

Nitric Oxide Enhances Salt Tolerance in Ginger Seedlings Through Phytohormone Homeostasis and Starch and Sugar Metabolism

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Abstract

Background: Nitric oxide (NO) is a key signaling molecule regulating plant physiological and biochemical processes as well as stress responses. In our study, the NO donor sodium nitroprusside (SNP) significantly promoted ginger seedling growth under salt stress, while treatment with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) reversed these effects, confirming the critical role of NO in enhancing salt tolerance. Meanwhile, NO enhanced antioxidant enzyme activities and reduced ROS accumulation to alleviate oxidative damage. To further investigate the molecular mechanisms by which NO alleviates salt stress in ginger seedlings, transcriptomic analysis was performed on ginger leaves.

Results: This analysis identified 2,252 DEGs under salt stress, which were enriched in 55 KEGG pathways, primarily including plant hormone signal transduction and starch and sucrose metabolism pathways. Specifically, further analysis showed that 19 DEGs were enriched in plant hormone signal transduction, while 16 DEGs were enriched in starch and sugar metabolism. NO treatment increased the levels of indole-3-acetic acid (IAA), gibberellin 3 (GA3) and trans-zeatin (ZR), while reducing the levels of abscisic acid (ABA), methyl jasmonate (MeJA) and brassinosteroids (BR). Moreover, NO promoted the accumulation of soluble sugars and sucrose and enhanced the activities of key enzymes involved in starch and sucrose metabolism, thereby effectively improving osmotic adjustment. All of these SNP-induced effects were reversed by cPTIO.

Conclusions: Taken together, our results demonstrate that NO improves antioxidant capacity, maintains endogenous phytohormone balance and enhances osmotic adjustment, ultimately alleviating salt stress damage in ginger seedlings.

1. Introduction

Salt stress is one of the major abiotic stresses threatening global agricultural production, which seriously inhibits crop growth, development and yield [1, 2]. Simultaneously, salt stress causes osmotic imbalance, ionic toxicity and oxidative damage in plants, thereby disrupting normal cellular metabolism, inhibiting photosynthetic efficiency and accelerating tissue senescence, which ultimately leads to a significant decline in crop yield and quality [3, 4, 5]. In plants, salt stress results in stunted growth and leaf yellowing, leaf wilting, root damage, reduced chlorophyll content, decreased photosynthetic rates and reduced branching, which seriously inhibits the plant growth and development [6]. Therefore, improving the tolerance of plants to salt stress has currently become a pressing issue.

In recent years, numerous studies have demonstrated that nitric oxide (NO), a widely distributed gaseous signaling molecule in plants, exerts crucial regulatory functions throughout the entire process of plant growth and development. The production of NO in plants primarily depends on enzymatic and non-enzymatic pathways [7]. Enzymatic pathways are mainly mediated by nitrate reductase (NR) and nitric oxide synthase-like (NOS-like) enzymes and non-enzymatic pathways rely on the chemical reduction of nitrite [8]. It not only participates in physiological processes such as seed germination, root

development, fruit ripening and postharvest preservation, but also plays a key role in plant responses to abiotic stress [9, 10]. Currently, a large number of studies have confirmed that NO treatment can effectively delay postharvest senescence, mitigate damage from adverse stress and reduce the incidence of fruit diseases [11, 12, 13]. Moreover, NO promotes sugar interconversion in peaches by regulating the activity of key enzymes involved in sugar metabolism, thereby maintaining the balance of soluble sugars and delaying the fruit softening and senescence [14]. These multiple pathways collectively maintain NO homeostasis in plants and participate in regulating various physiological processes and stress responses. It is worth noting that many studies have indicated that exogenous NO application can protect cell membrane structure and enhance osmotic regulation by eliminating reactive oxygen species (ROS), maintaining ion homeostasis and improving antioxidant capacity [15, 7]. Moreover, NO at appropriate concentrations can regulate the biosynthesis of endogenous plant hormones and the metabolism of osmoregulatory substances [16, 17], thereby alleviating the growth inhibition and physiological damage caused by stresses such as salt, drought and low temperature [18].

Notably, NO interacts with various plant hormones, playing a crucial role in regulating plant growth and development as well as responses to abiotic stress. The synergistic action of NO with hormones such as ethylene (ETH), auxin (AUX), abscisic acid (ABA), and jasmonic acid (JA) is extensively involved in plant stress responses and growth regulation [19, 20, 21, 22]. For example, endogenous NO interacts with ABA to enhance antioxidant enzyme activity and maintain chlorophyll content in cut roses, thus delaying cut flower senescence. [23]. NO and GA act synergistically to improve grape seed viability and germination, thereby promoting seedling growth [24]. The combined application of NO and strigolactones (SLs) increases photosynthetic pigment content and antioxidant capacity, ultimately enhancing salt tolerance in tomato seedlings [25]. On the other hand, starch and sugar metabolism in plants is central processes for energy supply and osmotic regulation. These metabolites not only provide the material and energy foundation for plant growth and development but also play crucial roles in maintaining cell turgor, scavenging ROS and transducing stress signals [26]. However, salt stress accelerates starch degradation and inhibits the activity of key enzymes involved in sugar synthesis, ultimately disrupting the distribution of photosynthetic products and causing an imbalance in soluble sugar levels, which severely impairs the normal plant growth and development as well as physiological processes related to stress resistance [27].

Therefore, this study identifies key genes in ginger seedlings associated with plant hormone signal transduction and starch and sugar metabolism during the process by which NO alleviates salt stress. The aims were to further elucidate the molecular mechanisms of NO through which NO enhances the salt tolerance of ginger seedlings by regulating hormone signaling and starch and sugar metabolism. This provides a theoretical basis for revealing the molecular mechanisms by which NO enhances the salt tolerance of ginger seedlings.

2. Materials and Methods

2.1 Plant Materials and Treatment

Zingiber officinale Roscoe cv. *Fengtou* was utilized as the plant material in this study. The ginger seed rhizomes were provided by the Hubei Key Laboratory of Spices & Horticultural Plant Germplasm Innovation & Utilization, Yangtze University. Healthy rhizome with plump buds and uniform size were selected and sown in pots measuring 25 cm in diameter at the top, 20 cm at the bottom and 30 cm in height. After the ginger was grown for 2 months, plants with a height of 50 cm and 7–9 true leaves were selected for the experiment. The experiment comprised five treatments: CK (distilled water), NaCl (20 g·L⁻¹), NaCl+SNP (150 μmol·L⁻¹) (a NO donor), NaCl+cPTIO (NO scavenger, 2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide potassium) (1 μmol·L⁻¹) and cPTIO. The concentrations were determined according to the results of preliminary experiments. Seedlings were cultured in a controlled environment maintaining a temperature of 26 °C and a photoperiod of 16 h light / 8 h dark. Samples were collected 7 days after treatment and immediately stored at -80 °C for subsequent physiological and molecular analyses.

2.2 Measurement of Growth Parameters

The morphological parameters of ginger seedlings in different treatment groups were determined. A camera (Canon EOS-77D) was used to photograph the overall morphology of ginger seedlings in each treatment group. Root systems were scanned using a root scanner (Model J221A, Indonesia) to obtain images. Seedling height, leaf length, and leaf width were measured using a ruler, while stem diameter was measured using a Vernier caliper. Morphological traits including plant height, stem diameter, leaf length and leaf width were determined, and mean values were calculated from five ginger seedlings per replicate.

2.3 Determination of H₂O₂, O₂⁻, MDA Contents and Antioxidant Enzyme Activities

The H₂O₂ content and O₂⁻ production rate in different ginger tissues were determined according to the method of Zhao et al. (2025) [28]. Briefly, 0.1 g of ginger tissue was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) on an ice bath. The homogenate was transferred to a centrifuge tube and centrifuged at 12,000×g for 15 min. Then, a 1 mL of the supernatant was collected and mixed with 1 mL of potassium phosphate buffer and 2 mL of potassium iodide (KI) solution. After thorough mixing, the absorbance was measured at 390 nm, and the H₂O₂ content and O₂⁻ production rate was calculated using a standard curve. Malondialdehyde (MDA) content was determined following the protocol described by Fan et al. (2023) [29]. The activities of POD and SOD were quantified using the procedures reported by Zhang et al. (2025) [30]. Catalase (CAT) activity was measured using a CAT assay kit (Solarbio, Beijing, China).

2.4 RNA Extraction and Transcriptome Sequencing Analysis

Total ribonucleic acid (RNA) was extracted from leaves of ginger seedlings under 150mM NaCl and 150mM NaCl+150 $\mu\text{mol}\cdot\text{L}^{-1}$ SNP treatments using a total RNA extraction kit (Coolaber, Beijing, China). Qualified RNA samples were sent to Wuhan Benagen Tech Solutions Co. Ltd. for library construction and Illumina sequencing. Raw reads generated after sequencing were subjected to quality control to remove low-quality sequences and contaminants, yielding high-quality clean reads. Subsequently, clean reads were mapped to the reference genome using Hisat2. Transcripts were assembled with StringTie, and the DESeq2 package was employed to identify differentially expressed genes (DEGs) with the threshold set at $|\log^2\text{FC}| > 1$ and $P < 0.05$. Finally, the Goseq R package and KOBAS 2.0 software were utilized to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, respectively, on DEGs with a corrected P value < 0.05 to explore their biological functions and involved metabolic pathways.

2.5 Quantitative Real-Time PCR (qRT-PCR) Analysis

To verify the accuracy of the RNA-seq results, 18 DEGs were randomly selected for qRT-PCR validation. Specific primers were designed using Primer Premier 5.0 (Table S1). The RBP gene was used as the internal reference for data normalization [31]. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a CFX 96 Real-Time PCR System (Bio-Rad, USA). The relative expression levels of candidate genes were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method [31].

2.6 Determination of Endogenous Hormone Contents

Endogenous plant hormone contents were determined using a modified method from Zhu et al. (2020) [32] and Yang et al. (2001) [33]. Frozen ginger leaves were thoroughly ground in liquid nitrogen and homogenized in ice-cold 80% (v/v) methanol containing 1 mmol L^{-1} BHT. After incubation at 4 °C for 16 h and two rounds of extraction, the supernatants were combined and purified using a C18 solid-phase extraction column. The eluate was dried under vacuum and reconstituted with dilution buffer. The contents of IAA, GA, ABA, MeJA, BR and ZR were quantified using ELISA kits, and hormone levels were calculated based on standard curves and expressed as ng g^{-1} fresh weight (FW).

2.7 Determination of Soluble Sugar, Starch and Sucrose Contents and Related Enzyme Activities

Total soluble sugar and sucrose contents were determined by the anthrone-sulfuric acid colorimetric method [34]. Starch content was measured according to Zhang et al. (2008) [35] using the residue

remaining after soluble sugar extraction. Activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) were quantified using kits from Solarbio (Beijing, China), with all operations conducted as directed in the product protocols. ADP-glucose pyrophosphorylase (AGPase) activity was assayed as described by Chullai et al. (2025) [36], and α -amylase activity was determined following the protocol of Souleyre et al. (2004) [37]. All assays were performed following standardized procedures to ensure accuracy and reliability.

