0.32	0.90820	-2.62	0.03463
Stilbene synthase	VIT_16s0100g00840	CHS	1.58	0.00000	-1.22	0.07250	-1.78	0.00000
Chalcone synthase	VIT_05s0136g00260	CHS	-1.48	0.04403	0.26	0.11752	-1.92	0.05836

Control group is Control; 0.2 mmol · L⁻¹ + 15 mmol · L⁻¹ N is 0.2Se + 15 N; 0.2 mmol · L⁻¹ Na₂SeO₃ is 0.2Se. FDR < 0.05000 indicates significant differences.
FC, fold change.

Table 6. Differential expression results of phenylpropanoid biosynthesis.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control		0.2Se versus Control		0.2Se versus 0.2Se + 15N	
			Log ₂ FC	FDR	Log ₂ FC	FDR	Log ₂ FC	FDR
Phenylalanine ammonia lyase	VIT_16s0039g01170	PAL	3.31	0.01280	-0.38	1.00000	-3.78	0.00447
Uncharacterised protein	VIT_12s0055g00810	E1.11.1.7	-0.78	0.00031	-2.28	0.00000	-1.49	0.00000
Phenylalanine ammonia lyase	VIT_16s0039g01100	PAL	2.40	0.03015	0.04	0.98827	-2.37	0.03448
Unnamed protein product	VIT_06s0004g01430	E3.2.1.21	0.55	0.62314	3.13	0.00000	2.61	0.00000
Phenylalanine ammonia lyase	VIT_16s0039g01120	PAL	2.05	0.13924	-2.61	1.00000	-4.62	0.00953
Peroxidase	VIT_12s0055g00810	E1.11.1.7	-0.02	0.92531	-2.00	0.00000	-1.96	0.00000
Trans-cinnamate 4-monoxygenase	VIT_06s0004g08150	CYP73A	-1.24	0.00488	-0.13	0.51475	1.14	0.00104
Putative beta-glucosidase	VIT_19s0014g04750	E3.2.1.21	-1.46	0.00000	-3.54	0.00000	-2.06	0.00005
Glycosyltransferase	VIT_16s0022g01970	UGT72E	-1.15	0.02177	-3.40	0.00000	-2.23	0.00028
Unnamed protein product	VIT_13s0064g01750	E3.2.1.21	-0.89	0.10202	-1.99	0.00000	-1.08	0.00000
Probable cinnamyl alcohol dehydrogenase	VIT_18s0001g14910	CAD	0.06	0.84734	-1.21	0.00000	-1.25	0.00000
Peroxidase	VIT_13s0067g02360	E1.11.1.7	-2.14	0.00000	-4.01	0.00000	-1.85	0.00000
Peroxidase	VIT_10s0116g01780	E1.11.1.7	-0.63	0.14670	-1.71	0.00000	-1.06	0.00000
Phenylalanine ammonia lyase	VIT_16s0039g01300	PAL	1.34	0.16736	-1.43	0.36247	-2.75	0.01649
Phenylalanine ammonia lyase	VIT_11s0016g01520	PAL	0.61	0.55273	-2.11	0.04852	-2.70	0.00120
Peroxidase	VIT_16s0100g00090	E1.11.1.7	0.94	0.15751	-1.77	0.00308	-2.69	0.00000
Phenylalanine ammonia lyase	VIT_16s0039g01110	PAL	1.78	0.11341	-1.22	0.54672	-2.99	0.01490
Unnamed protein product	VIT_06s0004g01420	E3.2.1.21	-0.44	0.35440	0.82	0.12460	1.27	0.00000
Peroxidase	VIT_08s0040g02200	E1.11.1.7	-1.02	0.08130	0.01	0.68626	1.06	0.04688
Peroxidase	VIT_12s0055g00990	E1.11.1.7	2.58	0.10764	-2.51	1.00000	-5.02	0.00337
Cytochrome P450 CYP73A100	VIT_11s0065g00350	CYP73A	0.11	0.88529	-1.52	0.00240	-1.62	0.00002
Peroxidase	VIT_16s0022g02470	E1.11.1.7	0.73	0.00275	-1.76	0.00000	-2.47	0.00000
Berberine bridge enzyme	VIT_10s0003g05420	K22395	-0.63	0.26053	-2.19	0.00000	-1.54	0.00000
Peroxidase	VIT_18s0072g00160	E1.11.1.7	-2.60	0.00004	-0.34	0.06431	2.28	0.00000
Peroxidase	VIT_07s0191g00050	E1.11.1.7	0.01	0.98307	1.02	0.00000	1.03	0.00000
Peroxidase	VIT_10s0003g00650	E1.11.1.7	-0.61	0.29475	-2.91	0.00001	-2.27	0.00104
Peroxidase	VIT_12s0059g02420	E1.11.1.7	-0.69	0.05056	-1.93	0.00000	-1.22	0.00439
Unnamed protein product	VIT_13s0064g01640	E3.2.1.21	-0.46	0.74547	1.16	0.15059	1.65	0.02022

