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OsNAC74 modulates rice growth and salt tolerance via hormone signaling pathways

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NAM, ATAF1/2, and CUC2 (NAC) transcription factors are a unique family of plant proteins crucial for regulating plant biotic and abiotic stress responses. This study focuses on *OsNAC74*, upstream regulatory factors of amino acid permease gene *OsAAP6* in rice that influences root regulates root growth, development, and salt tolerance by participating in hormone signaling pathways. *OsNAC74* mutations result in a slower germination rate and reduced root length in rice seeds. Under varied salt concentrations, *OsNAC74* homozygous mutants exhibited significantly lower survival rates than wild-type plants. Additionally, the expression of salt stress response genes was significantly downregulated in these mutants. The transcriptome sequencing revealed that differentially expressed genes are primarily involved in plant hormone signaling pathways, including abscisic acid, jasmonic acid, and auxin. In the *OsNAC74* homozygous mutant roots, auxin concentrations significantly increased, while abscisic acid, gibberellin, and jasmonic acid concentrations significantly decreased. The expression levels of hormone-related genes aligned with these changes in root hormone concentrations. These findings reveal that *OsNAC74* can positively regulate salt tolerance in rice and play an important role in rice seed germination and root development. Highlighting its extensive potential for application in rice breeding, providing important genetic resources for molecular design breeding of high-quality, multi-resistant rice varieties.

Keywords Rice, *OsNAC74*, Growth and development, Salt stress, Hormone signal transduction

Rice (*Oryza sativa* L.) is one of the world's most crucial food crops. It is a pillar of agricultural production in many countries and a staple for millions[1,2]. Over half of the global population depends on rice, with this figure reaching over two-thirds in China[3]. As the population grows, so does the demand for rice, particularly high-quality rice[4]. Soil salinization is a severe global ecological issue that hinders crop growth and development[5]. It is caused by rising groundwater levels, rainwater and river carrying, and human activities such as agriculture and industrial emissions, eventually causing soil salinization. Globally, about 20% of arable land and nearly half of irrigated land are being affected by salt stress, and the spatial distribution of saline soil shows significant imbalances. In the Asian region, China has the largest distribution of saline soil[5]. The process of salinization not only leads to continuous loss of soil nutrients, but also destroys the stability of soil aggregate structure, posing a serious threat to the normal growth and development of crops. This will lead to a decline in agricultural productivity and even trigger food security issues. Therefore, exploring and leveraging the genetic resources related to rice's salt tolerance is vital for continuously improving its yield and quality[6,7].

The NAM, ATAF1/2, and CUC2 (NAC) family represents a distinct and large family of plant transcription factors in plants, playing a pivotal role in plant growth, development, and environmental stress responses[8]. Studies have demonstrated that NAC transcription factors regulate various aspects of plant growth and development, including boundary cell formation, lateral root development, flowering, and secondary cell wall synthesis[9]. In *Arabidopsis thaliana*, the NAC transcription factor *XVP* regulates the balance between xylem

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formation and cambium cell division through interaction with BAK1[10]. In wheat, *TaNAC1* participates in the jasmonic acid signaling pathway, and its overexpression significantly increases lateral root formation[11]. Similarly, the overexpression of the NAC transcription factor *NTL8* in *Arabidopsis thaliana* and *PwNAC2* in Picea (spruce) significantly delayed flowering in transgenic plants[12]. Additionally, NAC family transcription factors play a crucial role in plant response to salt stress. The mechanisms underlying plant salt stress tolerance involve multi-layered synergistic effects: Maintaining ionic homeostasis through selective K^+ uptake, vacuolar compartmentalization of Na^+, and salt excretion via specialized structures[13]; regulating osmotic balance by accumulating organic solutes such as proline and betaine; eliminating reactive oxygen species (ROS) through activation of antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT); orchestrating stress responses via hormone signaling pathways mediated by abscisic acid (ABA) and ethylene; optimizing gene expression through SOS pathways, transcription factors, and epigenetic modifications; and developing morphological adaptations like succulent leaves[14]. These coordinated strategies collectively enable plants to withstand salt damage. In tobacco, NtNAC053 confers tolerance to drought and salt stress by inducing stress-responsive genes and antioxidant systems[15]. Overexpression of *GmNAC035* significantly enhances its salt tolerance in *Arabidopsis*[16]. Overexpression of *TaNAC29* enhances the antioxidant system of wheat and reduces H_2O_2 accumulation and membrane damage, thereby significantly enhancing the salt tolerance of wheat[17]. To date, 151 *NAC* gene family members have been identified in the rice genome[18]. Recent studies have highlighted their significant roles in rice growth, development, and stress responses. For example, *OsNAC103* negatively regulates rice plant height[19], while *ONAC095* negatively regulates drought resistance but positively regulates cold tolerance[20]. Overexpression of *OsNAC58* enhances white leaf blight resistance[21], whereas *OsNAC2* regulates leaf senescence through the ABA pathway[22]. Additionally, Overexpression of *ONAC016* can significantly enhance salt tolerance in rice[23].