2.8 Statistical Analysis

The experiment was arranged in a completely randomized design with three biological replicates. Data were analyzed via one-way analysis of variance (ANOVA) using SPSS 26.0 software. Significant differences were determined by Duncan's multiple range test at $p < 0.05$. Results are presented as means $\pm$ SE or SD. Heatmaps were constructed using TBtools.

3. Results

3.1 The Effect of NO on the Growth of Ginger Seedlings under Salt Stress

To investigate the effects of NO on the growth and development of ginger seedlings under salt stress, we treated salt-stressed ginger seedlings with the NO donor SNP and the NO scavenger cPTIO. As shown in Fig. 1, salt stress significantly inhibited the growth and development of ginger seedlings. Compared with the CK, the ginger seedlings showed obvious leaf yellowing and stunting (Fig. 1A). At the same time, root development was significantly shorter, and the plant height, stem diameter, leaf length and leaf width of ginger seedlings were significantly reduced (Figs. 1B–F). Application of SNP significantly reversed the growth inhibition caused by salt stress compared to NaCl treatment, improving the growth parameters of ginger seedlings. Furthermore, compared to the NaCl+SNP treatment, the addition of cPTIO under salt stress further reduced the plant height, stem diameter, leaf length, and leaf width of ginger seedlings. Therefore, these results indicate that NO effectively promotes the growth and development of ginger seedlings under salt stress. Notably, there was no significant difference between the cPTIO treatment and CK.

3.2 The Regulatory Role of NO on the Antioxidant System of Ginger Seedlings under Salt Stress

To further investigate the effects of NO on the antioxidant capacity of ginger seedlings under salt stress, the activities of antioxidant enzymes and the levels of ROS and MDA were detected. Fig. 2 shows that, compared with the CK, NaCl treatment alone significantly increased the levels of H_2O_2 , O_2^- and MDA in the roots, stems, and leaves of ginger seedlings, whereas the NaCl+SNP treatment reduced their

accumulation (Figs. 2A-C). Conversely, following NaCl+cPTIO treatment, the levels of H_2O_2 , O_2^- and MDA were significantly upregulated in comparison with NaCl treatment alone. These results indicate that NO plays a key role in alleviating salt stress-induced oxidative damage in ginger seedlings.

Simultaneously, compared with the CK, the activities of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in ginger seedlings were significantly increased under NaCl treatment (Figs. 2D-F). Among these, POD activity was significantly increased in roots, stems and leaves. The NaCl+SNP treatment further significantly increased POD, SOD and CAT activities in all tissues, particularly exhibiting the most prominent activation of POD and CAT activities in leaves, suggesting that SNP can efficiently scavenge ROS by activating the antioxidant system. Similarly, the activities of these antioxidant enzymes were significantly lower in the NaCl+cPTIO treatment than in the NaCl+SNP treatment. These results indicate that NO can enhance the antioxidant capacity of ginger seedlings under salt stress by scavenging excess ROS and reducing membrane lipid peroxidation, thereby alleviating oxidative damage caused by salt stress.

3.3 The Identification and Functional Classification of DEGs during NO Alleviation of Salt Stress

To investigate the molecular mechanisms by which NO alleviates salt stress in ginger seedlings, this study performed RNA-seq analysis on ginger seedlings treated with NaCl and NaCl+SNP. As shown in Table S2, the raw reads for all six samples were approximately 40 million, and the number of clean reads obtained after quality control filtering showed negligible loss compared to the original reads. Regarding quality metrics, the average percentages of Q20 and Q30 values for all samples reached 99.05% and 96.71%, respectively. Furthermore, the GC content (GCpct) of these samples remained stable between 47.12% and 48.72%. These results indicate the high sequencing quality of RNA-seq analysis and are suitable for further analysis.

As shown in Fig. 3, RNA-seq analysis of the NaCl vs. NaCl + SNP treatment identified the total of 2,252 differentially expressed genes (DEGs), including 805 up-regulated genes and 1,447 down-regulated genes (Figs. 3A-C; Table S3). These results demonstrate that SNP treatment significantly modulates the transcriptome profile of ginger under salt stress, with these DEGs being predominantly enriched in multiple key metabolic and signaling pathways. Furthermore, these DEGs were significantly enriched in the three major categories of biological processes (BP), cellular components (CC) and molecular functions (MF) (Fig. 3D; Table S4). For the BP category, DEGs were mainly concentrated in metabolism-related processes, with the highest enrichment observed in terms such as phosphorus metabolic process (188), phosphate-containing compound metabolic process (182), and phosphorylation (158). In the CC category, DEGs were most significantly enriched in membrane (718), followed by cell periphery (164), plasma membrane (141), and Golgi membrane (102). Regarding the MF category, DEGs were highly enriched in binding functions such as purine nucleotide binding (354), purine ribonucleotide binding (350), and adenylyl nucleotide binding (327).

Integrating KEGG pathway enrichment analysis, our study assigned 2,252 DEGs to 55 pathways (Fig. 3E; Table S5). Additionally, the top 20 most significantly enriched KEGG pathways represented the majority of DEGs. As further depicted in Fig. 3E, the significantly enriched DEGs were predominantly annotated to pathways including plant-pathogen interaction, plant hormone signal transduction, biosynthesis of secondary metabolites, starch and sucrose metabolism and MAPK signaling pathway. Consequently, these results strongly indicate that NO plays a pivotal role in regulating these key pathways during NO-induced the alleviation of salt stress in ginger seedlings.

3.4 The Analysis and Validation of DEGs in Plant Hormone Signal Transduction during NO Alleviation of Salt Stress

In order to verify the regulatory role of NO in plant hormone signaling pathways during the alleviation of salt stress, we analyzed the expression patterns in ginger seedlings of key genes involved in plant hormone signaling (map04075). The RNA-seq results showed that, in the comparison of NaCl and NaCl+SNP treatments, 19 DEGs were identified as differentially expressed in the AUX, cytokinin (CTK), gibberellin (GA), ABA, ETH, brassinosteroid (BR), JA and salicylic acid (SA) signaling pathways (Fig. 4A, Table S6). Specifically, *AUX1*, *LAX*, *IAA*, *ARF* and *GH3* are critical genes involved in auxin signaling. Compared with the NaCl treatment, the expression levels of *AUX*, *LAX* and *IAA* were significantly down-regulated under the NaCl+SNP treatment. On the contrary, the expression levels of *ARF* and *GH3* were significantly up-regulated by SNP. Regarding the GA signaling pathway, the expression levels of *PIF* and *PIL* were significantly down-regulated, whereas *PIF3* showed a significant up-regulation trend. Furthermore, the expression levels of genes *ABF* and *ABF-1* in ABA signaling pathway, as well as *JAZ* and *MYC2-1* related to JA signaling, were significantly reduced under NaCl+SNP treatment. Moreover, DEGs associated with the CTK, BR, and SA signaling pathways also were significantly regulated in response to NO treatment. These results indicate that NO regulates the expression of key genes in multiple hormonal signaling pathways, thus enhancing the tolerance of ginger seedlings to salt stress.

To further validate the reliability of the transcriptomic data, we selected 9 key genes involved in plant hormone signaling pathways (*IAA*, *ARF*, *GH3*, *PIF*, *PIL*, *PIF3*, *ABF*, *ABF-1*, *JAZ* and *MYC2*) for qRT-PCR validation (Fig. 4B). The results were highly consistent with the RNA-seq analysis. Compared with the NaCl treatment, the NaCl + SNP treatment significantly down-regulated the expression of *IAA*, *PIF*, *PIL*, *ABF*, *ABF-1* and *MYC2*, while the expression of *ARF*, *GH3*, *PIF3*, and *JAZ* was significantly upregulated. However, in the NaCl+cPTIO treatment group, the regulatory effects of SNP were significantly reversed. In summary, NO plays a key role in regulating the transcription of plant hormone-related genes, thereby effectively enhancing salt stress tolerance in ginger seedlings.

3.5 The Analysis and Confirmation of DEGs Involved in Starch and Sucrose Metabolism during NO Alleviation of

Salt Stress

According to the KEGG results, 16 DEGs were collectively enriched in the starch and sucrose metabolism pathway (map00500) (Fig. 5A, Table S7). These included genes annotated as hexokinase (*HK*, *HK-1*), beta-glucosidase (*E3.2.1.21*, *bglB*, *E3.2.1.21-1*), glucan endo-1,3-beta-glucosidase (*GN1_2_3-1*, *GN5_6*, *GN1_2_3*, *GN4*), trehalose 6-phosphate synthase/phosphatase (*TPS*, *otsB*, *otsB-2*, *otsB-1*), sucrose synthase (*SUS*), granule-bound starch synthase (*WAXY*) and alpha-amylase (*AMY*, *amyA*, *malS*). Among these, compared to NaCl treatment, NaCl + SNP significantly up-regulated the expression levels of 6 key genes, including *HK*, *E3.2.1.21*, *otsB*, *HK-1*, *E3.2.1.21-1* and *AMY*, *amyA*, *malS*. On the contrary, the expression levels of *bglB*, *GN1_2_3-1*, *GN5_6*, *TPS*, *SUS* and *WAXY* were significantly suppressed by the NaCl+SNP treatment. Collectively, NO modulates the transcription of key genes involved in starch and sucrose metabolism, helping to maintain the carbon balance and enhance the salt tolerance in ginger seedlings.

To validate the expression of DEGs, this study selected 9 key DEGs from the starch and sucrose metabolic pathways (*E3.2.1.21*, *otsB*, *HK-1*, *GN1_2_3*, *GN4*, *SUS*, *WAXY*, *otsB-1*, and *AMY*, *amyA*, *malS*) for qRT-PCR validation (Fig. 5B). The results showed that the expression trends of these genes were highly consistent with those from the RNA-seq analysis. Compared with the NaCl treatment, the expression levels of *E3.2.1.21*, *otsB*, *HK-1* and *otsB-1* were significantly elevated under NaCl+SNP treatment, while those of *GN1_2_3*, *GN4*, *SUS*, *WAXY* and *AMY*, *amyA*, *malS* were significantly reduced. Further analysis revealed that in the NaCl+cPTIO treatment, the regulatory effects of SNP were also significantly reversed, while the expression levels of these genes showed no significant differences compared to the NaCl treatment. This further confirms that NO may effectively alleviate the salt stress-induced physiological damage and enhance the salt tolerance in ginger seedlings by regulating starch and sucrose metabolism at transcriptional levels.