Continued

Table 6. Continued.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control		0.2Se versus Control		0.2Se versus 0.2Se + 15N	
			Log ₂ FC	FDR	Log ₂ FC	FDR	Log ₂ FC	FDR
Unnamed protein product	VIT_11s0016g01640	PAL	2.70	0.12995	-1.38	1.00000	-4.04	0.03834
Unnamed protein product	VIT_03s0038g01330	E2.3.1.133	1.20	0.00000	0.10	0.66635	-1.09	0.00000
4-coumarate – CoA ligase	VIT_02s0109g00250	4CL	2.14	0.00939	0.06	0.97506	-2.07	0.01062
Uncharacterised protein	VIT_06s0004g06110	bglX	-0.90	0.37906	0.31	0.22344	1.23	0.00000
Peroxidase	VIT_12s0055g01010	E1.11.1.7	1.89	0.32080	-2.98	1.00000	-4.86	0.02264
Probable mannitol dehydrogenase	VIT_04s0044g00190	CAD	0.15	0.60019	-1.12	0.00000	-1.25	0.00000
Probable cinnamyl alcohol dehydrogenase	VIT_03s0180g00250	CAD	-3.13	0.00000	-6.10	0.00000	-2.98	0.01763

Control group is Control; 0.2 mmol · L⁻¹ + 15 mmol · L⁻¹ N is 0.2Se + 15 N; 0.2 mmol · L⁻¹ Na₂SeO₃ is 0.2Se. FDR < 0.05000 indicates significant differences. FC, fold change; PAL, phenylalanine ammonia lyase.

the increase was more after Se + N application than after Se application. This may be because Se fertiliser could regulate the electron transfer in plant photosynthesis and respiration, thus improving the photosynthetic capacity of crops and increasing N accumulation. Additionally, when an appropriate concentration of N fertiliser was supplied, flavonoid content in grape leaves increased. This was consistent with previous studies (Cao et al., 2012; Aly et al., 2015) and may be attributed to the fact that N increases the activities of some enzymes involved in flavonoid synthesis.

Flavonoid and phenylpropane biosynthesis in grape after Na₂SeO₃ treatment

Flavonoids are low-molecular-weight secondary metabolites synthesised by plants and play an important role in their growth and development (Shi and Xie, 2014). They can increase the uptake of elements such as N and P by roots (Pei et al., 2020) and regulate seed germination, root growth, and photosynthetic pigment synthesis (Tan et al., 2019). Additionally, when plants are subjected to adverse conditions, large amounts of flavonoids are accumulated in plants to remove reactive oxygen species, activate defense-related signalling pathways, and improve the resistance of plants to adverse conditions (Landi et al., 2015). In this study, we performed transcriptome sequencing of grape leaves using a high-throughput sequencing platform (Illumina), and several DEGs related to flavonoid biosynthesis were enriched in the three comparison groups. In the flavonoid metabolism, stilbene synthase (CHS/ST) is involved in the first step of the catalytic reaction, and the expression level of its gene directly affects the quantity of flavonoid metabolites generated (Yeou et al., 2021). In 0.2Se + 15N versus Control, most DEGs related to CHS/ST were significantly upregulated, resulting in the accumulation of flavonoids in grape leaves. In 0.2Se versus Control, DEGs related to CHS/ST were not significantly upregulated or downregulated; however, the absolute number of downregulated unigenes was more. This led to lower flavonoid content in grape leaves in the 0.2Se group than in the Control.

