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Comprehensive transcriptome analysis of *Asparagus officinalis* in response to varying levels of salt stress

Shuangshuang Wen¹, Jiali Ying¹, Youju Ye¹, Yunfei Cai¹ and Renjuan Qian^{1*}

Abstract

Background Salt stress is a major abiotic factor that affects the distribution and growth of plants. *Asparagus officinalis* is primarily resistant to salt stress and is suitable for cultivation in saline-alkali soil.

Results The study integrated the morphology, physiological indexes, and transcriptome of *A. officinalis* exposed to different levels of NaCl, with the aim of understanding its biological processes under salt stress. The findings indicated that exposure to salt stress led to decreases in the height and weight of *A. officinalis* plants. Additionally, the levels of POD and SOD, as well as the amounts of MDA, proline, and soluble sugars, showed an increase, whereas the chlorophyll content decreased. Analysis of the transcriptome revealed that 6,203 genes that showed differential expression at different salt-stress levels. Various TFs, including FAR1, MYB, NAC, and bHLH, exhibited differential expression under salt stress. KEGG analysis showed that the DEGs were primarily associated with the plant hormone signal transduction and lignin biosynthesis pathways.

Conclusion These discoveries provide a solid foundation for an in-depth exploration of the pivotal genes, including *Aux/IAA*, *TCH4*, *COMT*, and *POD*, among others, as well as the pathways involved in asparagus's salt stress responses. Consequently, they have significant implications for the future analysis of the molecular mechanisms underlying asparagus's response to salt stress.

Keywords Salt stress, *Asparagus officinalis*, Transcriptome, Plant hormone signal transduction, Lignin biosynthesis

Introduction

High levels of salt in the soil can have detrimental effects on plants, including osmotic stress, oxidative stress, ion toxicity, and nutrient deficiencies, all of which can hinder plant growth and lead to reduced crop yield and quality [1, 2]. Presently, more than 20% of irrigated land worldwide is negatively affected by salt stress [3–5].

Saline-alkali soil is an important cultivated land reserve, which limits the distribution of plants. Therefore, planting salt tolerant cultivated crops in salinized land can effectively alleviate the pressure on land demand [6].

A. officinalis, a perennial plant, is part of the *Asparagus* genus within the Asparagaceae family [7]. *Asparagus* has high nutritional value and medical value and is highly popular in the international market [8, 9]. *Asparagus* is rich in vitamins, minerals, amino acids, saponins and rutin [10, 11], and possesses effects on preventing cancer, lowering blood lipids and preventing coronary heart disease. *Asparagus* is believed to have strong salt tolerance, as it can grow normally in moderate saline-alkali

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soil below 0.3%, concerning one of the favored vegetable species for the utilization of saline-alkali land [12]. Nevertheless, the growth of asparagus seedlings is still negatively impacted by salt stress, highlighting the importance of research to enhance asparagus resistance to salt stress. However, there remains a dearth of data regarding the biological processes involved in reaction to salt stress of asparagus. Changes in protective proteins and metabolites are regulated by activating transcriptional cascades after salt stress [13]. RNA sequencing (RNA-seq) is a valuable tool for identifying potential genes and pathways involved in plant responses to salt stress [14]. Extensive research has been conducted on gene expression changes caused by salt stress [15–17]. Salt-tolerant genes are enriched in various pathways, including the phenylpropanoid, plant hormone signal transduction, glutathione metabolism, and MAPK signal pathways [3, 6, 18–20].

A. officinalis seedlings were utilized in this research and subjected to varying levels of sodium chloride (NaCl) treatment. The morphology, physiological indexes and transcriptome analysis were conducted to investigate the pivotal genes and pathways of *A. officinalis* under salt stress. Findings of this study will offer important

information for improving the growth of crops that are more resilient to salt stress.

Results

Morphological changes of *A. officinalis* seedlings under salt stress

In order to investigate how *A. officinalis* reacts to salt stress, asparagus seedlings that were four months old were exposed to different NaCl concentrations (0 for control, 1.0% for P1 and 2.0% for P2), and the resulting morphological changes in *A. officinalis* were documented (Fig. 1). Following a 12-day treatment, it was observed that the growth of the *A. officinalis* plant was notably stunted by 1.0 and 2.0% NaCl compared to the control group (Fig. 1A). Additionally, the *A. officinalis* seedlings showed signs of wilting under the 2.0% NaCl condition. The plant height and fresh weight (FW) of the stem were significantly decreased by the treatments with 1.0 and 2.0% NaCl (Fig. 1B and C). Significantly, as the salt concentration increased, the growth of *A. officinalis* was significantly restricted.

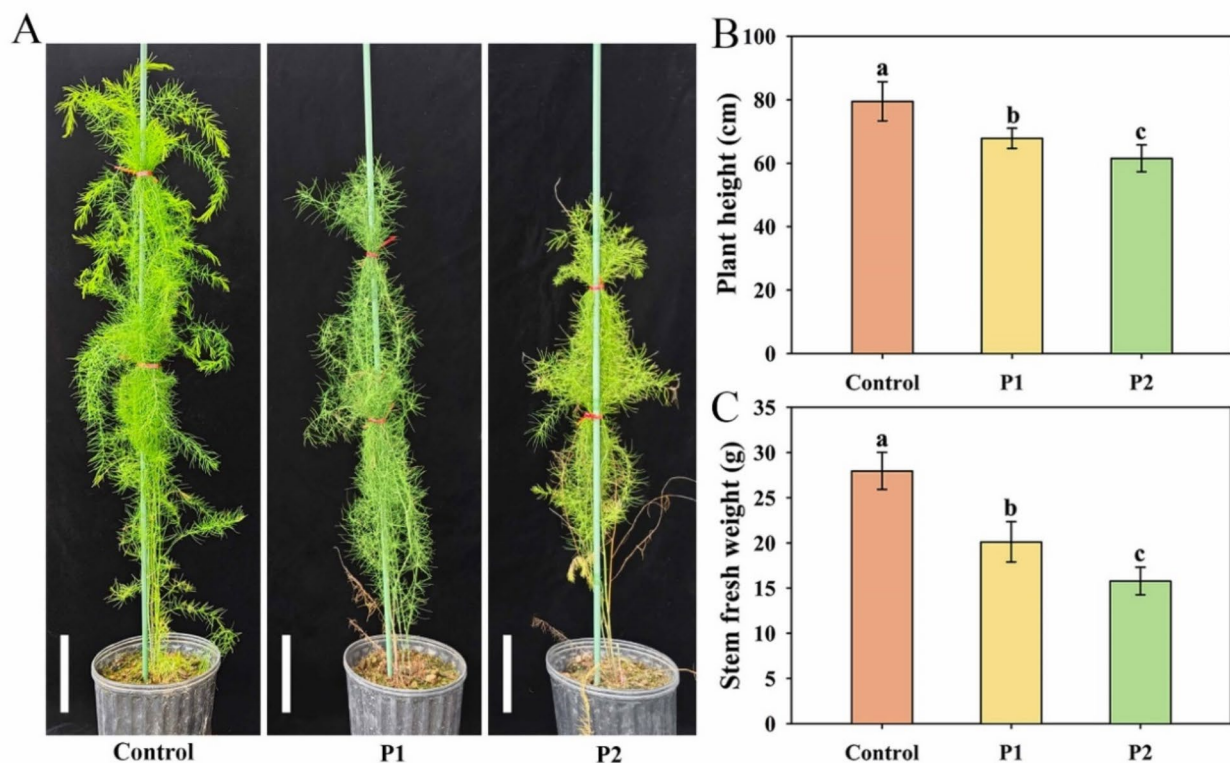


Fig. 1 Phenotypic characteristics changes of *A. officinalis* under salt stress for 12 days. **(A)** Phenotypes of seedlings under 0 (Control), 1.0% (P1), and 2.0% (P2) NaCl treatments. **(B–C)** Plant height and fresh weight (FW) of the stem. The experiment was conducted three times independently. The data showed averages and standard errors, with distinct letters indicating statistical significance ($p < 0.05$). Bar = 10 cm