3.6 The Changes in Endogenous Hormone Contents during NO Alleviation of Salt Stress

To further verify whether NO regulates endogenous plant hormones, this study systematically measured the accumulation of key plant hormones under salt stress in ginger seedlings after NO treatment (Fig. 6). The findings indicate that, compared with the CK, NaCl treatment significantly suppressed the levels of IAA, GA₃ and trans-Zeatin Riboside (ZR), while significantly increasing the synthesis of ABA, Methyl Jasmonate (MeJA) and BR. In contrast to NaCl treatment, the levels of IAA, GA₃ and ZR were significantly elevated, while the levels of ABA, MeJA and BR were significantly down-regulated in NaCl+SNP treatment. Subsequent analysis revealed that in the NaCl+cPTIO treatment, the regulatory effects of SNP on IAA, GA₃ and ZR, ABA and MeJA levels were significantly reversed. Furthermore, there were no significant differences in hormone levels between the cPTIO treatment and CK. Overall, NO treatment significantly maintains the balance of plant hormones, thereby alleviating the salt stress in ginger seedlings.

3.7 The Changes in Starch, Sucrose Contents and Key Enzyme Activities during NO Alleviation of Salt Stress

To elucidate the regulatory role of NO in starch and sugar metabolism during the salt stress response in ginger seedlings, this study analyzed soluble sugars, sucrose, starch content and key metabolic enzyme activities in seedlings in different treatments. As shown in Fig. 7, compared with the CK, the NaCl-treated ginger seedlings exhibited significantly higher levels of soluble sugars and sucrose and lower levels of starch content (Figs. 7A–C). In addition, the activities of sucrose phosphate synthase (SPS), sucrose synthase (SS) and α -amylase (α -AMY) were significantly enhanced under NaCl treatment, while AGPase activity was significantly inhibited (Figs. 7D–G). Compared with NaCl treatment, the NaCl+SNP-treated ginger seedlings showed an increase in the accumulation levels of soluble sugars, sucrose and starch. And the activities of SPS, SS, α -AMY and AGPase were significantly upregulated. By contrast, after NaCl + cPTIO treatment, the contents of soluble sugars, sucrose and starch and the activities of SPS, SS, α -AMY, and AGPase were significantly decreased compared to the NaCl + SNP treatment. Ginger seedlings treated with cPTIO alone showed no significant differences in soluble sugar, sucrose and starch content and metabolic enzymes activities compared to the CK. The above results indicate that NO regulates the accumulation of soluble sugars and sucrose by activating key enzymes in starch and sugar metabolism, thereby participating in the regulation of ginger seedlings in response to salt stress.

4. Discussion

Salt stress is one of the major abiotic stresses limiting plant growth and development, and it can significantly inhibit several key growth processes, including seed germination, root development, flowering and fruit set [38, 39, 40]. Currently, numerous studies have confirmed that exogenous application of gaseous signaling molecules can effectively alleviate the damage caused by abiotic stress to plants. As an important gaseous signaling molecule, NO can significantly improve growth inhibition in plants under stress conditions and enhance stress resistance, including salt stress [41]. The results of this study showed that salt stress significantly inhibited ginger seedling growth, and NO effectively alleviated salt damage and improved salt tolerance (Fig. 1). Wang et al. (2023) reported that NO significantly alleviated the inhibitory effects of salt stress on plant height, total root length and leaf area in tomato seedlings, thus effectively enhancing their salt tolerance [42]. Furthermore, Ren et al. (2020) found that NO effectively improves seed germination parameters in pak choi under salt stress, promotes radicle and plumule growth and consequently enhances early seedling growth [43]. In ginger seedlings, NO markedly reduced the accumulation of ROS, while enhancing the activity of antioxidant enzymes like POD and SOD, thereby effectively mitigating oxidative damage under salt stress. However, cPTIO reversed the protective effects of NO, confirming that NO is essential for maintaining antioxidant capacity and alleviating salt stress in ginger seedlings (Fig. 2). Similarly, treatment with NO could significantly increase the activity of antioxidant enzymes including SOD and CAT in barley leaves, promote ferritin accumulation, scavenge excess ROS and mitigate oxidative damage, thereby enhancing salt tolerance in barley [44]. Simultaneously, NO enhances salt tolerance in bermudagrass by increasing

the activity of antioxidant enzymes such as SOD and POD, reducing the accumulation of ROS and lipid peroxidation products, decreasing electrolyte leakage and maintaining cell membrane stability [45]. Furthermore, compared with NaCl+SNP treatment, NaCl+cPTIO treatment reversed the beneficial effects of NO, further verifying that NO promotes the growth in ginger seedlings under salt stress. Wang et al. (2023) [46] and Khatoon et al. (2025) [47] reached similar conclusions in tomato and wheat, respectively, showing that NO promotes plant growth under salt stress, while cPTIO reverses this effect. Collectively, NO enhances antioxidant enzyme activities and reduces ROS accumulation, thereby alleviating salt stress damage in ginger seedlings.

It is well known that plant hormones in plants include AUX, CTK, ABA, ETH, BR, JA, SA and SL [48]. Among these, hormones such as ETH, ABA, JA and SA play critical regulatory roles when plants are subjected to abiotic stress, greatly enhancing the tolerance to adverse environmental conditions [49, 50]. Numerous studies have demonstrated that under abiotic stresses such as drought, chilling and salt stress, plants regulate the gene expression through signal transduction by modulating endogenous hormone levels, ultimately enhancing stress resistance [51]. For example, drought stress markedly induces an increase in ABA levels, activating the stomatal closure and the expression of drought-responsive genes [52]. Chilling stress stimulates the upregulation of ETH synthesis [53]. Furthermore, JA and SA can also mediate the biosynthesis of plant secondary metabolites under drought stress [54]. Overall, these studies fully demonstrate that plant hormones play an indispensable role in plant stress response and adaptation. Our results showed that NO significantly increased IAA, GA₃ and ZR contents, while decreasing ABA, MeJA and BR levels in ginger seedlings under salt stress (Fig. 6), and markedly altered the expression of genes involved in plant hormone biosynthesis and signal transduction (Fig. 4). In our study, NO up-regulated *ARF* and *GH3* and down-regulated *AUX1*, *LAX* and *IAA* in ginger seedlings under salt stress. In *Arabidopsis thaliana*, salt stress induces NO accumulation, which inhibits root apical meristem growth by stabilizing Aux/IAA proteins and suppressing ARF-mediated auxin signaling [55]. Wang et al. (2023) indicated that GH₃-mediated auxin inactivation can delay lateral root development by regulating local auxin balance [42]. Therefore, we infer that NO participates in regulating lateral root growth and development by upregulating GH₃ expression, contributing to root system stability and enhancing salt tolerance in ginger seedlings. Taken together, these findings indicate that NO can improve the tolerance of ginger seedlings under salt stress by maintaining a dynamic balance of auxin metabolism and signaling. Furthermore, the key mechanisms by which GA₃ contributes to plant stress responses are gradually being elucidated and clarified. Additionally, NO may be involved in the processes of GA₃-induced alleviation of growth inhibition and mitigation of ion toxicity, thereby conferring salt tolerance to ginger seedlings. This suggests that the interaction between NO and GA₃ is a vital regulatory step in the plant response to salt stress [56]. Similarly, Huang et al. (2020) reported in rice that NO elevates GA₃ levels under salt stress, promoting plant growth and alleviating salt-induced growth inhibition [57]. Previous studies have indicated that genes encoding key enzymes in GA synthesis, such as GA3ox and GA20ox, directly regulate GA₃ synthesis and accumulation. This further provides a molecular rationale for the significant increase in endogenous GA₃ levels induced by NO under salt stress observed in this study [58]. Further studies have found that NO treatment under salt stress

reduces ABA levels in ginger seedlings, accompanied by a significant downregulation of *ABF* and *ABF-1* transcript levels. The *AREB/ABF-SnRK2* pathway plays a prominent positive regulatory role in ABA signaling and osmotic stress responses. *AREB/ABFs*, as members of the *bZIP* family of transcription factors, directly regulate the expression of downstream stress-responsive genes by binding to ABRE cis-acting elements [59]. Another study has shown that NO can reduce ABA levels by regulating the transcriptional levels of genes associated with the MAPK signaling pathway [60]. These results indicate that NO can alleviate salt stress-induced growth inhibition and physiological damage by down-regulating ABA levels and the expression of ABF family genes, thereby enhancing the salt tolerance of ginger seedlings. Simultaneously, NO effectively reduced MeJA levels and modulated the expression of *JAZ* and *MYC2* in ginger seedlings under salt stress. As a typical stress-responsive hormone, MeJA typically accumulates in large quantities under stress conditions and negatively affects normal plant growth. *JAZ* proteins are key negative regulators of JA signaling pathway and can block downstream JA signaling by inhibiting the activity of transcription factors such as *MYC2* [61]. Consistent with the decreased endogenous MeJA levels observed in this study, these findings indicate that NO can alleviate the growth inhibition of ginger seedlings caused by salt stress by regulating MeJA metabolism and the *JAZ-MYC2* signaling pathway, thereby markedly enhancing the salt tolerance of plants. In this study, NO significantly reduced endogenous BR levels in ginger seedlings while inducing the upregulation of *BAK1* expression and decreased the expression of three *BRI1* genes. BR is a key phytohormone that coordinates the balance between plant growth and stress resistance. BR signaling pathway is primarily initiated by the binding of *BRI1* receptors to the co-receptor *BAK1*, with *BAK1* acting as a co-receptor to enhance the efficiency of BR signaling transduction [62]. These results imply that NO might balance the growth and development of ginger seedlings in response to salt tolerance by regulating BR metabolism and the *BRI1-BAK1* signaling pathway. Interestingly, NO treatment significantly increased the endogenous ZR content in ginger seedlings under salt stress. *ARR-B*, a key gene in the ZR signaling pathway, exhibited a differential expression pattern after NO application. As an important active form of CTK, ZR participates in the regulation of cell division, growth and development and stress adaptation processes (Argueso et al. 2010). As core transcription factors in the CTK signaling pathway, the differential expression patterns of *ARR-B* family reflect the complex and precise regulation of CTK signaling by NO, which might contribute to the adaptation of ginger seedlings to salt stress. In summary, NO may effectively alleviate the growth inhibition and physiological damage caused by salt stress in ginger seedlings by synergistically regulating the dynamic balance of multiple endogenous phytohormone, further confirming that NO plays an indispensable role in the regulation of plant hormone signaling networks under salt stress.