Phenylpropane metabolism is a key pathway of secondary metabolism in plants (Douglas, 1996). PAL and 4CL are the two key enzymes in plants (Qiao et al., 2013) because they can promote plant cell differentiation and lignification and catalyse the formation of metabolites that are chemical barriers to pathogenic organisms and environmental stresses in plants (Heldt and Piechulla, 2011). In this study, in 0.2Se + 15N versus Control, seven DEGs related to PAL and one DEG related to 4CL were significantly upregulated, resulting in enhanced activities of PAL and 4CL and promoting the synthesis and accumulation of phenylpropanes. This is consistent with previous studies (Song et al., 2023). In 0.2Se versus Control, most unigenes were significantly downregulated, suggesting that the supply status of Se and N is closely related to the production and accumulation of phenylpropanes in grape.

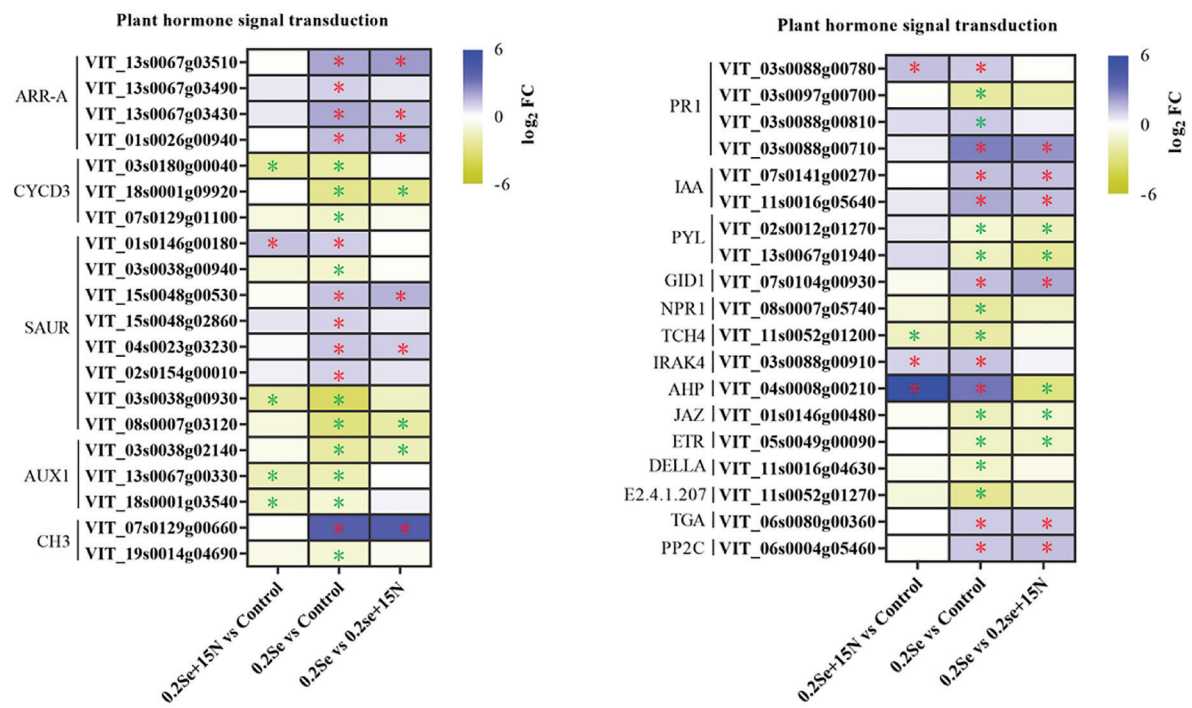


Figure 6. Key DEGs involved in plant hormone signal transduction in grape after Na₂SeO₃ treatment under various nitrogen conditions. ARR-A: two-component response regulator; CYCD3: cyclin-D3-1; SAUR: auxin-responsive protein; AUX1: auxin transporter-like protein; CH3: probable indole-3-acetic acid-amido synthetase; PR1: basic form of pathogenesis-related protein; IAA: auxin-induced protein; PYL: abscisic acid receptor; GID1: gibberellin receptor; NPR1: BTB/POZ domain and ankyrin repeat-containing protein; TCH4: probable xyloglucan endotransglucosylase/hydrolase protein; IRAK4: receptor-like cytosolic serine/threonine-protein kinase; AHP: histidine-containing phosphotransfer protein; JAZ: jasmonic acid protein; ETR: ethylene receptor; DELLA: DELLA protein GAI; E2.4.1.207: probable xyloglucan endotransglucosylase/hydrolase protein; TGA: transcription factor; PP2C: protein phosphatase 2C. *FDR < 0.05, where red represents significant upregulation and green represents significant downregulation.

Plant hormone signal transduction in grape after Na₂SeO₃ treatment under various nitrogen conditions