Our previous findings demonstrated that *OsNAC74* functions as an upstream regulator of *OsAAP6*, a key gene regulating protein content in rice seeds[24,25]. We created *OsNAC74* mutants using CRISPR-Cas9 gene editing technology and found that *OsNAC74* affects various quality traits of rice by regulating the expression levels of *OsAAP6* gene, protein, and starch metabolism related genes[25]. As a transcription factor of the NAC family, the regulatory mechanism of *OsNAC74* on rice growth, development, and stress response is still unclear. In this study, we discovered that *OsNAC74* significantly affects rice root development and contributes to the salt stress response in rice seedlings via the plant hormone signaling pathway. Therefore, these results are vital for the molecular design and breeding of high-quality, multi-resistant rice varieties in the later stage.

Materials and methods

Seed germination and phenotypic determination

OsNAC74 mutants (*Osnac74*–1, *Osnac74*–2, *Osnac74*–3) were obtained by CRISPR/Cas9 gene editing technology, and with wild was Zhonghua 11 (ZH11)[25]. The rice seeds harvested from plants without trait segregation in the T_2 generation of the *Osnac74* mutant were the seeds of the *OsNAC74* homozygous mutant. One hundred seeds were peeled and surface-disinfected with 75% ethanol for 2 minutes and 0.15% $HgCl_2$ for 15 min with shaking several times during this period, then rinsed 10 times with distilled water. The sterilized seeds were dried and sown on conventional ½ MS solid medium and ½ MS solid medium containing 5 μM GA, to investigate whether the changes in germination rate of *OsNAC74* homozygous mutant seeds are due to the GA signaling pathway. Germination rates were recorded every 12 h, and seedling heights above the ground were measured. The germination was defined as the presence of a 2 mm embryo on the seed coat, and the experiment was repeated thrice.

Salt stress test

The *OsNAC74* homozygous mutant seeds were inoculated onto ½ MS solid medium after disinfection. After seven days of growth, rice seedlings with consistent growth were selected and placed in hydroponic solution for seven days. Fourteen-day-old seedlings were inoculated into hydroponic solutions with 100 mM and 150 mM NaCl and incubated at 28°C. The nutrient solution was replaced every two days to prevent bacterial contamination[7].

Physiological index detection

A 3 cm middle segment of the primary leaf was placed in a centrifuge tube with 50 ml of NBT staining solution (1 $mg{\cdot}ml^{-1}$, pH=7.8, Coolaber, China), vacuumed at −0.1 MPa for 30 minutes, and incubated at room temperature for 60 minutes. The dye solution was discarded, and the leaves were decolorized with 95% ethanol in an 80℃ water bath until all green parts were removed. Take another fresh leaf and immerse it in DAB staining solution (1 $mg{\cdot}ml^{-1}$, pH=3.8, Coolaber, China), vacuumed for 30 min, incubated overnight at room temperature, and decolorized with 95% ethanol in an 80 °C water bath until clear.

Fresh rice leaves were separated from the midrib, cut into pieces, and mixed thoroughly. Chlorophyll extraction buffer ($V_{ethanol}$:$V_{acetone}$:V_{H2O} =4.5:4.5:1) was added, and the mixture was incubated at 4 °C in the dark for 12 h. Absorbance at 645 nm and 663 nm was measured using a spectrophotometer, with the buffer as a blank control, to calculate chlorophyll content. Rice leaves were also cut into pieces, combined with 3% sulfosalicylic acid solution, and extracted in a boiling water bath for 30 minutes. After cooling, glacial acetic acid and acidic ninhydrin solution were added, and the mixture was extracted in a boiling water bath for 30 minutes. Then, toluene was added; the mixture was shaken and allowed to stand. The upper extract was collected, using toluene as the blank control. The absorbance value at 520 nm was measured on a spectrophotometer, and the corresponding proline content was calculated. Fresh rice leaves were cut into pieces and added to a 3% trichloroacetic acid solution, then ground to homogeneity. The homogenate was centrifuged at 4℃ for 10 minutes at 3,000 rpm. The supernatant was mixed with 0.67% thiobarbituric acid solution and incubated in boiling water for 30 minutes.

After cooling, a 1.5 ml aliquot of supernatant and centrifuged at 12000 rpm. Absorbance at 450 nm, 532 nm, and 600 nm was measured, and malondialdehyde content was calculated. All experiments were repeated three times.