Starch and sucrose metabolism serve not only act as the primary energy sources for plants but also participate in abiotic stress response [27]. Under salt stress conditions, high concentrations of Na^+ can disrupt plant osmotic balance, causing tissue damage and even plant death [63]. Moreover, starch metabolism is increasingly recognized as a crucial regulatory mechanism for plants coping with abiotic stress. Under adverse conditions, plants usually reactivate starch degradation to release energy and carbon sources. The released sugars and other derivatives support plant growth under stress and

function as osmo protectants and compatible solutes to alleviate stress injury [64]. Meanwhile, sucrose and soluble sugars, as vital osmotic regulators, maintain cellular structural integrity, stabilize photosynthetic efficiency, scavenge reactive oxygen species, and mediate metabolic signaling. These functions further enhance membrane stability and antioxidant capacity, ultimately improving plant tolerance to various abiotic stresses [65]. Previous studies have shown that under salt stress, sucrose content in cotton increases with rising salt concentration, accompanied by significantly elevated SPS activity [66]. These observations are in line with our results, which NO significantly increased sucrose content and SPS activity under salt stress (Figs. 5, 7). In addition, drought stress induces a continuous increase in soluble sugar content and a marked decrease in starch content in tree leaves [12]. Similarly, drought stress promotes soluble sugar accumulation in soybean, enhances SPS and SS enzyme activities, and up-regulates the expression levels of *GmSPS1*, *GmSuSy2* and *GmA-INV* [67]. In the above-mentioned studies, the alterations in starch and sugar contents under abiotic stress are highly consistent with our results. This also further verify that changes in starch and sucrose metabolism-related metabolites and key enzyme activities represent important physiological and biochemical indicators of plant responses to abiotic stress. Furthermore, a previous study reported that NO could enhance salt tolerance in tomato plants by inducing massive accumulation of soluble sugars [68]. Likewise, in rice, exogenous NO significantly promotes sucrose accumulation and increases the enzymatic activities of SPS and SS under salt stress [57]. Ahmad et al. (2016) suggested that the abundant accumulation of soluble sugar also was induced by NO, thereby enhancing the salt tolerance of chickpea via osmotic adjustment [69]. Subsequent studies have revealed that NO treatment reduces starch content, promotes soluble sugar production and supplies energy for sorghum seed germination, thus strengthening salt tolerance during sorghum seed germination [70]. These findings are highly consistent with our conclusions, further confirming that NO enhances the plant salt tolerance by regulating the metabolic conversion from starch to sugar. In summary, NO effectively promotes an increase in sugar accumulation, reduces starch content and enhances the activity of relevant enzymes, thereby improving the salt tolerance in ginger seedlings.

5. Conclusions

In conclusion, this study systematically elucidated the alleviating effects of NO on salt stress in ginger seedlings. Through comparative transcriptomic analysis, a large number of key candidate genes involved in the salt stress alleviation process were identified. The results indicate that NO can significantly enhance antioxidant enzyme activity and decrease excess ROS production, alleviating the oxidant damage under salt stress. In addition, the dynamic balance and signaling transduction of phytohormone and starch and sugar metabolism were activated by NO in ginger seedlings under salt stress. These effects help maintain phytohormone homeostasis and promote the accumulation of soluble sugars and sucrose, thereby effectively mitigating salt stress damage (Fig. 8). This study provides important theoretical support for elucidating the molecular mechanisms underlying salt tolerance in ginger seedlings.

Declarations

Ethics approval and consent to participate

According to institutional guidelines, formal ethical approval was not required for this type of study.

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Author contributions

Yue Zhong: Writing - original draft. Data curation. **Shijia Zhu**: Data curation. **Jinlian Yuan**: Investigation. **Yiming Yang, Manli qin, Zehan Zhu** and **Xiang Ma**: Software. **Jiayu Zhai**: Methodology. **Shouhui Wei**: Investigation. **Lijuan Wei**: Methodology. Writing - review & editing. **Yiqing Liu**: Validation. Project administration. Funding acquisition.

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Data availability

Data will be made available on request.

Declarations

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.

Consent for publication

Not applicable.

References

1. Wang Y, Zhang R, Liu Y, Li R, Ge J, Deng S, Zhang X, Chen Y, Wei H, Dai Q. Rice response to salt stress and research progress in salt tolerance mechanism. Chinese Journal of Rice Science. 2022; 36: 105. <https://doi.org/10.16819/j.1001-7216.2022.210609>.

2. Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O. Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. *Front Plant Sci.* 2015; 6: 978. <https://doi.org/10.3389/fpls.2015.00978>.
3. Chaves MM, Flexas J, Pinheiro C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann Bot-London.* 2009; 103: 551–560. <https://doi.org/10.1093/aob/mcn125>.
4. Mbarki S, Sytar O, Cerda A, Zivcak M, Rastogi A, He X, Zoghlami A, Abdelly C, Brestic M. Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. In *Salinity Responses and Tolerance in Plants, Volume 1: Targeting Sensory, Transport and Signaling Mechanisms*. Cham: Springer International Publishing; 2018. p. 85–136. https://doi.org/10.1007/978-3-319-75671-4_4.
5. Seemann JR, Critchley C. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta.* 1985; 164: 151–162. <https://doi.org/10.1007/bf00396077>
6. Zhang Y, Li Y, de Zeeuw T, Duijts K, Kawa D, Lamers J, Munzert K, Li H, Zou Y, Jessica M, Yan J, Verstappen F, Wang Y, Gijsberts T, Wang J, Gigli-Bisceglia N, Engelsdorf T, van Dijk A, Testerink C. Root branching under high salinity requires auxin-independent modulation of *LATERAL ORGAN BOUNDARY DOMAIN 16* function. *The Plant Cell.* 2024; 36: 899–918. <https://doi.org/10.1093/plcell/koad317>
7. Wilson ID, Neill SJ, Hancock JT. Nitric oxide synthesis and signalling in plants. *Plant Cell Environ.* 2008; 31: 622–631. <https://doi.org/10.1111/j.1365-3040.2007.01761.x>
8. Timilsina A, Dong W, Hasanuzzaman M, Liu B, Hu C. Nitrate–nitrite–nitric oxide pathway: A mechanism of hypoxia and anoxia tolerance in plants. *Int J Mol Sci.* 2022; 23: 11522. <https://doi.org/10.3390/ijms231911522>
9. Khan M, Al Azzawi TNI, Ali S, Yun BW, Mun BG. Nitric oxide, a key modulator in the alleviation of environmental stress-mediated damage in crop plants: a meta-analysis. *Plants.* 2023; 12: 2121. <https://doi.org/10.3390/plants12112121>
10. Fancy NN, Bahlmann AK, Loake GJ. Nitric oxide function in plant abiotic stress. *Plant Cell Environ.* 2017; 40: 462–472. <https://doi.org/10.1111/pce.12707>
11. Sahu SK, Barman K, Singh AK. Nitric oxide application for postharvest quality retention of guava fruits. *Acta Physiol Plant.* 2020; 42: 156. <https://doi.org/10.1007/s11738-020-03143-8>
12. Wang Z, Wang C. Dynamics of nonstructural carbohydrates during drought and subsequent recovery: a global meta-analysis. *Agr Forest Meteorol.* 2025; 363: 110429. <https://doi.org/10.1016/j.agrformet.2025.110429>
13. Zheng Y, Hong H, Chen L, Li J, Sheng J, Shen L. *LeMAPK1*, *LeMAPK2*, and *LeMAPK3* are associated with nitric oxide-induced defense response against *Botrytis cinerea* in the *Lycopersicon esculentum* fruit. *J Agr Food Chem.* 2014; 62: 1390–1396. <https://doi.org/10.1021/jf404870d>

14. Sun Z, Li Y, Zhou J, Zhu SH. Effects of exogenous nitric oxide on contents of soluble sugars and related enzyme activities in 'Feicheng' peach fruit. *J Sci Food Agr.* 2011; 91: 1795–1800. <https://doi.org/10.1002/jsfa.4384>
15. Wani KI, Naeem M, Castroverde CDM, Kalaji HM, Albaqami M, Aftab T. Molecular mechanisms of nitric oxide (NO) signaling and reactive oxygen species (ROS) homeostasis during abiotic stresses in plants. *Int J Mol Sci.* 2021; 22: 9656. <https://doi.org/10.3390/ijms22179656>
16. Zhong Y, Wu X, Zhang L, Zhang Y, Wei L, Liu Y. The roles of nitric oxide in improving postharvest fruits quality: Crosstalk with phytohormones. *Food Chem.* 2024; 455: 139977. <https://doi.org/10.1016/j.foodchem.2024.139977>
17. Wei L, Zhang J, Wei S, Hu D, Liu Y, Feng L, Li C, Qi N, Wang C, Liao W. Nitric oxide enhanced salt stress tolerance in tomato seedlings, involving phytohormone equilibrium and photosynthesis. *Int J Mol Sci.* 2022; 23: 4539. <https://doi.org/10.3390/ijms23094539>
18. Siddiqui MH, Al-Whaibi MH, Basalah MO. Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma.* 2011; 248: 447–455. <https://doi.org/10.1007/s00709-010-0206-9>
19. Sun H, Feng F, Liu J, Zhao Q. The interaction between auxin and nitric oxide regulates root growth in response to iron deficiency in rice. *Front Plant Sci.* 2017; 8: 2169. <https://doi.org/10.3389/fpls.2017.02169>
20. Chen L, Sun S, Song CP, Zhou JM, Li J, Zuo J. Nitric oxide negatively regulates gibberellin signaling to coordinate growth and salt tolerance in *Arabidopsis*. *J. Genet. Genom.* 2022; 49: 756–765. <https://doi.org/10.1016/j.jgg.2022.02.023>
21. Zhang Q, Zhang L, Geng B, Feng J, Zhu S. Interactive effects of abscisic acid and nitric oxide on chilling resistance and active oxygen metabolism in peach fruit during cold storage. *J Sci Food Agric.* 2019; 99: 3367–3380. <https://doi.org/10.1002/jsfa.9554>
22. Hung KT, Kao CH. Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves. *J Plant Physiol.* 2004; 161: 43–52. <https://doi.org/10.1078/0176-1617-01178>
23. Deng Y, Wang C, Huo J, Hu W, Liao W. The involvement of NO in ABA-delayed the senescence of cut roses by maintaining water content and antioxidant enzymes activity. *Sci Hortic.* 2019; 247: 35–41 <https://doi.org/10.1016/j.scienta.2018.12.006>
24. Kara Z, Yazar K, Doğan O, Vergili E. Sodium nitroprusside and gibberellin effects on seed germination and seedling development of grapevine (*Vitis vinifera* L.) cvs. Ekşi Kara and Gök Üzümleri. *Erwerbs-Obstbau.* 2020; 62 Suppl 1: 61–68. <https://doi.org/10.1007/s10341-020-00497-8>
25. Liu H, Li C, Yan M, Zhao Z, Huang P, Wei L, Wu X, Wang C, Liao W. Strigolactone is involved in nitric oxide-enhanced the salt resistance in tomato seedlings. *J Plant Res.* 2022; 135: 337–350. <https://doi.org/10.1007/s10265-022-01371-2>
26. Dong S, Beckles DM. Dynamic changes in the starch-sugar interconversion within plant source and sink tissues promote a better abiotic stress response. *J Plant Physiol.* 2019; 234: 80–93. <https://doi.org/10.1016/j.jplph.2019.01.007>