Plant hormones not only participate in regulating growth and development, signal transduction, and adversity resistance but also regulate nitrogen metabolism in plants. ABA and IAA are closely related to signal transduction of nitrogen and directly affect the ability of plants to adapt to low-nitrogen stress (Chen et al., 2023). Pretreatment with IAA and GA can significantly increase nitrogen metabolism in *Morella rubra* Lour. under salt stress (Chakrabarti and Mukherji, 2003). In this study, in 0.2Se + 15N versus Control, only one auxin-related DEG encoding SAUR was significantly upregulated, whereas three auxin-related DEGs encoding SAUR and AUX1 were significantly downregulated. In 0.2Se + 15N versus Control, eight auxin-related DEGs encoding SAUR, AUX1, CH3, and IAA were significantly upregulated, whereas seven DEGs were significantly downregulated. Two ABA-related DEGs encoding PYL were significantly downregulated; one GA-related DEG encoding GID1 was significantly upregulated (VIT_07s0104g00930);

one JAZ-related DEG encoding JAZ was significantly downregulated (VIT_01s0146g00480). Hence, Na₂SeO₃ treatment had negligible effects on hormone metabolism of grape under appropriate nitrogen supply, which ensured healthy and rapid plant growth. However, under nitrogen deficiency, Na₂SeO₃ treatment could greatly activate the expression of genes related to plant hormone metabolism, accelerate nitrogen metabolism by accumulating more auxin and GA and reducing ABA accumulation, and improve the adaptation of plants to nitrogen deficiency stress. This was consistent with previous studies (Chakrabarti and Mukherji, 2003; Chen et al., 2023). Hence, Se could regulate the expressions of genes related to hormone metabolism under nitrogen deficiency to promote the adaptation of grape plants to low-nitrogen environment.

Starch and sucrose metabolisms in grape after Na₂SeO₃ treatment under various nitrogen conditions

Carbon assimilated by photosynthesis in leaves is used for the formation of starch in chloroplasts or transported to the cytoplasm for the synthesis of sucrose. Most