Transcriptome sequencing

Fourteen-day-old *OsNAC74* homozygous mutant and wild-type seedlings with consistent growth were cultivated in 100 mM NaCl solution for 7 days. Total RNA was extracted from seedling roots, and its concentration, purity, and integrity were assessed. A cDNA library was constructed and tested for quality. Sequencing was performed in PE150 mode using the Illumina NovaSeq6000 sequencing platform. Filtered data were aligned with the reference genome. Differentially expressed genes (DEGs) were identified using Fold Change ≥ 2 and FDR < 0.01. These genes were annotated and analyzed using COG, GO, and KEGG databases (www.kegg.jp/kegg/kegg1.html). Three biological replicates were taken for each sample.

RNA extraction and real-time quantitative polymerase chain reaction

Total RNA was extracted from leaf and root tissues of *OsNAC74* homozygous mutants and wild-type, and reverse transcribed to cDNA. Real-time quantification was conducted with a 2×M5 HiPer SYBR Premix EsTaq Quantitative Polymerase Chain Reaction Kit and a Quantitation Analyzer. The reaction system comprised a total volume of 20 μL: 10 μL of 2×M5 HiPer SYBR Premix EsTaq, 0.5 μl each of upstream and downstream primers (Table S1–2, 1 $\mu mol \cdot L^{-1}$), 2 μL of cDNA template (50 $ng \cdot \mu L^{-1}$), and 7 μl of ddH_2O. The reaction procedure is as follows: 30 s at 94℃, 15 s at 94℃, 30 s at 60℃, repeated for 40 cycles. Quantification was normalized using β-actin as the internal reference gene, and the relative expression levels of genes were normalized using the calculation method of $2^{-\Delta\Delta CT}$. All data settings were repeated three times.

Results

OsNAC74 participates in regulating rice seed germination

Previous studies have demonstrated that the *OsNAC74* mutation significantly reduces seed length and grain width in rice[25]. We hypothesized that the altered seed size in *OsNAC74* homozygous mutants might influence seed germination. To test whether *OsNAC74* regulates seed germination, we performed germination experiments using *OsNAC74* homozygous mutant seeds. The results revealed that, compared with the wild type, the *OsNAC74* homozygous mutants exhibited no significant difference in shoot length after germination but showed a significant reduction in germination rate (Fig. 1A-B). Since gibberellin plays a critical role in seed dormancy and germination, we applied exogenous gibberellin to examine whether the reduced germination rate in *OsNAC74* homozygous mutants was associated with the gibberellin signaling pathway. After treatment with 5 μM gibberellin, the germination rate of *OsNAC74* homozygous mutant seeds increased significantly but remained lower than that of the wild type (Fig. 1C). These findings suggested that *OsNAC74* may regulate rice seed germination through the gibberellin signaling pathway.

The *OsNAC74* mutation reduced the salt tolerance of rice seedlings

To determine whether the NAC transcription factor *OsNAC74* is involved in stress responses in rice, we conducted salt stress experiments on *OsNAC74* mutant seedlings. Under 100 mM and 150 mM NaCl treatments, the survival rate of *OsNAC74* homozygous mutant seedlings was significantly lower than that of the wild type (Fig. 2A-C). Furthermore, RT-qPCR was used to detect the expression levels of genes related to salt stress response. The results showed that after salt treatment, the expression levels of peroxisome membrane protein gene *OsPEX11*, lectin gene *OsJRL*, potassium channel protein *OsAKT1*, cation transporter gene *OsHKT1*, as well as ABA response genes *OsSalt* and *OsWSI18* were significantly reduced in the *OsNAC74* homozygous mutants (Fig. 2D-I).

Plant cells typically produce large amounts of ROS under salt stress[26]. To assess ROS levels, used nitroblue tetrazolium (NBT) and 3,3′-diaminobenzidine (DAB) as chromogenic substrates to detect hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) levels in the leaves of *OsNAC74* homozygous mutants and wild-type rice following salt stress treatment. The staining results revealed that the NBT- and DAB-stained areas in the leaves of *OsNAC74* homozygous mutants were significantly larger than those in the wild type. Moreover, higher salt concentrations resulted in larger staining areas (Fig. 3A-B). These findings indicated that H_2O_2 and O_2^- accumulation in the leaves of *OsNAC74* homozygous mutants is significantly higher than in the wild type, with greater accumulation observed at higher salt concentrations. Subsequently, we also measured physiological indicators associated with salt stress. The results showed that the *OsNAC74* homozygous mutants had significantly reduced chlorophyll a, chlorophyll b, and total chlorophyll content in the leaves (Fig. 3C-E). In contrast, proline and malondialdehyde contents were significantly higher (Fig. 3F-G). These findings suggested that *OsNAC74* homozygous mutants experience more severe cellular damage under varying salt concentrations, leading to increased sensitivity of rice seedlings to salt stress.