Physiological indexes of *A. officinalis* under different salt concentrations and stress duration

Physiological indexes associated with stress reactions were assessed to explore the impact of salt stress on the development of *A. officinalis*. The results demonstrated that salt stress had a significant impact on peroxidases (POD) and superoxide dismutases (SOD) activities, as well as malondialdehyde (MDA), proline, soluble protein, and total chlorophyll content (Fig. 2). Under varying NaCl concentrations and durations of stress, all of the above parameters, with the exception of chlorophyll content, showed varying degrees of increase compared to the control group (Fig. 2A-E). The amount of chlorophyll decreased significantly when exposed to various salt treatments, with a more pronounced reduction observed with 2.0% NaCl compared to 1.0% NaCl (Fig. 2F). These results indicate that there were differences in physiological responses of *A. officinalis* seedlings under different salt concentrations and stress duration, which may closely relate to the level of gene expression. Samples collected at 9 days after treatment were selected for transcriptome analysis based on the physiological indexes.

Transcriptome profile and correlation analysis of *A. officinalis* under salt stress

RNA-seq was conducted on the cladodes of seedlings to investigate the molecular mechanisms of *A. officinalis* in response to salt stress. Nine cDNA libraries were created, and sequencing data were collected (Additional File 1). The average number of raw reads from nine libraries

was 22.79 million, while the mean number of clean reads obtained after filtering out adapters and low-quality sequences was 22.64 million. In addition, GC content ranged from 47.34 to 48.38%. All samples displayed Q20 greater than 97.21%, and Q30 values over 92.97%. Overall, the transcriptome sequencing data was excellent and is suitable for additional analysis.

The gene expression distribution of the P2 sample was generally higher than that of the control and P1 samples, as shown in the Additional File 2 A. Principal Component Analysis (PCA) indicated that control, P1 and P2 formed separate clusters, indicating high reliability among the samples (Additional File 2B). The Pearson Correlation Coefficients (PCC) for control, P1 and P2 in *A. officinalis* exceeded 0.86, indicating strong correlations among the three treatment groups (Additional File 2 C).

DEGs analysis of *A. officinalis* under salt stress

Comparisons between control and P1, control and P2, and P1 and P2 libraries were conducted for differentially expressed genes (DEGs) analysis. Gene expression profiling in all samples revealed the presence of transcripts being expressed in various treatments under salt stress conditions (Fig. 3 and Additional File 3). We created volcano plots for the three comparison groups to determine the number of DEGs that were significantly regulated (Fig. 3A-C). The quantity of DEGs peaked in the comparison between the control and P2 group, suggesting that the alterations in transcription levels in *A. officinalis* under 2.0% NaCl exceeded those under 1.0% NaCl.

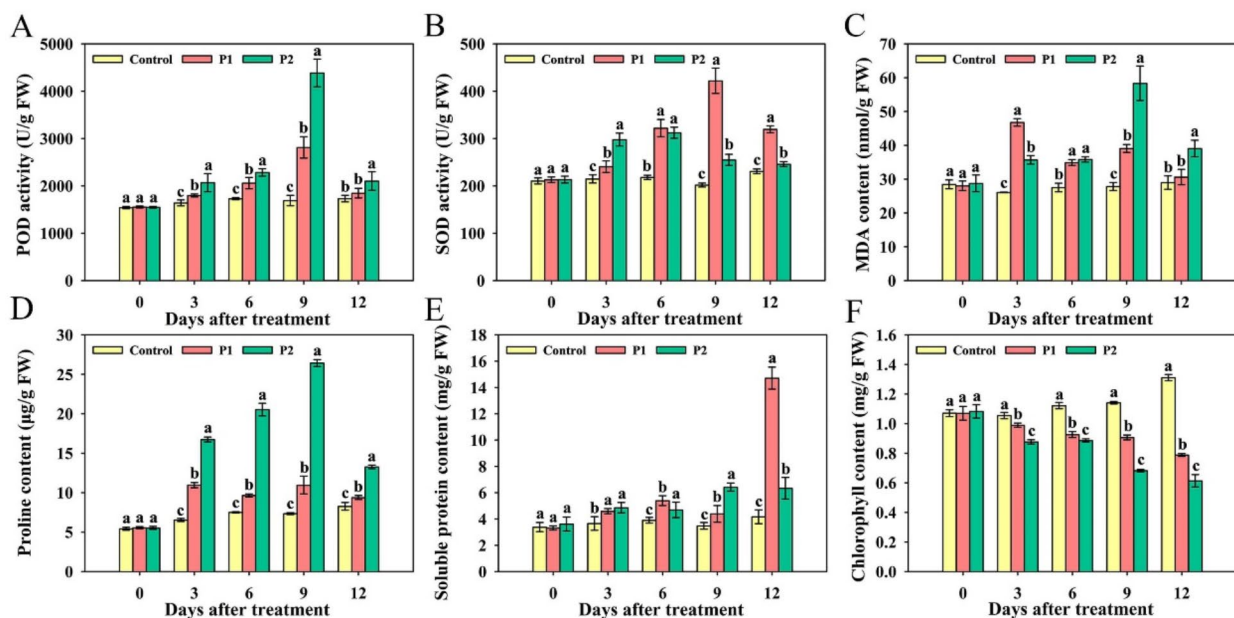


Fig. 2 Physiological indexes and enzyme activity of *A. officinalis*. The enzyme activities of POD (A), SOD (B) and the contents of MDA (C), proline (D), soluble protein (E) and chlorophyll (F) in *A. officinalis* under 0 (Control), 1.0% (P1), and 2.0% (P2) NaCl treatments (0, 3, 6, 9, and 12 days). Three replicates were performed. The data showed averages and standard errors, with distinct letters indicating statistical significance ($p < 0.05$)