27. Chen L, Meng Y, Bai Y, Yu H, Qian Y, Zhang D, Zhou Y. Starch and sucrose metabolism and plant hormone signaling pathways play crucial roles in *aquilegia* salt stress adaption. *Int J Mol Sci.* 2023; 24: 3948. <https://doi.org/10.3390/ijms24043948>
28. Zhao X, Yang Y, Zhang Y, Liu H, Lin T, Liu Y, Wei L, Liu Y. Homeostasis of phytohormones is involved in cold resistance of *Zanthoxylum bungeanum* maxim. *Plant Physiol Biochem.* 2025; 110543. <https://doi.org/10.1016/j.plaphy.2025.110543>
29. Fan A, Wan C, Liu H, Xiong X, Nong Y, Kahramanoğlu İ, Yang R, Zeng L. Melatonin treatment maintains the quality and delays senescence of postharvest cattails (*Typha latifolia* L.) during cold storage. *Food Chem X.* 2023; 19: 100796. <https://doi.org/10.1016/j.fochx.2023.100796>
30. Zhang L, Wu X, Zhong Y, Yang Y, Wei S, Sun C, Wei L, Liu Y. Hydrogen sulfide enhances the disease resistance of ginger to rhizome rot during postharvest storage through modulation of antioxidant response and nitric oxide-mediated S-nitrosylation. *Postharvest Biol Technol.* 2025; 220: 113321. <https://doi.org/10.1016/j.postharvbio.2024.113321>
31. Li G, Ma J, Yin J, Guo F, Xi K, Yang P, Cai X, Jia Q, Li L, Liu Y, Zhu Y. Identification of reference genes for reverse transcription-quantitative PCR analysis of ginger under abiotic stress and for postharvest biology studies. *Front Plant Sci.* 2022; 13: 893495. <https://doi.org/10.3389/fpls.2022.893495>
32. Zhu Y, Jiang X, Zhang J, He Y, Zhu X, Zhou X, Gong H, Yin J, Liu Y. Silicon confers cucumber resistance to salinity stress through regulation of proline and cytokinins. *Plant Physiol Biochem.* 2020; 156: 209–220. <https://doi.org/10.1016/j.plaphy.2020.09.014>
33. Yang J, Zhang J, Wang Z, Zhu Q, Wang W. Hormonal changes in the grains of rice subjected to water stress during grain filling. *Plant Physiol.* 2001; 127: 315–323. <https://doi.org/10.1104/pp.127.1.315>
34. Xiao Y, Wu Y, Huang Z, Guo M, Zhang L, Luo X, Xia H, Zhang X, Liang D, Lv X, Wang J, Lin L. Mechanism of induced soluble sugar accumulation and organic acid reduction in plum fruits by application of melatonin. *BMC Plant Biol.* 2024; 24: 1208. <https://doi.org/10.1186/s12870-024-05949-x>
35. Zhang L, Häusler RE, Greiten C, Hajirezaei MR, Haferkamp I, Neuhaus HE, Flügge U, Ludewig F. Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnol J.* 2008; 6: 453–464. <https://doi.org/10.1111/j.1467-7652.2008.00332.x>
36. Chullai LR, Kynshi BL, Syiem MB. 2025. Glucose 6-phosphate enrichment augments AGPase enzyme activity and biomass production in the cyanobacterium *Nostoc muscorum* Meg 1. *J Appl Phycol.* 2025; 37: 823–835. <https://doi.org/10.1007/s10811-024-03433-5>
37. Souleyre EJ, Iannetta PP, Ross HA, Hancock RD, Shepherd LV, Viola R, Taylor MA, Davies HV. Starch metabolism in developing strawberry (*Fragaria × ananassa*) fruits. 2004; 121: 369–376. <https://doi.org/10.1111/j.0031-9317.2004.0338.x>
38. Hu M, Shi Z, Zhang Z, Zhang Y, Li H. Effects of exogenous glucose on seed germination and antioxidant capacity in wheat seedlings under salt stress. *Plant Growth Regul.* 2012; 68: 177–188. <https://doi.org/10.1007/s10725-012-9705-3>

39. Dang TVT, Cho HS, Lee S, Hwang I. Salt stress-accelerated proteasomal degradation of *LBD11* suppresses ROS-mediated meristem development and root growth in *Arabidopsis*. *Plant Commun.* 6(4). <https://doi.org/10.1016/j.xplc.2025.101241>
40. Haque MSS, Rahman F, Naimuzzaman M, Jannat F, Mahmud M. Responses of nitrobenzene on growth and yield attributes in okra to salinity stress. *J Agric Sci Engineering.* 2024; 6: 49–59. <https://doi.org/10.48309/jase.2024.425212.1041>
41. Shang JX, Li X, Li C, Zhao L. The role of nitric oxide in plant responses to salt stress. *Int J Mol Sci.* 2022; 23: 6167. <https://doi.org/10.3390/ijms23116167>
42. Wang C, Wei L, Zhang J, Hu D, Gao R, Liu Y, Feng L, Gong W, Liao W. Nitric oxide enhances salt tolerance in tomato seedlings by regulating endogenous S-nitrosylation levels. *J Plant Growth Regul.* 2023; 42: 275–293. <https://doi.org/10.1007/s00344-021-10546-5>
43. Ren Y, Wang W, He J, Zhang L, Wei Y, Yang M. Nitric oxide alleviates salt stress in seed germination and early seedling growth of pakchoi (*Brassica chinensis* L.) by enhancing physiological and biochemical parameters. *Ecotoxicol Environ Saf.* 2020; 187: 109785. <https://doi.org/10.1016/j.ecoenv.2019.109785>
44. Li QY, Niu HB, Yin J, Wang MB, Shao HB, Deng DZ, Chen XX, Ren JP, Li YC. Protective role of exogenous nitric oxide against oxidative-stress induced by salt stress in barley (*Hordeum vulgare*). *Colloid Surface B.* 2008; 65: 220–225. <https://doi.org/10.1016/j.colsurfb.2008.04.007>
45. Liu A, Fan J, Gitau MM, Chen L, Fu J. Nitric oxide involvement in bermudagrass response to salt stress. *J Am Soc Hortic Sci.* 2016; 141: 425–433. <https://doi.org/10.21273/JASHS03794-16>
46. Wang Q, De Gernier H, Duan X, Xie Y, Geelen D, Hayashi KI, Xuan W, Geisler M, ten Tusscher K, Beeckman T, Vanneste S. *GH3*-mediated auxin inactivation attenuates multiple stages of lateral root development. *New Phytol.* 2023; 240: 1900–1912. <https://doi.org/10.1111/nph.19284>
47. Khatoon S, Kumari S, Gandhi M, Nagarwal K, Sahoo RN, Iqbal N, Khan MIR. Appraising diverse metrics of nitric oxide in salt stress tolerance of high yielding wheat genotypes. *Nitric Oxide.* 2025; 156: 82–93. <https://doi.org/10.1016/j.niox.2025.03.005>
48. McSteen P, Zhao Y. Plant hormones and signaling: common themes and new developments. *Dev Cell.* 2008; 14: 467–473. <https://doi.org/10.1016/j.devcel.2008.03.013>
49. Panozzo A, Bolla PK, Barion G, Botton A, Vamerali T. Phytohormonal regulation of abiotic stress tolerance, leaf senescence and yield response in field crops: a comprehensive review. *BioTech.* 2025; 14: 14. <https://doi.org/10.3390/biotech14010014>
50. Waadt R, Sella CA, Hsu PK, Takahashi Y, Munemasa S, Schroeder JI. Plant hormone regulation of abiotic stress responses. *Nat Rev Mol Cell Biol.* 2022; 23: 680–694. <https://doi.org/10.1038/s41580-022-00479-6>
51. Gupta S, Kaur R, Upadhyay A, Chauhan A, Tripathi V. Unveiling the secrets of abiotic stress tolerance in plants through molecular and hormonal insights. *3 Biotech.* 2024; 14: 252. <https://doi.org/10.1007/s13205-024-04083-7>

52. Zhu JK. Abiotic stress signaling and responses in plants. *Cell*. 2016; 167: 313–324. <https://doi.org/10.1016/j.cell.2016.08.029>
53. Sun X, Zhao T, Gan S, Ren X, Fang L, Karungo SK, Wang Y, Liang C, Li S, Xin H. Ethylene positively regulates cold tolerance in grapevine by modulating the expression of *ETHYLENE RESPONSE FACTOR 057*. *Sci Rep*. 2016; 6: 24066. <https://doi.org/10.1038/srep24066>
54. Ghassemi-Golezani K, Farhangi-Abriz S. Plant Responses to Exogenous Salicylic and Jasmonic Acids Under Drought Stress. In: Aftab, T., Yusuf, M., eds) *Jasmonates and Salicylates Signaling in Plants. Signaling and Communication in Plants*. Springer, Cham. 2021. https://doi.org/10.1007/978-3-030-75805-9_4
55. Liu W, Li RJ, Han TT, Cai W, Fu ZW, Lu YT. Salt stress reduces root meristem size by nitric oxide-mediated modulation of auxin accumulation and signaling in *Arabidopsis*. *Plant Physiol*. 2015; 168: 343–356. <https://doi.org/10.1104/pp.15.00030>
56. Sehrawat A, Deswal R. S-Nitrosylation in Abiotic Stress in Plants and Nitric Oxide Interaction with Plant Hormones. In: *Mechanism of Plant Hormone Signaling under Stress*. Vol 1; 2017. p. 399–411. <https://doi.org/10.1002/9781118889022.ch16>
57. Huang J, Zhu C, Hussain S, Huang J, Liang Q, Zhu L, Cao X, Kong Y, Li Y, Wang L, Li J, Zhang J. Effects of nitric oxide on nitrogen metabolism and the salt resistance of rice (*Oryza sativa* L.) seedlings with different salt tolerances. *Plant Physiol Biochem*. 2020; 155: 374–383. <https://doi.org/10.1016/j.plaphy.2020.06.013>
58. He H, Liang G, Lu S, Wang P, Liu T, Ma Z, Zuo C, Sun X, Chen B, Mao J. Genome-wide identification and expression analysis of *GA2ox*, *GA3ox*, and *GA20ox* are related to gibberellin oxidase genes in grape (*Vitis vinifera* L.). *Genes*. 2019; 10: 680. <https://doi.org/10.3390/genes10090680>
59. Fujita Y, Yoshida T, Yamaguchi-Shinozaki K. Pivotal role of the *AREB/ABF-SnRK2* pathway in *ABRE*-mediated transcription in response to osmotic stress in plants. *Physiol Plant*. 2013; 147: 15–27. <https://doi.org/10.1111/j.1399-3054.2012.01635.x>
60. Wei L, Zhang J, Wei S, Wang C, Deng Y, Hu D, Li H, Gong W, Pan Y, Liao W. Nitric oxide alleviates salt stress through protein S-nitrosylation and transcriptional regulation in tomato seedlings. *Planta*. 2022; 256: 101. <https://doi.org/10.1007/s00425-022-04015-w>
61. Wasternack C, Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Ann Bot-London*. 2013; 111: 1021–1058. <https://doi.org/10.1093/aob/mct067>
62. Clouse SD. Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. *Plant Cell*. 2011; 23: 1219–1230. <https://doi.org/10.1105/tpc.111.084475>
63. El Moukhtari A, Cabassa-Hourton C, Farissi M, Savouré A. How does proline treatment promote salt stress tolerance during crop plant development? *Front Plant Sci*. 2020; 11: 1127. <https://doi.org/10.3389/fpls.2020.01127>