OsNAC74 participates in regulating salt tolerance in rice through the plant hormone pathway

To investigate the molecular mechanism by which *OsNAC74* regulates salt tolerance in rice, we performed transcriptome sequencing on the roots of *OsNAC74* mutants rice seedlings treated with 100 mM NaCl for seven days. After sequencing, filter to remove those containing adapters and low-quality sequences. Following filtering, the clean data for each sample accounted for more than 96.24% of the total data, with a Q30 base proportion exceeding 90.15%. The high-quality clean sequences were aligned to the reference rice genome, achieving an alignment efficiency ranging from 88.29% to 93.00%. These results indicate that our transcriptome sequencing data is accurate and reliable, and the selected reference genome assembly is suitable for subsequent analyses. Differential gene expression (DGE) was determined by False Discovery Rate (FDR) and Fold Change (FC) as criteria, with FC≥2 and FDR < 0.01 used for screening. A total of 1014 differentially expressed genes (DEGs)

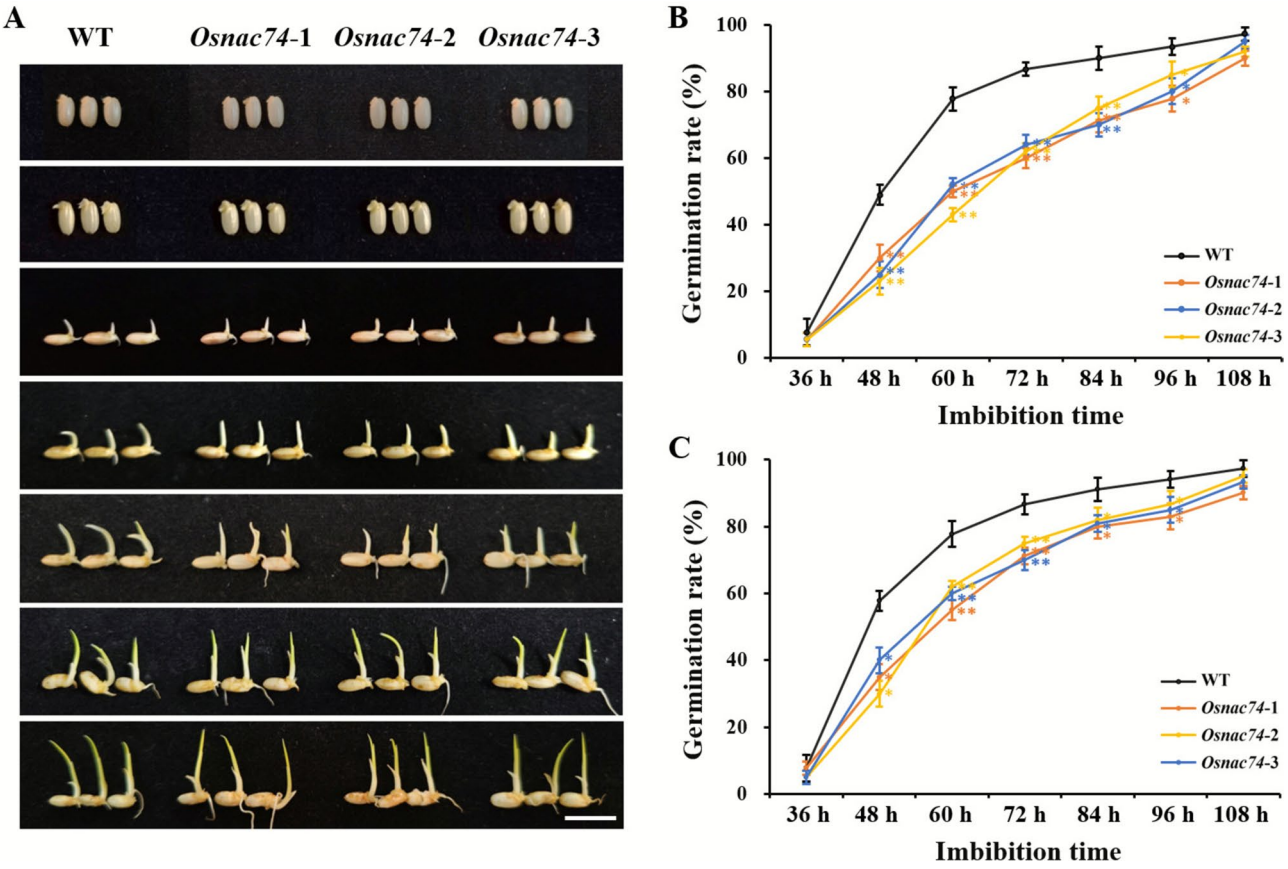

Fig. 1. Seed germination experiment of *OsNAC74* homozygous mutants. (**A**) Seed germination phenotype of *OsNAC74* homozygous mutants. (**B**) Statistics on the germination rate of seeds under normal conditions. (**C**) Germination rate of seeds treated with 5 μM exogenous GA. Record germination rate every 12 h. Significant differences determined using a two-tailed *t*-test: $^{**}P \leq 0.01$, $^{*}P \leq 0.05$. WT: wild type. Bars: 1 cm. Error bars represent the standard error of the mean.

were identified in the *OsNAC74* homozygous mutants, including 669 genes that were significantly up-regulated and 345 genes that were significantly down-regulated (Fig. 4A).