Table 7. Differential expression results of plant hormone signal transduction.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control		0.2Se versus 0.2se + 15N	
			Log ₂ FC	FDR	Log ₂ FC	FDR
Two-component response regulator	VIT_13s0067g03510	ARR-A	-0.18	0.83317	2.04	0.00000
Cyclin-D3-1	VIT_18s0001g09920	CYCD3	-0.05	0.89851	-2.63	0.00000
Unnamed protein product	VIT_07s0129g01100	CYCD3	-0.79	0.00000	-1.32	0.00000
Gibberellin receptor GID1B	VIT_07s0104g00930	GID1	-0.48	0.06023	1.42	0.00000
Regulatory protein NPR5	VIT_08s0007g05740	NPR1	-0.86	0.34463	-2.23	0.00791
Auxin-responsive protein SAUR71	VIT_01s0146g00180	SAUR	1.37	0.00002	1.14	0.00003
Unnamed protein product	VIT_03s0038g02140	AUX1	-0.53	0.07953	-2.14	0.00000
Basic form of pathogenesis-related protein	VIT_03s0088g00780	PR1	1.42	0.00000	1.18	0.00099
Auxin transporter	VIT_13s0067g00330	AUX1	-1.64	0.00000	-1.76	0.00000
Indole-3-acetic acid-amido synthetase	VIT_19s0014g04690	GH3	-0.65	0.17485	-1.06	0.02835
Xyloglucan endotransglucosylase/hydrolase	VIT_11s0052g01200	TCH4	-1.45	0.00058	-2.11	0.00000
Hypothetical protein DKX38	VIT_03s0088g00910	IRAK4	1.03	0.00000	1.32	0.00000
Auxin-induced protein 6B	VIT_03s0038g00940	SAUR	-0.91	0.15417	-1.17	0.04523
Histidine-containing phosphotransfer protein	VIT_04s0008g00210	AHP	6.05	0.00000	3.12	0.01971
Auxin transporter	VIT_18s0001g03540	AUX1	-1.34	0.00000	-1.04	0.00000
Auxin-responsive protein SAUR36	VIT_15s0048g00530	SAUR	-0.34	0.12180	1.35	0.00000
Cyclin-D3-1	VIT_03s0180g00040	CYCD3	-2.26	0.00000	-2.19	0.00000
Basic form of pathogenesis-related protein	VIT_03s0097g00700	PR1	-0.27	0.93243	-2.22	0.00000
Pathogenesis-related protein	VIT_03s0088g00810	PR1	0.82	0.00000	1.21	0.00000
Two-component response regulator ORR9 isoform X1	VIT_13s0067g03490	ARR-A	0.51	0.00025	1.05	0.00000
Basic form of pathogenesis-related protein	VIT_03s0088g00710	PR1	0.47	0.84569	2.85	0.00775
Auxin-responsive protein IAA9 isoform X1	VIT_11s0016g05640	IAA	0.52	0.41001	1.93	0.00000
Jasmonate-zim-domain protein	VIT_01s0146g00480	JAZ	-0.38	0.37895	-1.53	0.00028
Ethylene receptor	VIT_05s0049g00090	ETR	-0.02	0.96813	-1.35	0.00000
DELLA protein SLR1	VIT_11s0016g04630	DELLA	-0.49	0.00519	-1.16	0.00000
Xyloglucan endotransglucosylase/hydrolase	VIT_11s0052g01270	E2.4.1.207	-0.87	0.26567	-2.51	0.00023
Transcription factor TGA9	VIT_06s0080g00360	TGA	-0.05	0.94243	1.17	0.00036
Two-component response regulator ORR9	VIT_13s0067g03430	ARR-A	0.53	0.76430	1.96	0.00000
Two-component response regulator ARR6	VIT_01s0022g00940	ARR-A	-0.07	0.86455	1.46	0.00000

Continued

Table 7. Continued.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control			0.2Se versus Control			0.2Se versus 0.2se + 15N		
			Log ₂ FC	FDR		Log ₂ FC			Log ₂ FC	FDR	
Auxin-responsive protein SAUR32	VIT_15s0048g02860	SAUR	0.59	0.32414		1.03	0.00000		0.46	0.11100	
Auxin-responsive protein SAUR50	VIT_04s0023g03230	SAUR	0.14	0.87585		1.25	0.00574		1.13	0.00406	
Auxin-responsive protein	VIT_07s0141g00270	IAA	0.03	0.93143		1.42	0.00000		1.41	0.00000	
Auxin-responsive protein SAUR36	VIT_02s0154g00010	SAUR	0.38	0.32725		1.02	0.00028		0.65	0.20123	
Unnamed protein product	VIT_02s0012g01270	PYL	0.58	0.24239		-1.04	0.00037		-1.61	0.00000	
Auxin-induced protein 6B	VIT_03s0038g00930	SAUR	-2.16	0.00509		-3.62	0.00014		-1.45	0.34481	
Abscissic acid receptor PYL4	VIT_13s0067g01940	PYL	0.89	0.01109		-1.46	0.00418		-2.34	0.00000	
Probable indole-3-acetic acid-amido synthetase	VIT_07s0129g00660	GH3	-0.13	1.00000		4.03	0.04928		4.18	0.03700	
Auxin-induced protein	VIT_08s0007g03120	SAUR	-0.82	0.54502		-2.97	0.04849		-2.14	0.23987	
Unnamed protein product	VIT_06s0004g05460	PP2C	-0.16	0.88910		1.24	0.00000		1.42	0.00771	