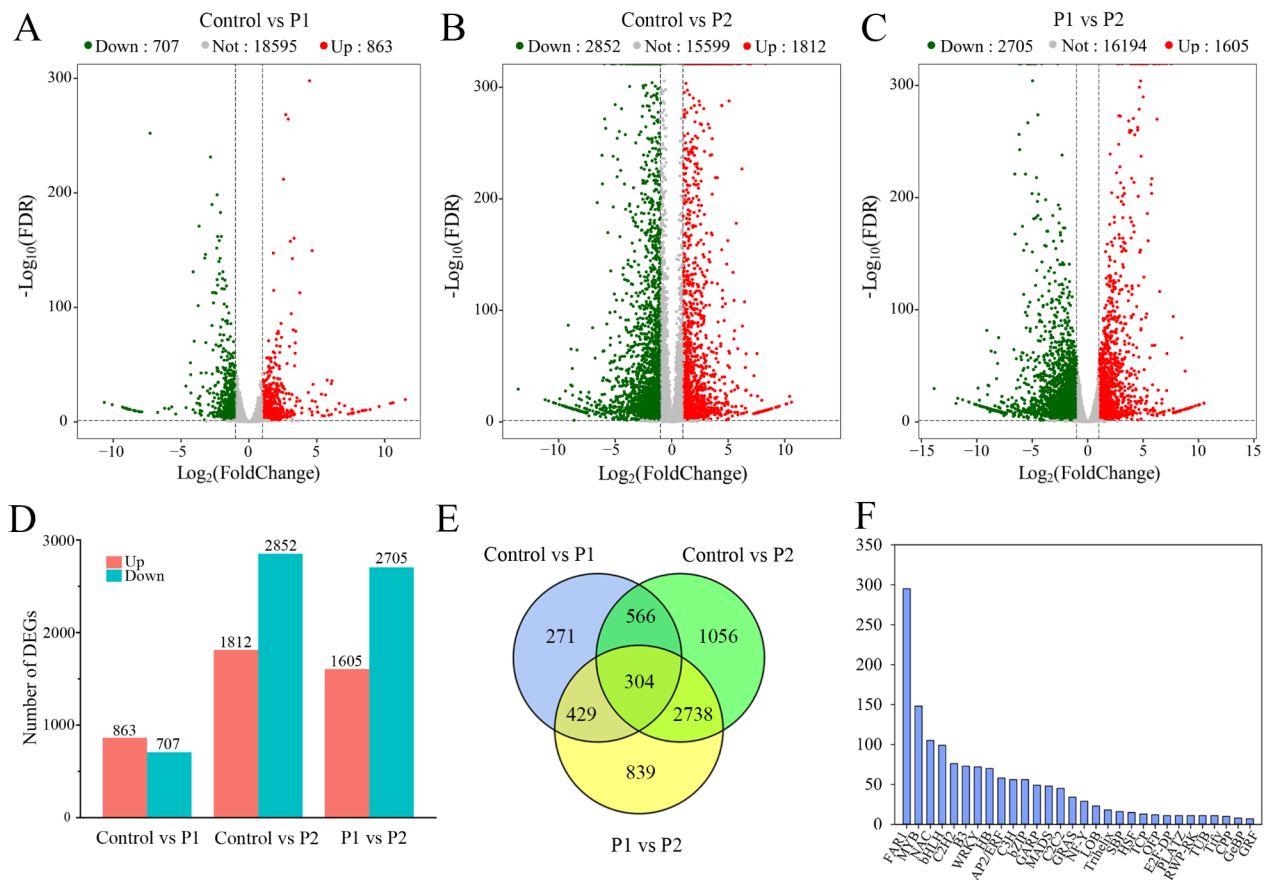


Fig. 3 DEGs of *A. officinalis* under salt treatments. (A–C) Analysis of DEGs using volcano plots. (D) Number of DEGs that were either upregulated or down-regulated. (E) Venn diagrams of DEGs. (F) Transcription factor families

In comparison to the control group, the P1 and P2 treatment groups showed 1,570 DEGs (863 upregulated and 707 downregulated) and 4,664 DEGs (1,812 upregulated and 2,852 downregulated), respectively. Additionally, the P2 treatment group had 4,310 DEGs (1,605 upregulated and 2,705 downregulated) compared to the P1 treatment group (Fig. 3D). Venn diagram displayed the distribution of DEGs among various groups. 6,203 DEGs were enriched in all treatment groups, with a higher number of DEGs found in the comparison between the control and P2 groups compared to that between the control and P1 groups (Fig. 3E). A sequence comparative analysis was conducted to examine the TFs expressed in *A. officinalis* under various treatments, and 51 TF families were identified (Fig. 3F). The top eight TFs in different comparative groups included FAR1, MYB, NAC, bHLH, C2H2, B3, WRKY and HB. Biological processes related to salt stress in *A. officinalis* may explain the variations in TFs expression level.

Gene Ontology (GO) annotation analysis of DEGs among different treatments

GO annotation analysis was conducted to gain an understanding of the biological roles of DEGs under salt stress conditions (Fig. 4). The findings demonstrated that DEGs showed significant enrichment in biological processes and molecular functions. Analysis of GO revealed varying numbers of enriched terms among the three treatments as shown in Additional File 4. Comparing the control to P1, control to P2 and P1 to P2 in terms of GO categories, 2,861, 10,177, and 9,016 terms were categorized as cellular components, 1,267, 4,163, and 3,782 were categorized as molecular functions, and 2,640, 8,288, and 7,619 were categorized as biological processes (Additional File 4). As shown in Fig. 4, the primary enrichment of molecular functions was in categories related to ‘catalytic activity’, ‘binding’, and ‘transcription regulator activity’. The biological process was primarily characterized by enrichment in categories such as ‘cellular process’, ‘metabolic process’, and ‘response to stimulus’. While the primary enrichment of the cellular component was related to ‘cell’, ‘cell part’ and ‘organelle’.