Functional annotation using the COG database was performed on the selected DEGs to explore their classification and functional inference. Revealed their primary involvement in carbohydrate transport and metabolism, cell wall/membrane biosynthesis, secondary metabolite biosynthesis, transport and metabolism, and signal transduction mechanisms (Fig. 4B). GO (Gene Ontology) functional annotation and enrichment analysis showed that these genes were predominantly located in nucleosomes, nuclei, and membranes. Their molecular functions included sequence-specific DNA binding, amino acid transmembrane transport activity, DNA-binding transcription factor activity, and protein kinase activity. The genes were primarily associated with biological processes such as injury response, jasmonic acid-mediated signaling pathways, regulation of abscisic acid, water-deficiency response regulation, and defense response (Fig. 4C, Fig. S1–Fig. S2). To further investigate the metabolic pathways associated with the DEGs in the *OsNAC74* mutants, KEGG (https://www.kegg.jp/kegg/kegg1.html) pathway annotation was conducted[27,28]. The results showed that these genes were predominantly enriched in pathways such as plant hormone signaling and the MAPK signaling pathway (Fig. 4D).

Both KEGG metabolic pathway enrichment analysis and GO functional enrichment analysis revealed that the DEGs are closely associated with plant hormone regulation. Thereupon, we hypothesized that *OsNAC74* might regulate salt tolerance in rice seedlings by modulating plant hormone signaling pathways. To test this, we treated *OsNAC74* homozygous mutants and wild-type seedlings with 100 mM NaCl for seven days and subsequently measured hormone concentrations in rice seedling roots. The results showed that, after seven days of treatment with 100 mM NaCl, auxin concentrations in the roots of *OsNAC74* homozygous mutant seedlings were significantly increased, whereas abscisic acid, jasmonic acid, and gibberellin concentrations were significantly decreased (Fig. 5). Additionally, we examined the expression levels of 16 hormone-related genes in the roots, which aligned with the observed hormone concentration patterns (Fig. S3). These findings suggested that *OsNAC74* regulates salt tolerance in rice seedlings by participating in plant hormone signaling pathways.

OsNAC74 participates in regulating root development of rice

Transcriptome sequencing analysis further indicated that *OsNAC74* is involved in the plant hormone signaling pathway (Fig. 4D), and the auxin concentration in the mutant roots increased significantly following salt