64. Krasensky J, Jonak C. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J Exp Bot.* 2012; 63: 1593–1608. <https://doi.org/10.1093/jxb/err460>
65. Ahmad F, Singh A, Kamal A. Osmoprotective Role of Sugar in Mitigating Abiotic Stress in Plants. *In Protective Chemical Agents in the Amelioration of Plant Abiotic Stress* (eds A. Roychoudhury and D.K. Tripathi). 2020. <https://doi.org/10.1002/9781119552154.ch3>
66. Peng J, Liu J, Zhang L, Luo J, Dong H, Ma Y, Zhao X, Chen B, Sui N, Zhou Z, Meng Y. Effects of soil salinity on sucrose metabolism in cotton leaves. *PLoS One.* 2016; 11: e0156241. <https://doi.org/10.1371/journal.pone.0156241>
67. Du Y, Zhao Q, Chen L, Yao X, Zhang H, Wu J, Xie F. Effect of drought stress during soybean R2–R6 growth stages on sucrose metabolism in leaf and seed. *Int J Mol Sci.* 2020; 21: 618. <https://doi.org/10.21203/rs.2.16721/v1>
68. Wu X, Zhu W, Zhang H, Ding H, Zhang HJ. Exogenous nitric oxide protects against salt-induced oxidative stress in the leaves from two genotypes of tomato (*Lycopersicon esculentum* Mill.). *Acta Physiol Plant.* 2011; 33: 1199–1209. <https://doi.org/10.1007/s11738-010-0648-x>
69. Ahmad P, Abdel Latef AA, Hashem A, Abd_Allah EF, Gucel S, Tran LSP. Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Front Plant Sci.* 2016; 7: 347. <https://doi.org/10.3389/fpls.2016.00347>
70. Yin MQ, Wang D, Wang JR, Lan M, Zhao J, Dong SQ, Song XE, Alam SY, Xiang Y, Wang YG, Wen YY. Effects of exogenous nitric oxide on seed germination and starch transformation of sorghum seeds under salt stress. 2019; 4119–4128. <https://doi.org/10.3864/j.issn.0578-1752.2019.22.016>

Figures

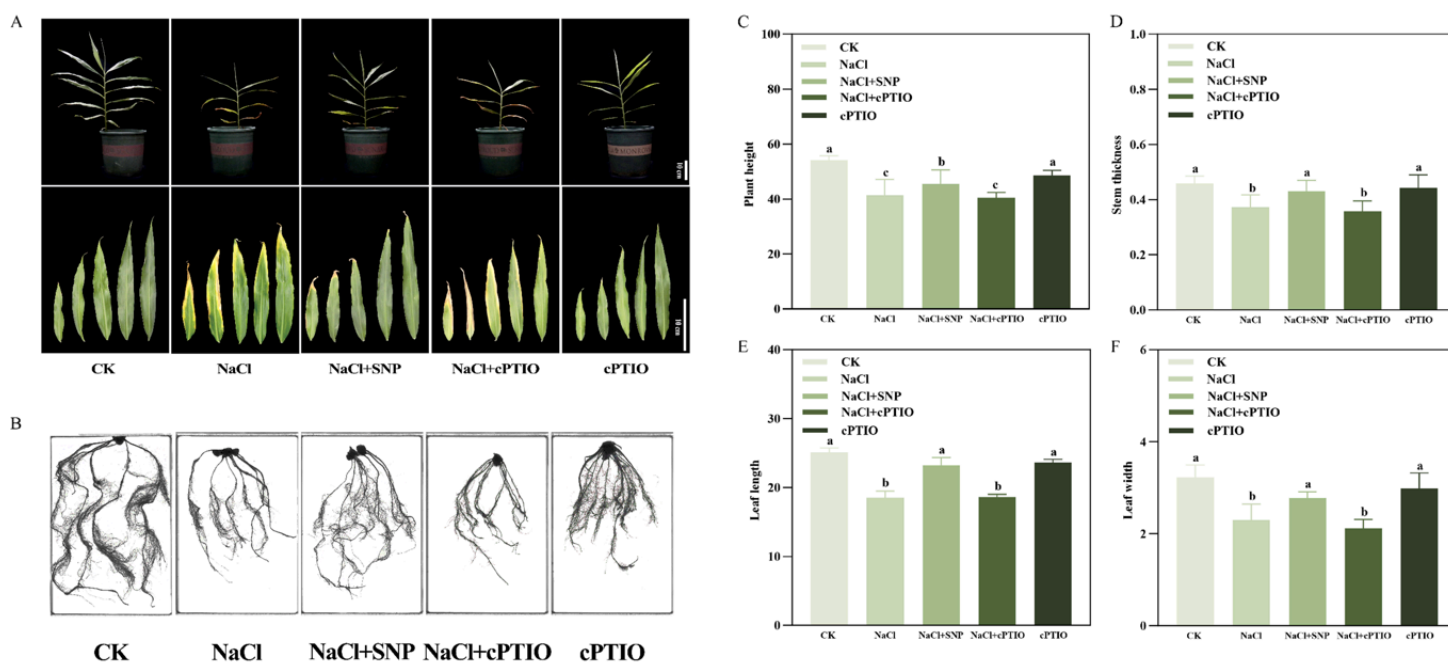


Figure 1

Effects of NO on the phenotype and growth indices of ginger seedlings under salt stress after 7 days of treatment. (A) Phenotypic characteristics of whole plants and leaves in different treatments. (B) Root morphology of ginger seedlings under different treatments. (C) Plant height. (D) Stem diameter. (E) Leaf length. (F) Leaf width. Values (mean±SE) are the means of three independent experiments (n=15). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

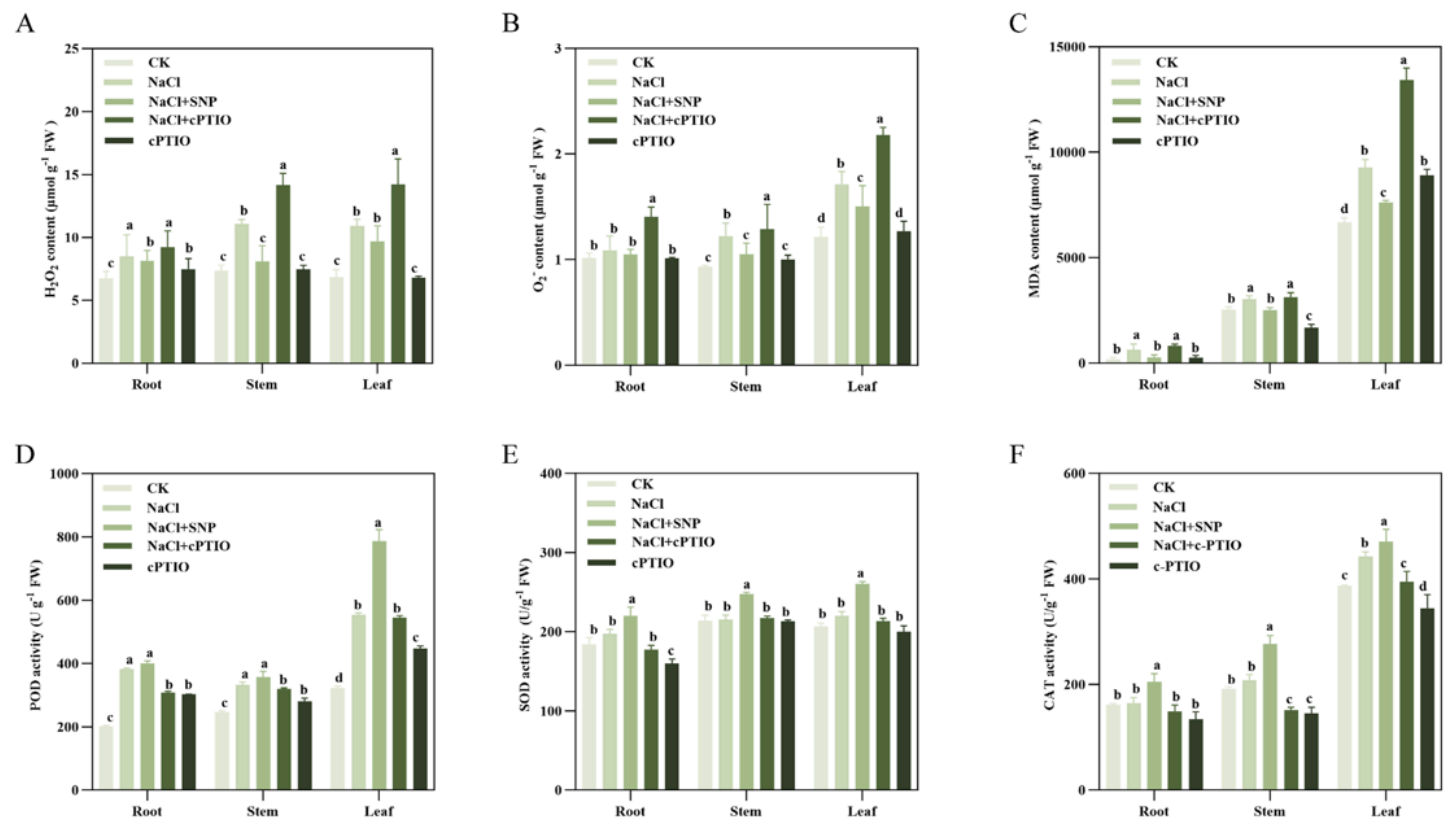


Figure 2

Effects of NO on oxidative damage and antioxidant enzyme activities in ginger seedlings under salt stress. (A) H_2O_2 content. (B) O_2^- content. (C) MDA content. (D) POD activity. (E) SOD activity. (F) CAT activity. Values (mean±SE) are the means of three independent experiments (n=15). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