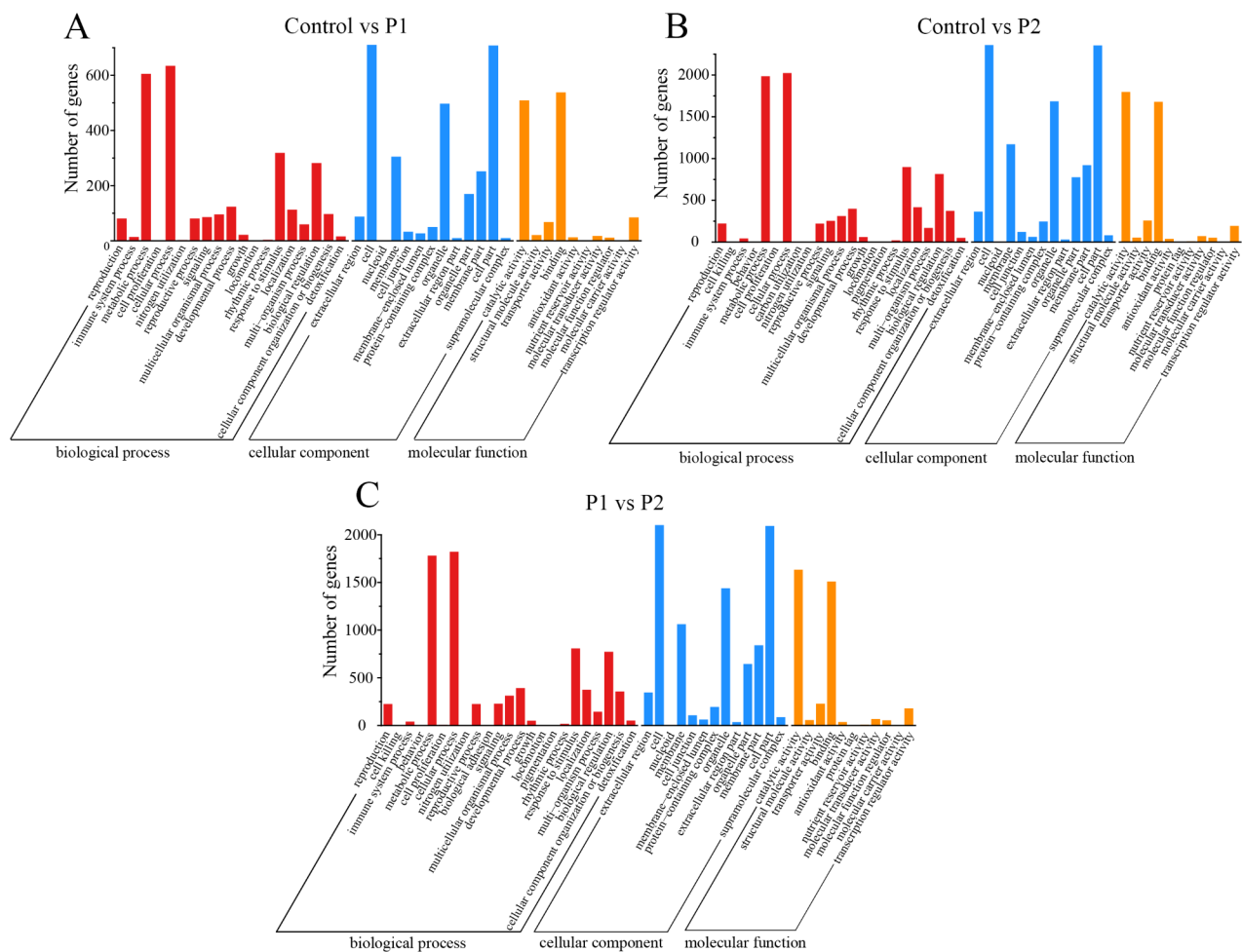


Fig. 4 Histogram of annotation analysis of Gene Ontology exposed to salt stress for different concentration in (A) Control vs. P1, (B) Control vs. P2 and (C) P1 vs. P2

Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis of DEGs among different treatments

In order to fully grasp the biological pathways affected by various NaCl treatments, KEGG pathway enrichment analyses were conducted, revealing 20 key pathways enriched in comparisons between control and P1, control and P2, and P1 and P2, as shown in Fig. 5 and Additional File 5. DEGs in the control vs. P1 group were notably enriched in pathways such as ‘vitamin B6 metabolism’, ‘stilbenoid, diarylheptanoid and gingerol biosynthesis’, and ‘starch and sucrose metabolism’ (Fig. 5A). DEGs in the control vs. P2 group showed significant enrichment in pathways such as ‘valine, leucine and isoleucine degradation’, ‘starch and sucrose metabolism’ and ‘photosynthesis-antenna proteins’ (Fig. 5B). DEGs in the P1 vs. P2 group showed significant enrichment in pathways such as ‘zeatin biosynthesis’, ‘valine, leucine and isoleucine degradation’ and ‘starch and sucrose metabolism’ (Fig. 5C). The findings suggested that the DEGs in these pathways could have significant impacts on the reaction of *A. officinalis*

to salt stress. However, a significant amount of DEGs were found to be enriched in phenylpropanoid biosynthesis and plant hormone signal transduction pathways under various salt treatments, suggesting that these pathways play crucial roles in stress response and warrant further investigation.

DEGs related to the pathways of plant hormone signal transduction

Plant hormones play a crucial role in controlling the growth and development of plants, as well as their response to salt stress [21]. Figure 6 and Additional File 6 display DEGs involved in the signaling transduction pathways of plant hormones. In the case of auxin signaling, 2 genes encoding *auxin resistant 1* (*AUX1*) and *auxin/indole-3-acetic acid* (*Aux/IAA*) were upregulated, while 2 genes encoding *Aux/IAA* were downregulated in 1.0% NaCl treatment. In the case of 2.0% NaCl treatment, 1 gene encoding *gretchen hagen 3* (*GH3*) was upregulated, and 15 genes encoding *auxin response factor* (*ARF*),

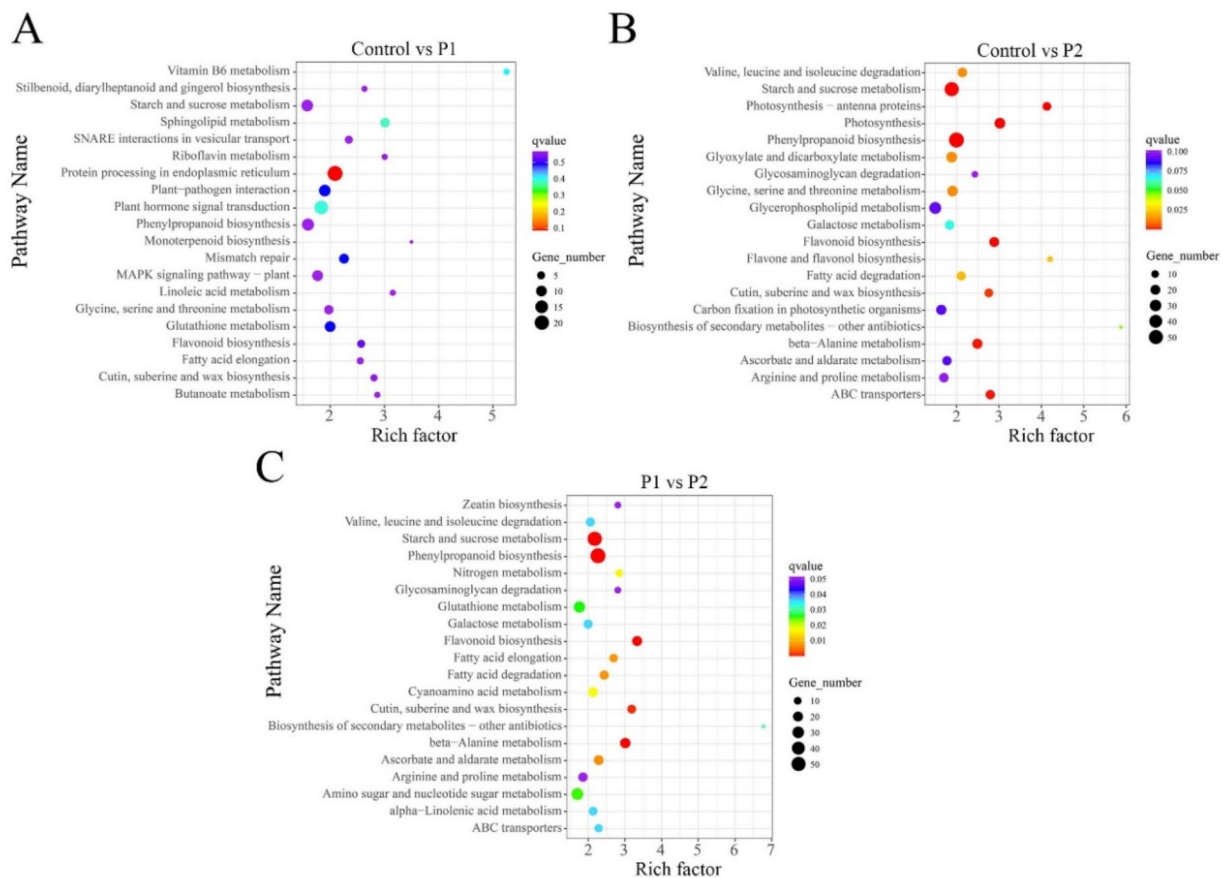


Fig. 5 Analysis of the top 20 KEGG pathways in *A. officinalis* across various pairwise groups. **(A)** Control vs. P1. **(B)** Control vs. P2. **(C)** P1 vs. P2

AUX1, *Aux/IAA*, and *small auxin-up RNA (SAUR)* were downregulated. For cytokinin (CTK) signaling, 2 genes encoding *histidine phosphotransfer protein (AHP)* were found to be downregulated in response to 1.0% NaCl treatment, whereas 1 gene encoding *AHP* was upregulated and 5 genes encoding *type-A response regulators (A-ARR)*, *type-B response regulators (B-ARR)*, and *AHP* were downregulated in 2.0% NaCl treatment. During salt stress, the expression of *pathogenesis-related protein 1 (PR1)* and *TGACG-binding factor (TGA)* homologs increased in salicylic acid (SA) signaling. In the scenarios involving brassinosteroid (BR), ethylene (ETH), jasmonic acid (JA), and abscisic acid (ABA) signaling pathways, numerous DEGs were also found.