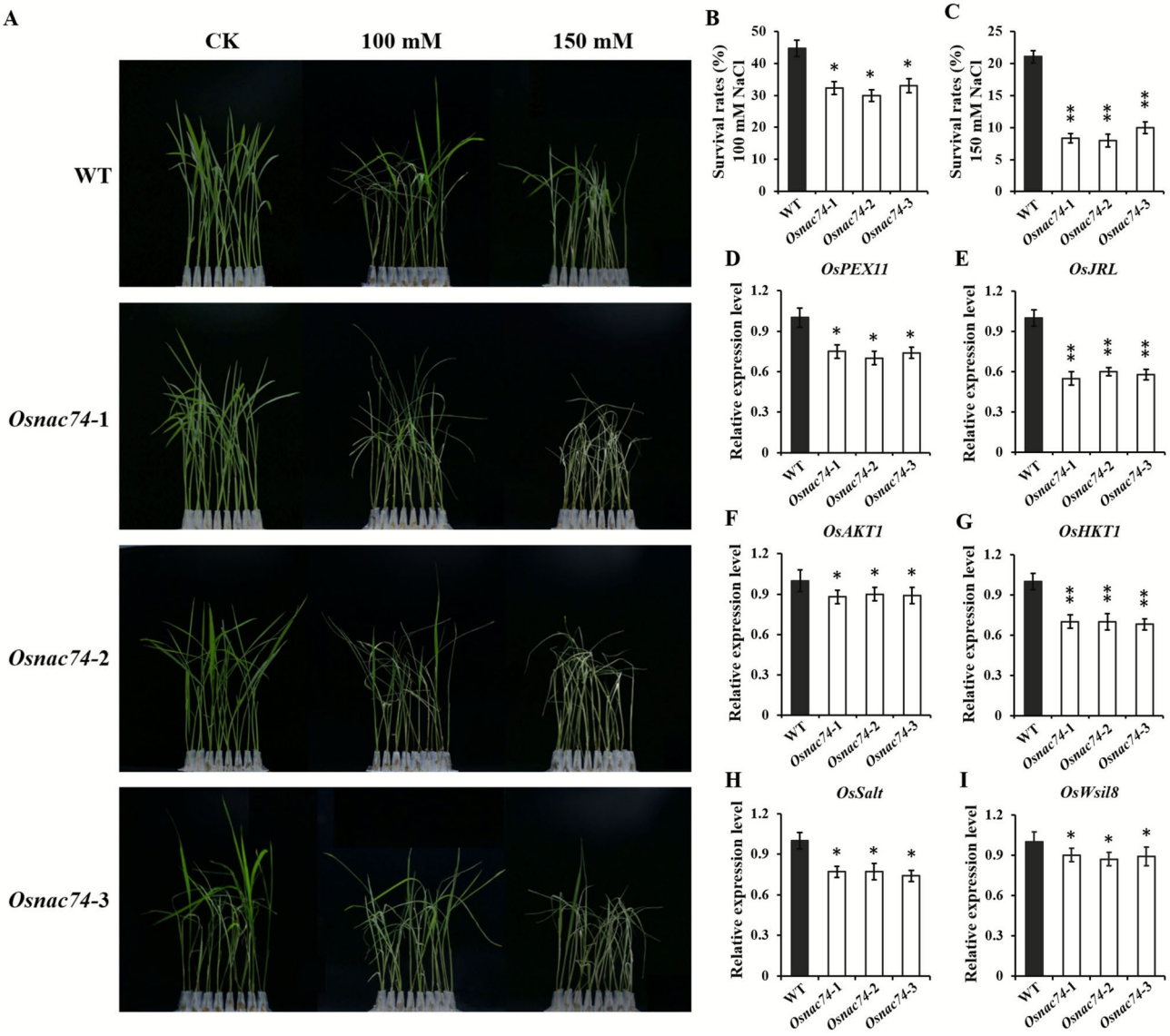

Fig. 2. Salt stress test and gene expression analysis in *OsNAC74* homozygous mutants. (**A**) Phenotype of *OsNAC74* mutants under salt stress, all curled leaves are considered dead. (**B**) Survival rate of rice seedlings under 100 mM NaCl. (**C**) Survival rate of rice seedlings under 150 mM NaCl. (**D-I**) RT-qPCR analysis of salt stress-related gene expression. Significant differences determined using a two-tailed *t*-test: $^{**}P \leq 0.01$, $^{*}P \leq 0.05$. WT: wild type. Bars: 2 mm. Error bars represent the standard error of the mean.

treatment (Fig. 5A). Previous studies have demonstrated that auxin plays a critical role in regulating plant root development[29]. Based on these findings, we speculated that *OsNAC74* might regulate root development in rice through the auxin pathway. To verify this hypothesis, we investigated the root growth and development of *OsNAC74* homozygous mutants and wild-type seedlings grown under normal conditions for seven days. Compared with the wild type, the *OsNAC74* homozygous mutant seedlings exhibited significantly shorter main root and lateral root lengths (Fig. 6A-C), while the number of lateral roots remained unchanged (Fig. 6D). These results suggested that *OsNAC74* regulates root growth and development in rice through the auxin pathway and that the reduced root length in *OsNAC74* homozygous mutants may be a key factor contributing to their decreased salt tolerance.

Discussion

Amino acid permease (AAP), a critical member of the amino acid transporter family, plays a key role in amino acid transport between various plant organs[30]. Among these, *OsAAP6*, a rice amino acid permease gene previously isolated and cloned by us, positively regulates the protein content and nutritional quality of rice seeds. Using a yeast one-hybrid assay, we identified OsNAC74 as an upstream regulator of *OsAAP6*[25]. The *OsNAC74* mutation resulted in reduced protein content and smaller grain size in rice seeds, indicating that *OsNAC74* influences both the nutritional and physical quality of rice seeds by regulating *OsAAP6* and