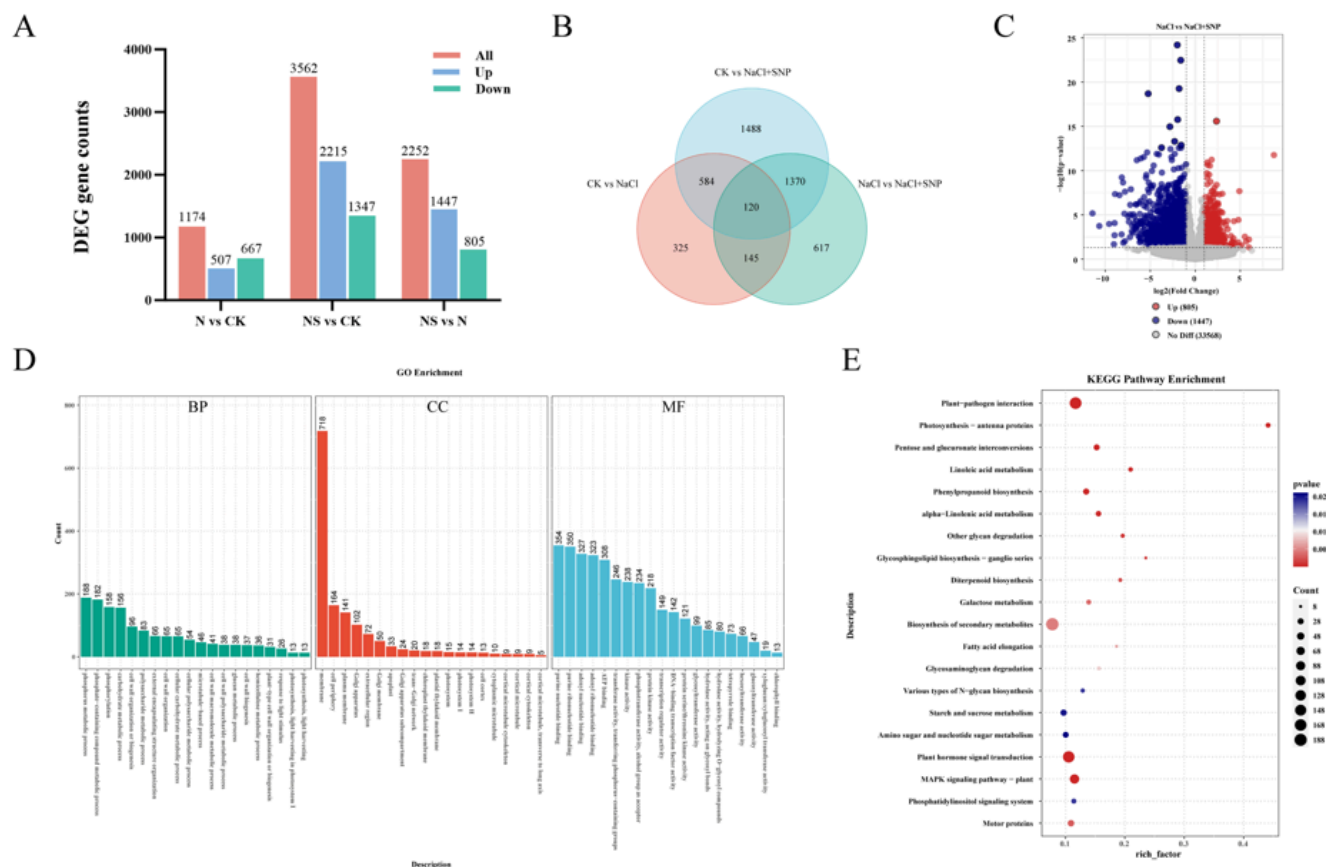


Figure 3

Transcriptome profiling analysis during NO alleviation of salt stress in ginger seedlings. (A) Number of DEGs in three comparison treatments. (B) Venn diagram showing the overlap of DEGs among the three comparison treatments. (C) The number of DEGs is shown by the volcano plot. (D) Functional gene ontology (GO) enrichment analysis of DEGs. GO terms are summarized in three categories: biological process, cellular component and molecular function. (E) The DEG enrichment in different KEGG pathways.

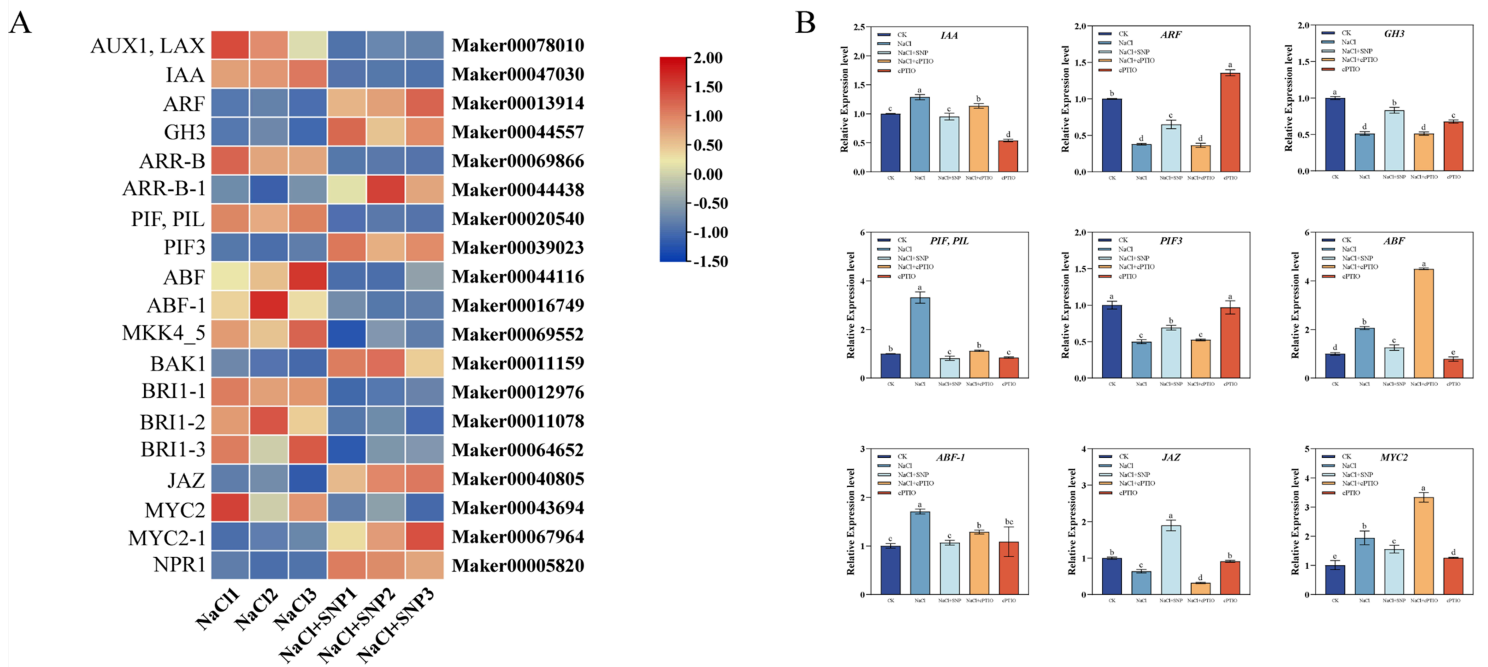


Figure 4

Expression analysis of plant hormone signaling-related DEGs in ginger seedlings under salt stress and NO treatment. (A) Heatmap of plant hormone signaling-related DEG expression in ginger seedlings under NaCl and NaCl+SNP treatments. (B) qRT-PCR validation of key plant hormone signaling genes. Different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

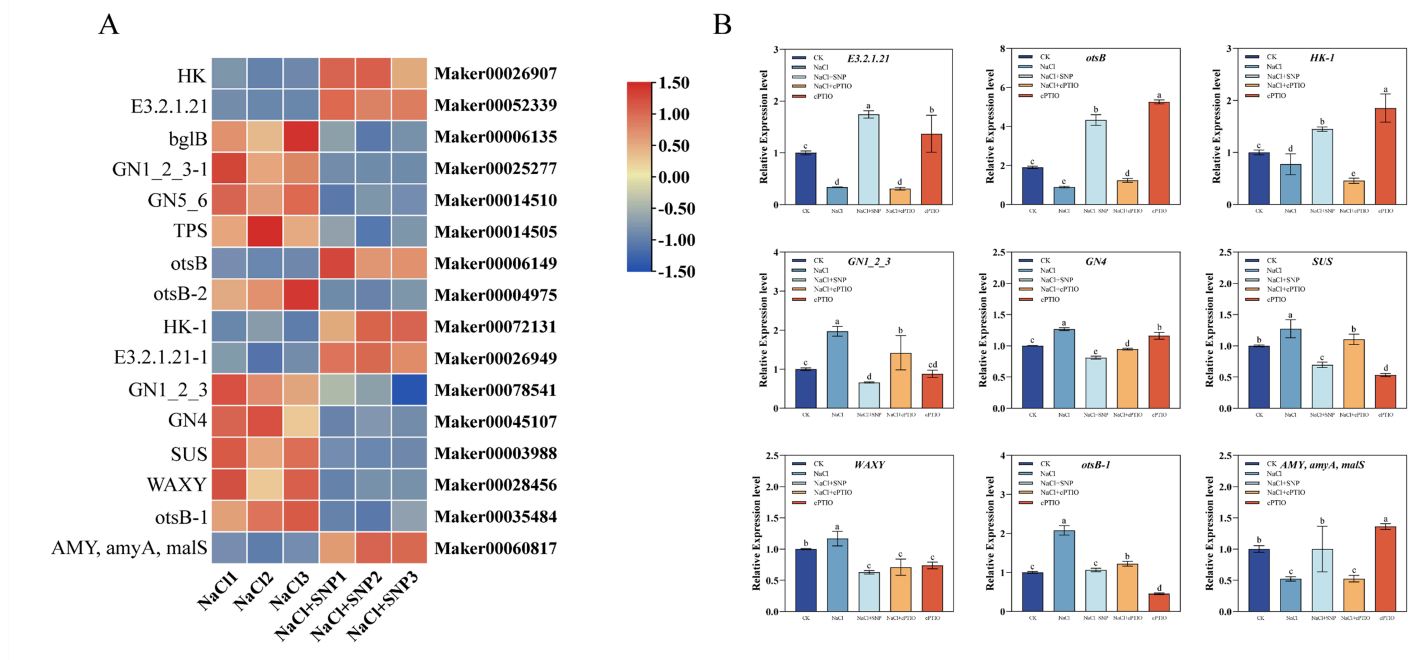


Figure 5

Expression analysis of starch and sucrose metabolism DEGs in ginger seedlings under salt stress and NO treatment. (A) Heatmap of starch and sucrose metabolism DEG expression in ginger seedlings under NaCl and NaCl+SNP treatments. (B) qRT-PCR validation of key starch and sucrose metabolism genes in ginger under salt stress and NO regulation. Values (mean±SE) are the means of three independent experiments (n=15). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