DEGs involved lignin biosynthetic pathways

Analyzing the phenylpropanoid biosynthesis pathways enriched in three groups is essential for studying the important genes related to salt stress. 30 DEGs related to lignin biosynthesis were identified after screening the DEGs involved in phenylpropanoid biosynthesis (Fig. 7 and Additional File 6). Lignin, the primary constituent of plant vascular tissue, is a polymer originating from

phenylpropanoids [22]. Within the pathway responsible for lignin creation, DEGs were identified including *phenylalanine ammonia-lyase (PAL)*, *caffeic acid O-methyltransferase (COMT)*, *caffeoyl-CoA O-methyltransferase (CCoAOMT)*, *cinnamoyl-CoA reductase (CCR)*, *peroxidase (POD)*, *cinnamyl alcohol dehydrogenase (CAD)*, and *4-coumarate-CoA ligase (4CL)*. In lignin biosynthesis, 5 genes encoding *CCoAOMT*, *COMT* and *POD* were upregulated, while 1 gene encoding *POD* was downregulated in 1.0% NaCl treatment. In the case of 2.0% NaCl treatment, 12 genes encoding *CAD*, *CCoAOMT*, *CCR*, and *POD* were upregulated, and 14 genes encoding *4CL*, *CCoAOMT*, *PAL*, and *POD* were downregulated.

Validation of the RNA-Seq data by quantitative real-time PCR (qRT-PCR)

To confirm the DEGs identified through RNA-seq analysis, qRT-PCR was conducted on a selection of 16 genes chosen from the plant hormone signaling transduction pathways (Fig. 8A) and 8 genes chosen from lignin biosynthetic pathways (Fig. 8B). The qRT-PCR findings were aligned with the transcriptome analysis, confirming the accuracy of RNA-seq data.

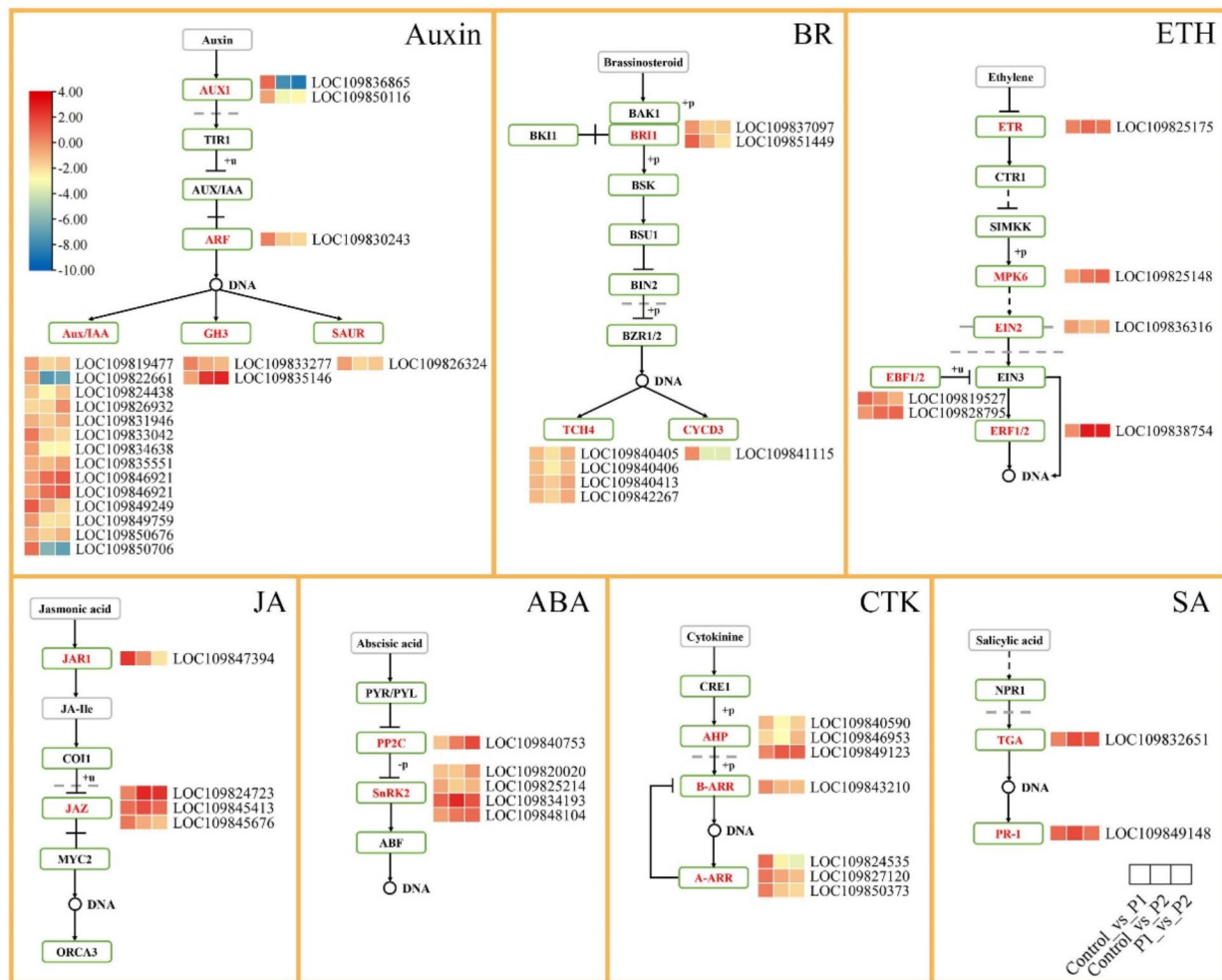


Fig. 6 DEGs in the plant hormone signaling transduction pathways when exposed to salt stress

Discussion

Plants that can withstand high levels of salt are suitable for surviving in saline-alkali soil. Planting salt-tolerant plants on saline-alkali soil could alleviate farmland pressure. Transcriptome analysis is crucial for identifying the essential genes of *A. officinalis* in responses to salt stress [23]. In this research, morphological, physiological changes, as well as transcriptome analysis were combined to explain the response mechanisms of *A. officinalis* under different concentrations of NaCl.

Morphological, physiological and transcriptome analyses of *A. officinalis* under salt stress

Morphological changes in different treatments under salt stress can be observed (Fig. 1A). The decrease in both plant height and FW of *A. officinalis* was significant, particularly when exposed to 2.0% NaCl concentrations, indicating that higher levels of NaCl have a more pronounced inhibitory impact on plant development (Fig. 1B and C). The physiologic alterations may also

indicate the impact of salt stress on plants (Fig. 2). Exposure to high levels of salt can trigger an overproduction of reactive oxygen species (ROS), resulting in oxidative stress and causing cell damage [24]. Yet, plants have the ability to reduce harm through their antioxidant system [25]. SOD and POD, two essential antioxidant enzymes, which are critical for maintaining ROS balance during salt stress by effectively counteracting ROS-induced damage and enhancing plant resistance to oxidative stress [26]. To mitigate the effects of salt stress on *A. officinalis*, the functions of SOD and POD were stimulated in this research (Fig. 2A and B). Comparable findings were also noted in various other plant species including tomato, wheat, radish and rice [27–30]. MDA, a lipid peroxidation product, can be utilized to assess the extent of cell membrane injury [25]. The study indicated that the MDA levels in *A. officinalis* were elevated under salt stress (Fig. 2C). Under stress conditions, the accumulation of salt resistant metabolites such as proline and soluble proteins are important in regulating cell membrane