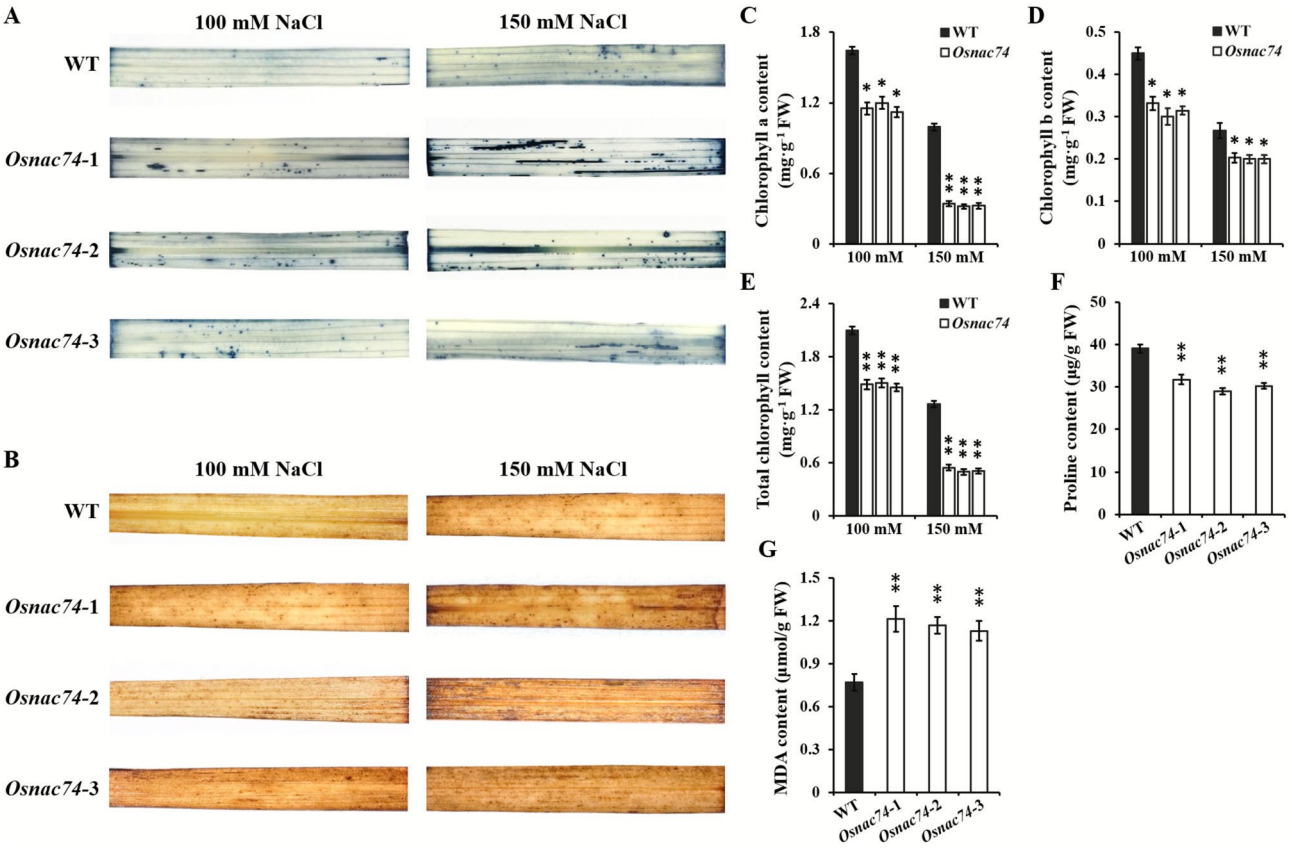

Fig. 3. Physiological indicators of salt stress in *OsNAC74* homozygous mutants. (**A**) NBT staining. (**B**) DAB staining. (**C**) Chlorophyll a content. (**D**) Chlorophyll b content. (**E**) Total chlorophyll content. (**F**) Proline content. (**G**) Malondialdehyde content. Significant differences determined using a two-tailed *t*-test: $^{**}P \leq 0.01$, $^{*}P \leq 0.05$. WT: Wild type. Bars: 5 mm. Error bars represent the standard error of the mean.

genes involved in protein and starch metabolism[25]. The seed germination experiments showed that *OsNAC74* mutations significantly decreased the germination rate of rice seeds, potentially due to the altered grain size observed in *OsNAC74* homozygous mutant seeds. Mature seeds regulate downstream transcription factors and regulatory elements associated with germination via gibberellin signaling, thereby breaking seed dormancy and promoting germination by stimulating cell elongation and division[31]. Notably, the germination rate of *OsNAC74* homozygous mutant seeds was significantly improved by exogenous application of gibberellin, suggesting that their slow germination is primarily caused by a reduction in gibberellin concentration within the seeds. These results suggested that *OsNAC74* regulates seed size through its involvement in the gibberellin signaling pathway and plays a vital role in rice seed germination.

Soil salinization is a global ecological issue that severely hinders the growth and development of cereal crops, leading to reduced food production. Therefore, to ensure food security and promote the sustainable development of agriculture, breeding new crop varieties with enhanced salt tolerance is essential. NAC transcription factors (NAC-TFs), a unique family of plant transcription factors, play critical roles in regulating plant responses to biotic and abiotic stresses[32]. For instance, ZmNAC84 directly binds to the *ZmCAT1* promoter, regulating its expression and improving maize salt tolerance[33]. Similarly, *GhNAC2-A06* is involved in cotton's drought stress response by regulating the expression of drought-related genes[34]. Overexpression of *TaNAC22* in wheat significantly enhances tolerance to cadmium stress[35], while *ONAC066* positively regulates disease resistance in rice by inhibiting the ABA signaling pathway, with its overexpression significantly enhancing resistance to rice blast and bacterial wilt diseases[36]. In this study, we found that the *OsNAC74* mutation significantly reduced salt tolerance in rice seedlings, with salt stress response-related genes significantly down-regulated in *OsNAC74* homozygous mutants. These findings indicated that *OsNAC74* negatively regulates rice salt tolerance by regulating the expression of salt stress-related genes.