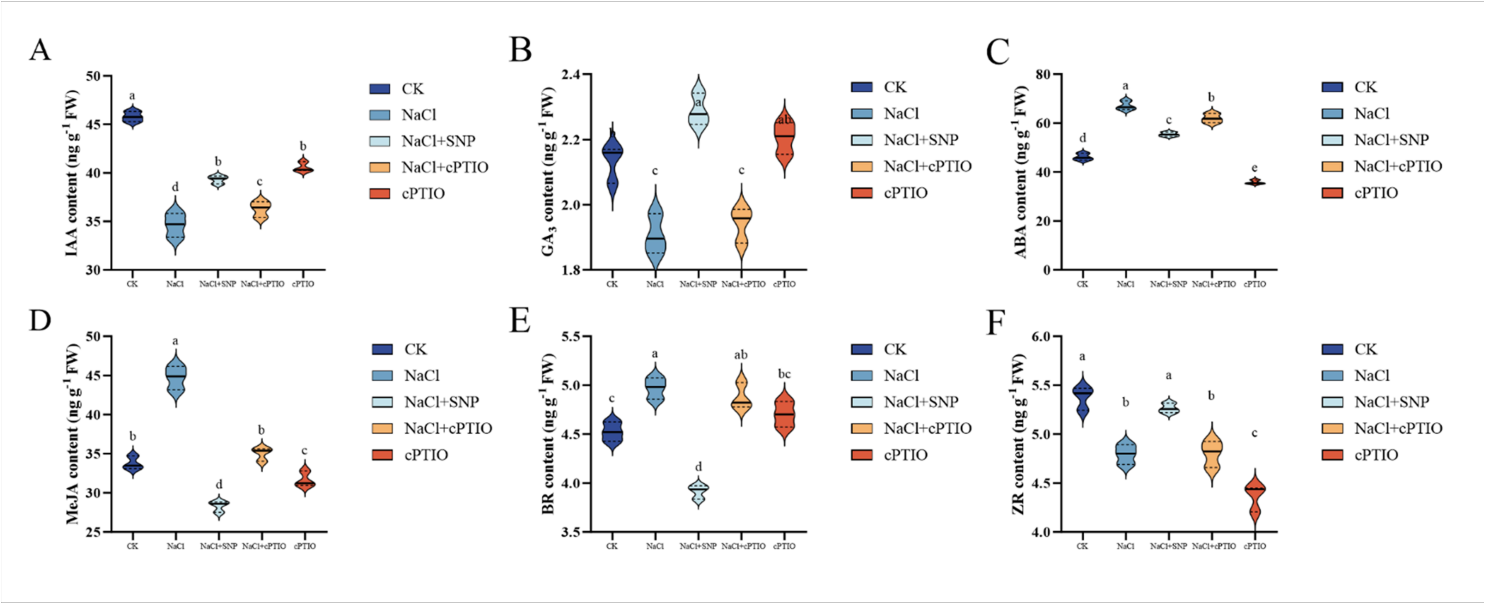


Figure 6

Changes in IAA, GA_3 , ABA, MeJA, BR and ZR content during NO alleviation of salt stress. (A) IAA content. (B) GA_3 content. (C) ABA content. (D) MeJA content. (E) BR content. (F) ZR content. Values (mean±SE) are the means of three independent experiments (n=15). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

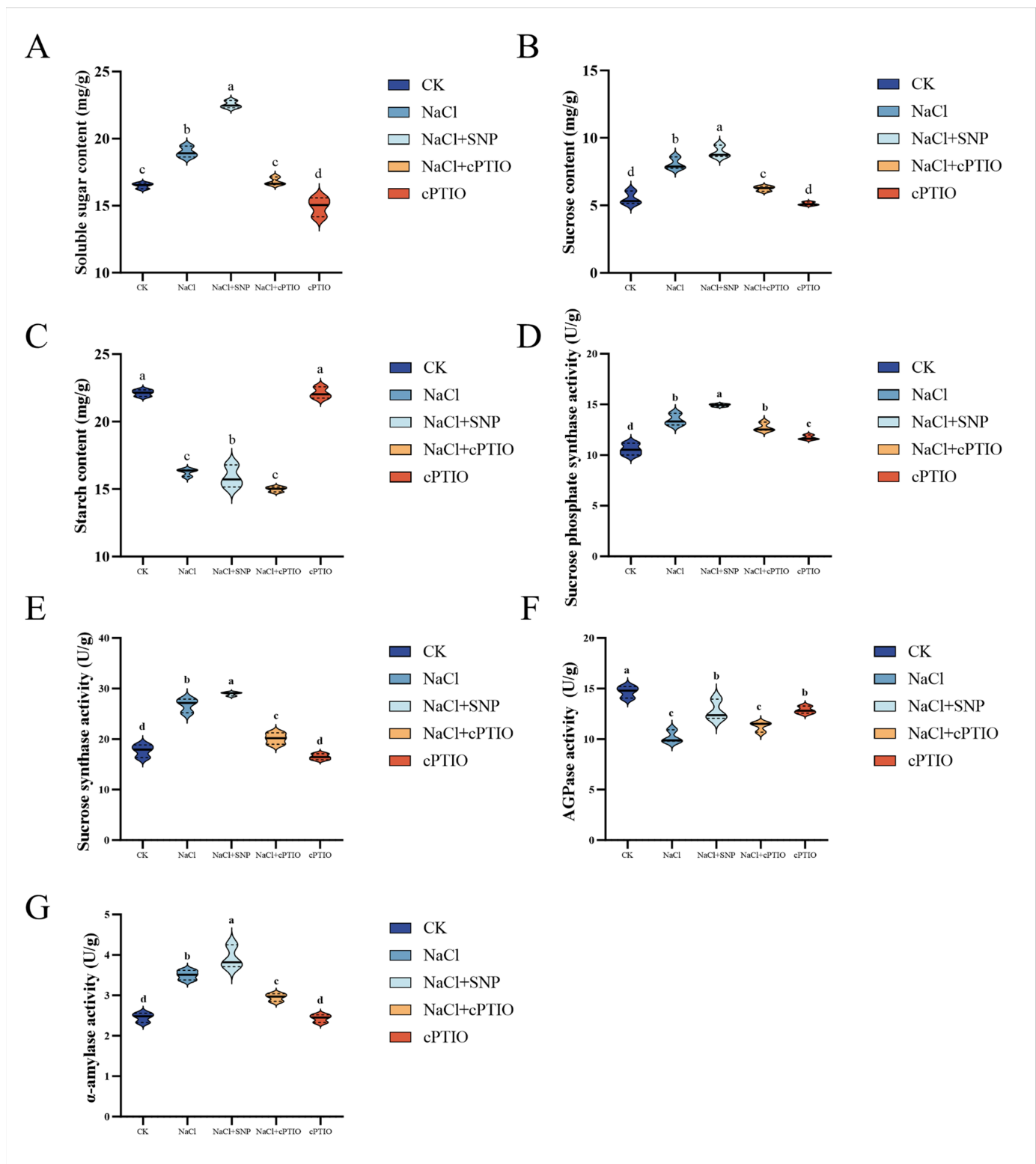


Figure 7

Changes in starch and sugar contents as well as key metabolic enzyme activities during NO-mediated alleviation of salt stress. (A) Soluble sugar content. (B) Sucrose content. (C) Starch content. (D) Sucrose phosphate synthase (SPS) activity. (E) Sucrose synthase (SS) activity. (F) AGPase activity. (G) α -AMY activity. Values (mean $\pm$ SE) are the means of three independent experiments (n=15). Different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

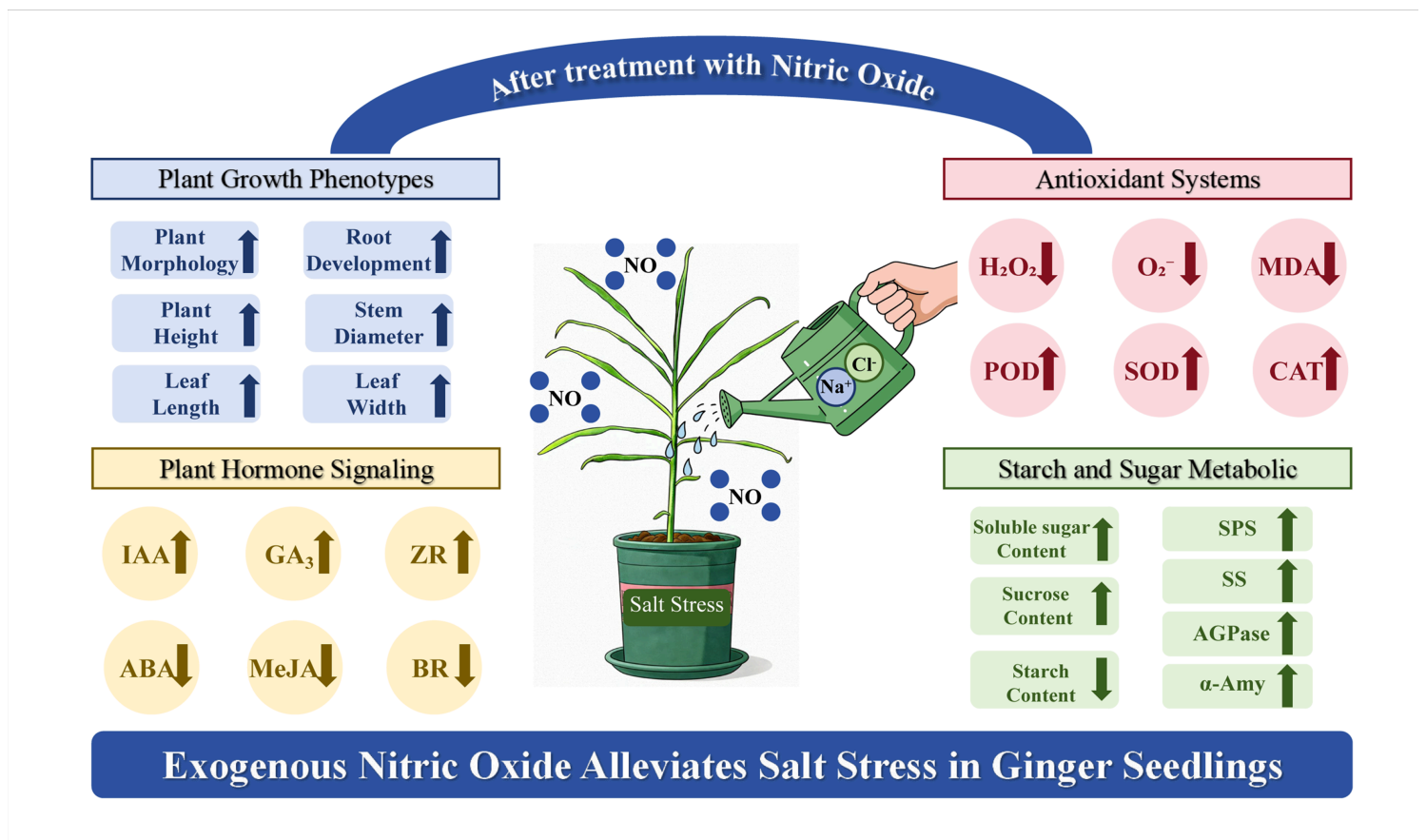


Figure 8

Comparative mechanisms of exogenous NO in ginger seedlings under salt stress.