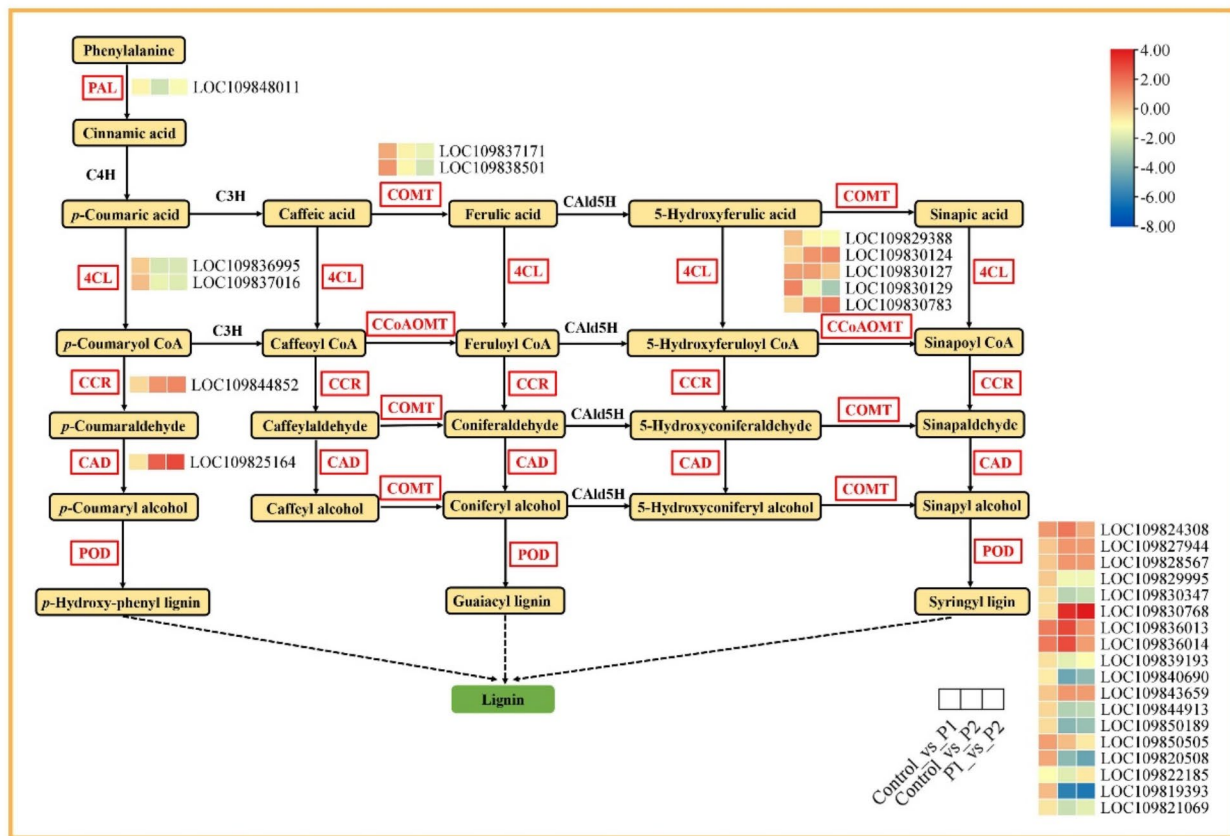


Fig. 7 DEGs in the lignin biosynthetic pathways when exposed to salt stress

permeability [31, 32]. *A. officinalis* showed a significant increase in proline and soluble protein content under salt stress (Fig. 2D and E), indicating that proline and soluble protein may participate in reducing the degree of damage to plants caused by salt stress. Under salt stress, the structures of chloroplasts are disrupted leading to the decrease of chlorophyll content [33, 34]. Here, we found the chlorophyll content in *A. officinalis* cladodes significantly decreased in NaCl treatments (Fig. 2F). The decrease in plant growth may be caused by the decrease in chlorophyll content under salt stress conditions.

To initially reveal the key salt-responsive molecular mechanisms of *A. officinalis* seedlings, the transcriptome analysis was performed. DEGs in 2.0% NaCl treatment of 9 days were maximum compared with control and 1.0% NaCl (Fig. 3). Among these, transcription factors such as WRKY75, WRKY40, NAC79, and ABR1 displayed increased expression levels as the salt concentration increases, whereas the expression of transcription factors like ERF109, WRKY71, TCP12, and WRKY41 were suppressed in response to elevated salt concentration. These genes potentially play significant roles in salt stress responses and can be further investigated through molecular biological approaches. Analysis of KEGG

enrichment revealed that the DEGs were primarily associated with the plant hormone signaling transduction and phenylpropanoid biosynthesis pathway (Fig. 5), suggesting their potential role in the mechanism of *A. officinalis* in combating salt stress.

Plant hormone signal transduction pathways in *A. officinalis* under salt stress

Plant hormones are known to have important functions in signaling transduction pathways mediated by abiotic stress [35–37]. Auxin is crucial for regulating plant growth and responding to stress [38]. Aux/IAA functions as a transcriptional inhibitor, controlling the activity of auxin response genes such as *IAA*, *GH3*, and *SAUR* through interaction with the ARF protein [39, 40]. The majority of DEGs related to auxin signaling transduction pathways were notably decreased in expression levels when exposed to elevated salt treatments, as shown in Fig. 6, which is consistent with previous studies [41]. ABA plays a crucial role in orchestrating intricate signal transduction pathways and serves as a vital plant defense hormone that enhances plant resilience in the face of abiotic stress [20, 42]. The pyrabactin resistance/pyr1-like (PYR/PYL) receptor could detect ABA and blocks the action of