Control group is Control; 0.2 mmol · L⁻¹ + 15 mmol · L⁻¹ N is 0.2Se + 15 N; 0.2 mmol · L⁻¹ Na₂SeO₃ is 0.2Se. FDR < 0.05000 indicates significant differences.

Table 8. Differential expression results of starch and sucrose metabolism.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control		0.2Se versus Control		0.2Se versus 0.2se + 15N	
			Log ₂ FC	FDR	Log ₂ FC	FDR	Log ₂ FC	FDR
Uncharacterised protein	VIT_06s0009g00810	E3.2.1.21	-0.78	0.30589	-2.28	0.00000	-1.49	0.00000
Unnamed protein product	VIT_06s0004g01430	E3.2.1.21	0.55	0.62314	3.13	0.00000	2.61	0.00000
Hypothetical protein	VIT_06s0004g00720	GN4	0.23	0.32455	-0.98	0.09370	-1.20	0.00000
Starch synthase, chloroplastic/amyloplastic	VIT_02s0025g02790	WAXY	0.98	0.08222	0.27	0.15132	1.28	0.00238
Alpha-trehalose-phosphate synthase	VIT_10s0003g02160	TPS	-0.06	0.78358	0.92	0.27515	1.00	0.00000
Endoglucanase	VIT_07s0005g00740	E3.2.1.4	-1.19	0.00000	-2.27	0.00000	-1.06	0.00000
Alpha-glucosidase	VIT_10s0092g00240	malZ	0.79	0.32308	-0.24	0.10826	-1.01	0.00000
Putative beta-glucosidase	VIT_19s0014g04750	E3.2.1.21	-1.46	0.00000	-3.54	0.00000	-2.06	0.00006
Probable fructokinase-5	VIT_18s0089g01230	E2.7.1.4	-0.06	0.88916	-3.53	0.00000	-2.01	0.00000
Alpha-amylose	VIT_18s0001g00560	AMY	-0.82	0.73105	0.63	0.58606	1.48	0.00000

Continued

Table 8. Continued.