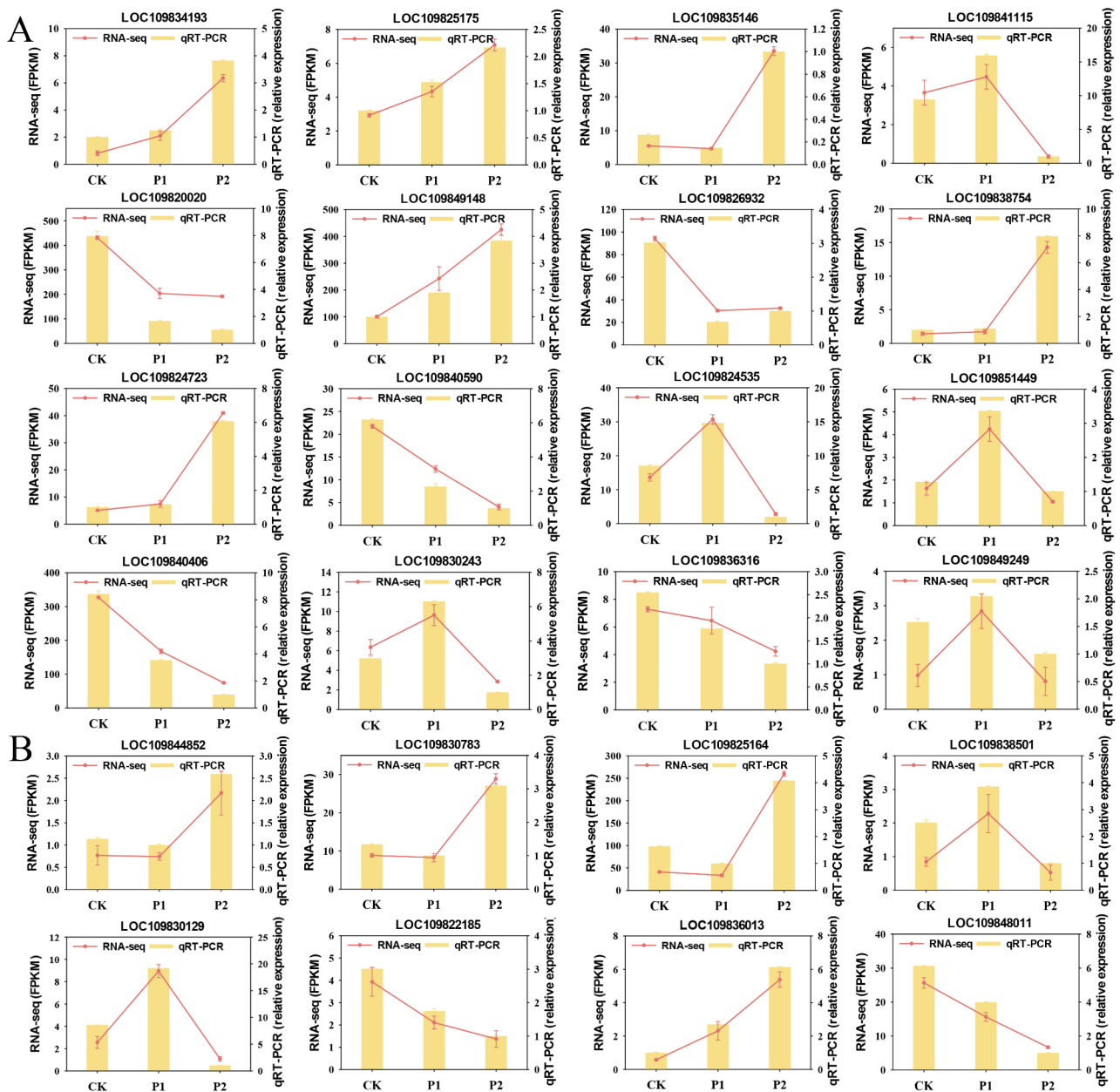


Fig. 8 Validation of DEGs using qRT-PCR. Comparisons of RNA-seq data (red lines) with qRT-PCR (yellow bars) of selected genes. **(A)** qRT-PCR validation of the genes involved in plant hormone signaling transduction pathway. **(B)** qRT-PCR validation of genes involved in lignin biosynthetic pathways

protein phosphatase 2 C (PP2C). This leads to the stimulation of downstream kinase SNF1-related protein kinase 2 (SnRK2), ultimately activating ABA-responsive genes [43, 44]. The salt treatment significantly regulated *PYR/PYL* and *SnRK2* in this study (Fig. 6), suggesting the effect of salt on the ABA signaling pathway. Numerous studies have shown that CTK has both positive and negative effects on plant stress resistance [45–47]. Our research found that the genes *AHP*, *B-ARR*, and *A-ARR* showed decreased expression levels under salt stress (Fig. 6), suggesting that CTK could play a role in the reaction of *A.*

offinialis seedlings to salt stress. An increase in ABA synthesis has been linked to a decrease in CTK levels during salt stress, serving as a strong defense mechanism for plants against salt stress [37, 48]. JA manages the main aspects of plant adaptation to environmental stress [49]. The study indicated a notable increase in the expression of *jasmonate acid resistant1 (JAR1)* and *jasmonate ZIM domain (JAZ)* genes in response to NaCl stress, as shown in Fig. 6, which is similar to previous findings [50]. Additionally, SA has the ability to control how plants react to stress [6]. The application of SA may enhance the ability

of plants to withstand high salt levels [51]. The findings of this research also reveal that the *TGA* and *PR1* genes showed a notable increase in expression levels under salt stress (Fig. 6). Additional hormones like BR may also have a significant impact on plant responses to salt stress [52]. There may be crosstalk between different plant hormones under salt stress, which requires further research.

Lignin biosynthetic pathways in *A. officinalis* under salt stress

The lignin biosynthesis pathways are a key element of the phenylpropanoid pathway. Lignin plays a crucial role in the structure of plant cell walls, increasing the ability of plants to withstand both biotic and abiotic stressors [53]. Lignin is produced from individual building blocks obtained from p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [3]. Previous studies have shown that plants could increase the degree of lignification to improve salt tolerance [54]. Some genes have been identified to be involved in the process of lignin production and storage, such as *trans-cinnamate monooxygenase* (*CYP73A*), *4CL*, *CCoAOMT*, *CAD*, *CCR*, *COMT*, *conifer-aldehyde-5-hydroxylase* (*CAld5H*), and *hydroxycinnamoyl-CoA shikimate/quinic acid hydroxycinnamoyl transferase* (*HCT*) [55–57]. Studies have indicated that salt stress can result in the accumulation of lignin, which is linked to the activity of enzymes such as *CCoAOMT*, *C4H*, *CCR*, *POD*, and *C3H* [58–60]. Under salt stress, *PheCAD2*, *PheCAD3*, and *PheCAD5* of moso bamboo (*Phyllostachys pubescens*) were highly upregulated, confirming their participation in salt stress responses [61]. *CrCOMT* was demonstrated to positively regulate stress responses in *Arabidopsis* under salt stress [62]. *CCoAOMT* was confirmed to play a role in both lignin production and response to salt stress in jute (*Corchorus* L.) [63]. During this research, the phenylpropanoid biosynthesis pathways showed a notable increase in activity when exposed to salt stress, leading to the identification of 30 DEGs related to lignin biosynthesis (Fig. 7). More DEGs were found in 2.0% NaCl than 1.0% NaCl, implying that the reactions of *A. officinalis* were more intricate under high salt concentrations. However, the transcription levels of structural genes involved in lignin biosynthesis pathways were complex under different concentrations of NaCl, which means that further investigation is required for explaining the lignin biosynthetic pathways under salt stress of *A. officinalis*.

When considering all the data, it appears that NaCl treatments may have an impact on the genes associated with the plant hormone signal transduction and lignin biosynthetic pathways (Fig. 9). This research has revealed that under 2.0% NaCl, the expression level of genes is more affected in *A. officinalis*. The existing findings may offer a theoretical foundation for comprehending the

molecular processes involved in the response of *A. officinalis* to varying levels of salt stress. However, the specific regulatory patterns and pathways under different salt concentrations still need further exploration.

Materials and methods

Plant materials and salt stress application

A. officinalis was grown in a greenhouse at Zhejiang Institute of Subtropical Crops in Zhejiang Province, China, with a light cycle of 16 h light and 8 h dark at a temperature of 25°C. *A. officinalis* was grown in containers with a blend of garden soil and peat in equal parts. Seedlings that were four months old and cultivated in a soil blend were utilized for this research.

NaCl solution was incrementally introduced to the soil until the salt level reached 0 (control), 1.0% (P1) and 2.0% (P2). After 0, 3, 6, 9, and 12 days of salt treatments, cladodes from *A. officinalis* seedlings were collected for measurement of physiological indexes and RNA-seq. After 12 days of treatment, the plant height and FW were both recorded. The experiments were performed with three independent biological replicates.

Detection and analysis of key physiological indexes

The height of the plant was determined by measuring from the base to the tip of the stem. The FW of *A. officinalis* above ground was weighed using balance. Physiological indexes including POD, SOD, MDA, proline content, soluble protein content and chlorophyll content were measured as reported [21–23]. The trials were conducted thrice with three sets of biological samples.

RNA extraction, transcriptome sequencing, and data processing

RNA-seq was performed using samples obtained from treatments with NaCl concentrations of 0, 1.0, and 2.0% after 9 days. The analysis was done to study the main changes of *A. officinalis* in response to different NaCl concentrations. Each treatment was conducted with three biological replicates.

RNA extraction from *A. officinalis* cladodes was performed using the RNAPrep Pure Plant Kit (TIANGEN, Beijing, China) for subsequent transcriptome analysis. Genepioneer Biotechnologies Co., Ltd (Nanjing, China) performed the creation of the cDNA library and the sequencing using NovaSeq 6000 platform (150 bp paired-end reads). The sequencing data can be accessed from NCBI (BioProject ID: PRJNA1074503).

Transcriptome data and gene annotation analysis

The raw data acquired by sequencing was filtered to obtain clean reads. At the same time, calculations were performed for Q20, Q30, and GC-content. Each sample's clean reads were individually aligned to the *A. officinalis* reference

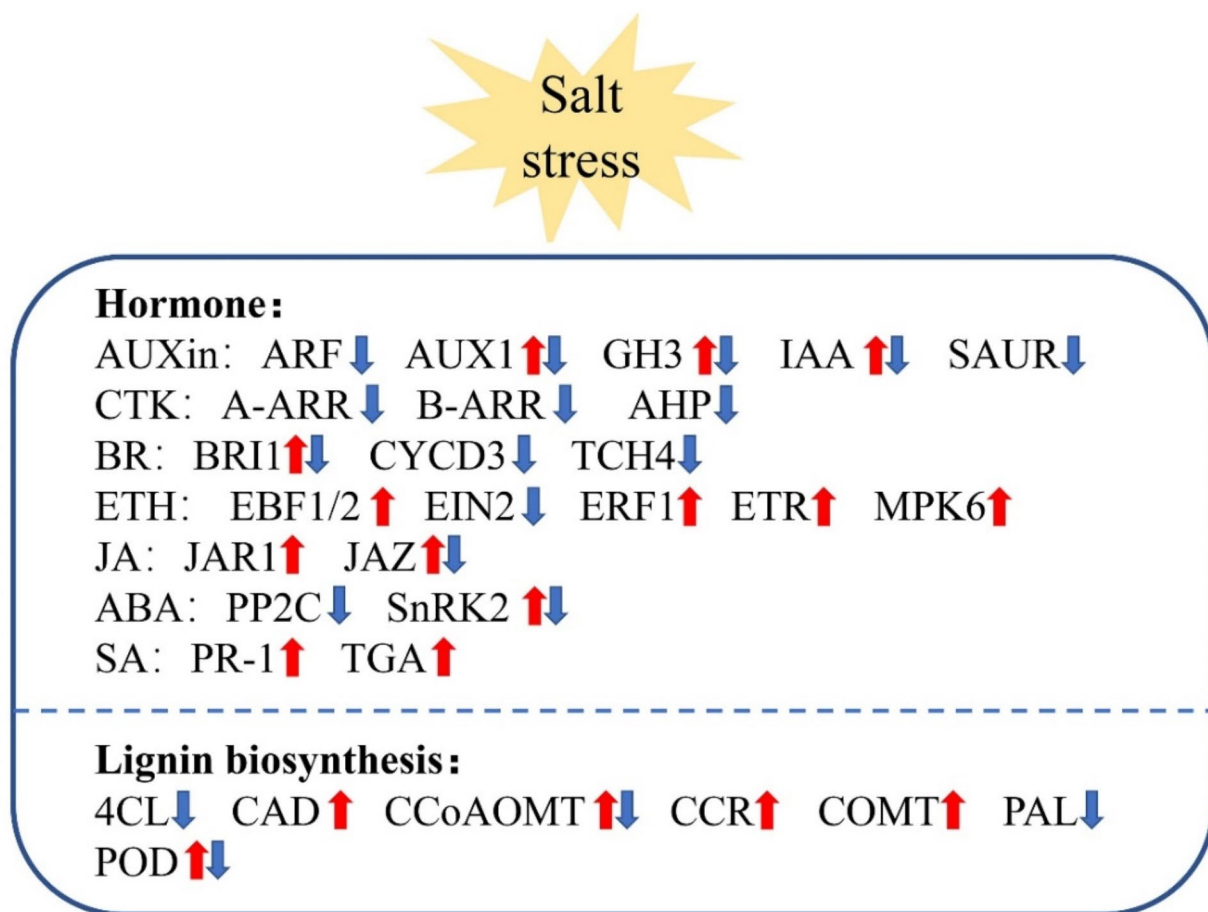


Fig. 9 Proposed model for how *A. officinalis* seedlings reacts to salt stress. The blue arrow indicates downregulated genes, while the red arrow indicates upregulated genes

genome (<https://www.ncbi.nlm.nih.gov/datasets/taxonomy/4686/>) using the HISAT2 software [24]. The level of gene expression was calculated using fragments per kilobase of transcript per million mapped reads (FPKM) [25]. Differentially expressed genes (DEGs) were detected at three different levels of NaCl (0%, 1.0%, and 2.0%) through the utilization of DESeq2 program [26]. DEGs function annotation utilized the KEGG and GO databases. The KOBAS online analysis database (<http://kobas.cbi.pku.edu.cn/home.do>) was used to conduct KEGG pathway analysis, while the R package clusterProfiler was utilized for GO enrichment analysis of DEGs [27–29]. PlantTFDB databases (<http://planttfdb.gao-lab.org/>) were used to predict transcription factors (TFs) [30].

qRT-PCR

24 genes were chosen at random for qRT-PCR analysis to confirm the accuracy of the RNA-seq. The FastKing RT Kit (TaKaRa, Dalian, China) was utilized for cDNA synthesis, followed by qRT-PCR using SYBR qPCR Master

Mix (Vazyme, Nanjing, China). Online software (<https://sg.idtdna.com/Scitools/Applications/RealTimePCR/default.aspx>) was used to design primers, and the information of primers was displayed in Additional File 7. The Light Cycler480 Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA) was used for qRT-PCR analysis and the program was as reported [31]. The $2^{-\Delta\Delta C_t}$ method was used for calculating the relative expression [32]. Three independent biological and technological replicates were used for each sample.

Statistical analysis

SPSS (version 23) was utilized for statistical analysis. Data mapping is displayed as average \pm SD, with significance ($p < 0.05$) taken into account.

Conclusions

In this study, the effects of various concentrations of NaCl on four-month-old *A. officinalis* seedlings were researched. The changes in gene expression levels were comprehensively

analyzed in the cladodes of *A. officinalis* seedlings. The findings suggest that *A. officinalis* exhibits diverse response mechanisms to varying levels of salt stress. Additional examination demonstrated that the genes related to the hormone signal transduction and lignin biosynthetic pathways in *A. officinalis* cladodes were be regulated when exposed to salt stress. The results offer comprehensive data that serves as a foundation for advancing our understanding of the molecular pathways activated in *A. officinalis* in response to salt stress, which is essential for developing salt-resistant genetic resources.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-024-05540-4>.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7

Author contributions

S.W: Design experiments, Investigation, Analyze the data, Write manuscripts. J.Y: Investigation, Analyze the data. Y.Y: Provide ideas, Investigation. Y.C: Investigation. R.Q: Provide ideas, Design experiments, Writing-review & editing. All authors reviewed the manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available in the NCBI SRA repository, with accession number PRJNA1074503 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1074503>). All data generated or analyzed during this study are included within the article and its additional files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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