Gene name	Gene ID	KO name	0.2Se + 15N versus Control		0.2Se versus Control		0.2Se versus 0.2se + 15N	
			Log ₂ FC	FDR	Log ₂ FC	FDR	Log ₂ FC	FDR
Beta-fructofuranosidase, soluble isoenzyme I isoform X1	VIT_16s0022g00670	INV	-0.35	0.19816	-2.12	0.00000	-1.75	0.00000
Unnamed protein product	VIT_13s0064g01750	E3.2.1.21	-0.89	0.41109	-1.99	0.00000	-1.07	0.00000
Putative alpha,alpha-trehalose-phosphate synthase	VIT_01s0026g00280	TPS	1.37	0.00000	-0.43	0.00279	-1.07	0.00000
Phosphotransferase	VIT_09s0002g03390	HK	0.97	0.48252	-2.70	0.23476	-3.66	0.027023
Sucrose synthase	VIT_11s0016g00470	E2.4.1.13	-0.39	0.23845	-1.80	0.00000	-1.40	0.00000
Endoglucanase	VIT_04s0008g02010	E3.2.1.4	1.03	0.08715	-0.14	0.88231	-1.15	0.04076
Sucrose synthase	VIT_17s0053g00700	E2.4.1.13	0.20	0.36444	-1.60	0.00000	-1.78	0.00000
Probable fructokinase	VIT_05s0102g00710	E2.7.1.4	-0.50	0.18347	-1.62	0.00000	-1.11	0.00000
Probable alpha,alpha-trehalose-phosphate synthase	VIT_12s0028g01670	TPS	-0.03	0.96391	-1.21	0.00261	-1.16	0.00280
Unnamed protein product	VIT_06s0004g01420	E3.2.1.21	-0.48	0.35446	0.82	0.11907	1.27	0.00000
Sucrose synthase	VIT_07s0005g00750	E2.4.1.13	0.68	0.06330	-0.34	0.15054	-1.00	0.00000
Unnamed protein product	VIT_07s0005g06660	WA.XY	-1.61	0.57698	1.65	0.22606	3.25	0.03323
Probable alpha,alpha-trehalose-phosphate synthase	VIT_17s0000g08010	TPS	3.15	0.00000	-0.11	0.88102	3.26	0.00000
Putative alpha,alpha-trehalose-phosphate synthase	VIT_06s0009g01650	TPS	1.20	0.18780	-0.97	0.51346	-2.15	0.02419
Unnamed protein product	VIT_13s0064g01640	E3.2.1.21	-0.46	0.74547	1.16	0.15059	1.65	0.02022
Uncharacterised protein	VIT_06s0004g06110	bgIX	-0.90	0.37906	0.31	0.22344	1.23	0.00000
Probable fructokinase-7 isoform X1	VIT_15s0048g01260	E2.7.1.4	0.77	0.29019	-0.75	0.06879	-1.50	0.00000
Sucrose synthase	VIT_04s0079g00230	E2.4.1.13	0.24	0.33133	-1.83	0.00000	-2.05	0.00000

Control group is Control; 0.2 mmol · L⁻¹ + 15 mmol · L⁻¹ N is 0.2Se + 15 N; 0.2 mmol · L⁻¹ Na₂SeO₃ is 0.2Se. FDR < 0.05000 indicates significant differences. FC, fold change.

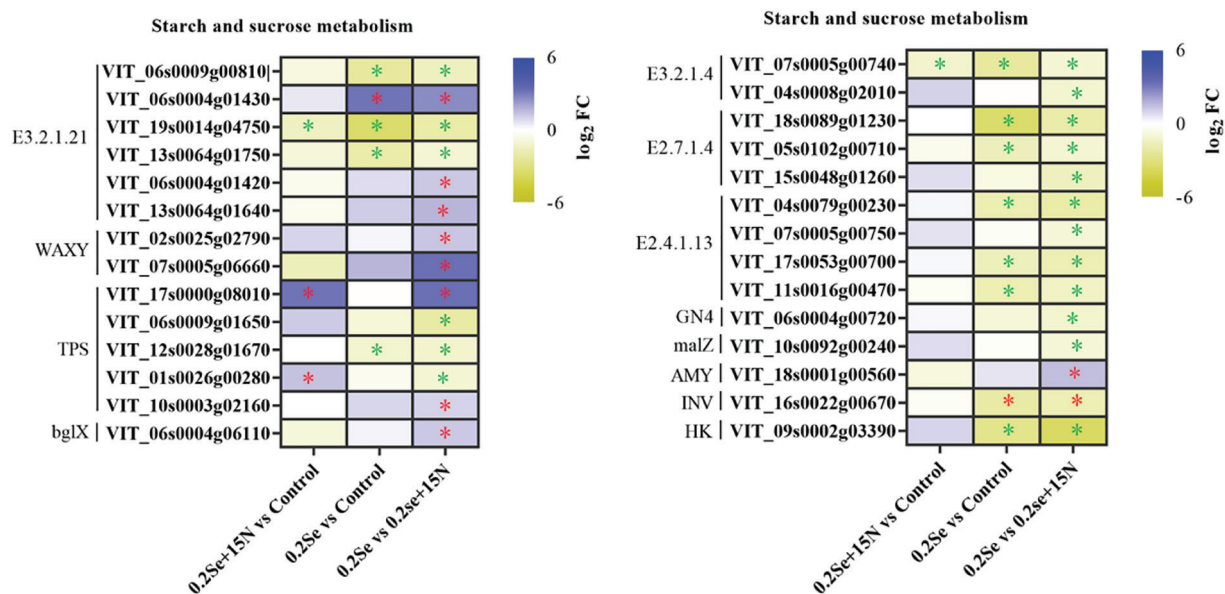


Figure 7. Key DEGs involved in starch and sucrose metabolism in grape after Na_2SeO_3 treatment under various nitrogen conditions. E3.2.1.21: beta-glucosidase; WAXY: granule-bound starch synthase; TPS: alpha-trehalose-phosphate synthase; bglX: xylan 1,4-beta-xylosidase; E3.2.1.4: endoglucanase; E2.7.1.4: fructokinase; E2.4.1.13: sucrose synthase; GN4: glucan endo-1,3-beta-glucosidase; malZ: alpha-glucosidase; AMY: alpha-amylase; INV: beta-fructofuranosidase; HK: hexokinase. * FDR < 0.05, where red represents significant upregulation and green represents significant downregulation.

photosynthetic products required for plant growth and development are supplied and transported in the form of sucrose. Sucrose phosphate synthase (SPS) is an important control point of the sucrose synthesis pathway and a key enzyme required for the entry of sucrose into various metabolic pathways. Its activity can reflect the status of sucrose biosynthesis pathways (Harbrun et al., 1981; Liu et al., 2005). Some studies reported that SPS is negatively correlated with starch accumulation and positively correlated with sucrose synthesis (Huber, 1983; Wang et al., 2000). In this study, in 0.2Se versus Control, three DEGs encoding SPS were significantly downregulated, suggesting that sucrose accumulation was proportional to the activity of SPS. Accumulation and conversion of starch and sucrose were closely related to growth and development of plants and activities of enzymes in plants (Vizzolo et al., 1996; Suthumchai et al., 2007). In 0.2Se versus Control, three DEGs encoding β -glucosidase, one DEG encoding alpha-trehalose-phosphate synthase, one DEG encoding endo-1,4- β -D-glucanohydrolase, two DEGs encoding fructokinase, three DEGs encoding SPS, one DEG encoding β -fructofuranosidase, and one DEG encoding hexokinase were significantly downregulated. This led to decreased activity of the relevant enzymes, resulting in decreased soluble sugar content. Additionally, most DEGs in 15N + 0.2Se versus Control were upregulated. Combined with Figure 1, it could be inferred that this phenomenon was due to the lower starch content and higher soluble sugar content in the Se + N groups than in the Se groups.

CONCLUSIONS

Grape plants grew more vigorously after Na_2SeO_3 treatment under appropriate nitrogen supply than under nitrogen deficiency. Particularly, in the 0.2Se + 15N group, the plant height, stem diameter, root volume, biomass, and other growth indexes and the contents of flavonoids, soluble sugar, total nitrogen, and soluble protein in grape leaves were the highest. The transcriptome analysis found that, under nitrogen supply conditions, Na_2SeO_3 treatment regulated the upregulation of some gene activities of stilbene synthase, PAL, 4CL, and α -trehalose phosphate synthase; under nitrogen deficiency conditions, genes encoding auxin and gibberellin were upregulated after Na_2SeO_3 treatment, while genes encoding ethylene, jasmonic acid, β -glucosidase, α -trehalose phosphate synthase, endoglucanase, fructokinase, sucrose synthase, and hexokinase were downregulated. The expression of these genes plays an important role in regulating the growth of grape plants. These findings indicated potential application of Na_2SeO_3 in crop production under nitrogen deficiency.

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AUTHOR CONTRIBUTIONS

Yongfu Zhang conceived and designed the experiments. Yongfu Zhang and Liling Mo analysed the data and wrote the paper. Xiaoqin Li, Kai Wang, Zuqin Qiao, and Zhao Liu performed the experiments. Yongfu Zhang and Liling Mo contributed equally.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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