Jasmonic acid is involved in regulating various signal transduction pathways in plants. It plays an important role in plant defense against biotic and abiotic stresses, such as salinity, drought, vernalization, and heavy metal exposure[37]. Additionally, as a growth regulator, jasmonic acid works alongside other plant hormones through a complex signaling cascade to balance plant growth and development under stress[38]. Abscisic acid is crucial for balancing plant endogenous hormones and regulating growth metabolism. It plays an essential and irreplaceable role in many physiological and biochemical processes during normal plant growth and environmental stress responses[39]. Transcriptome sequencing results in this study revealed that differentially expressed genes were

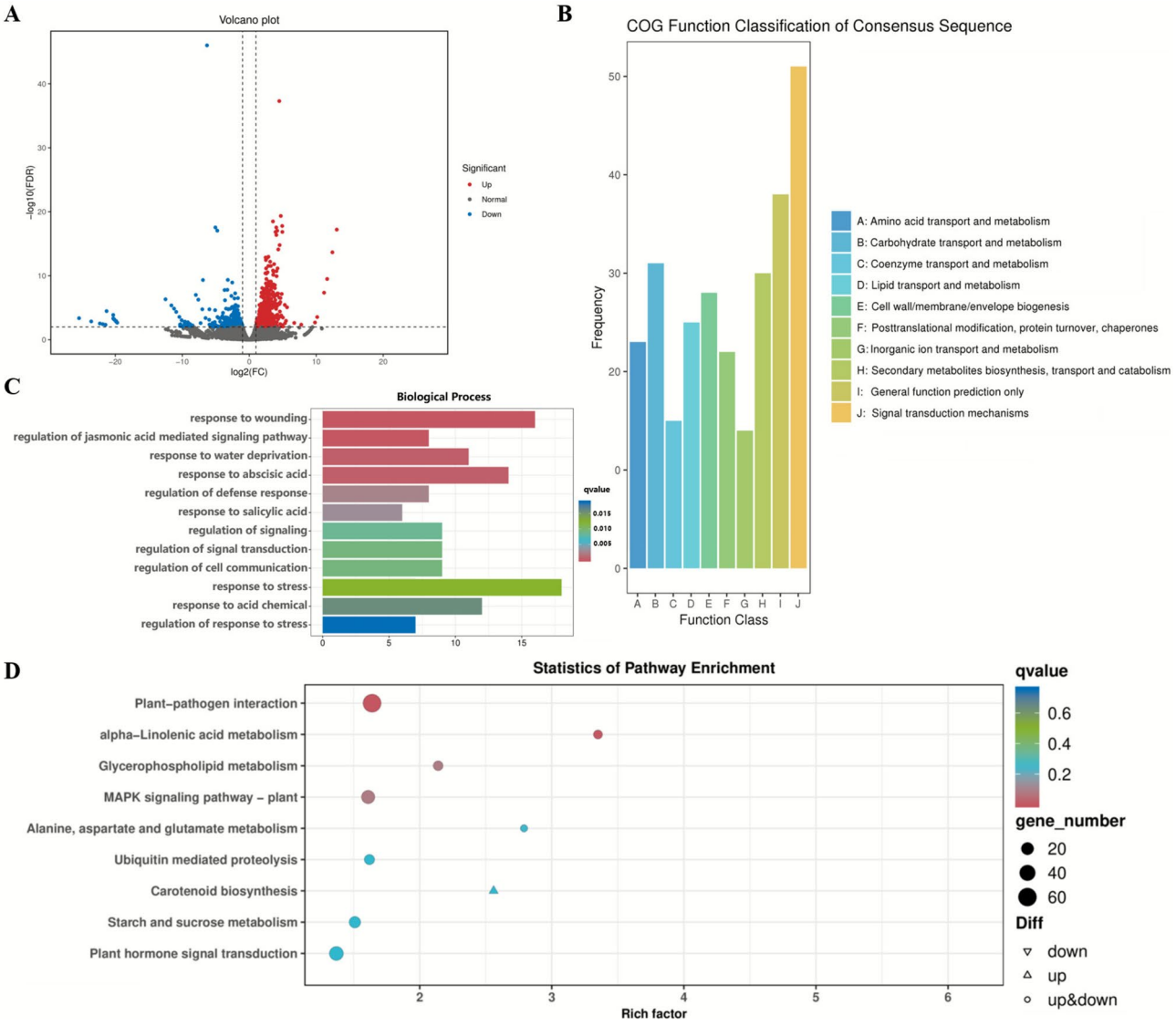

Fig. 4. Transcriptomic analysis of *OsNAC74* homozygous mutants under salt stress. (**A**) Volcano plot showing differentially expressed genes, where each dot represents a gene. (**B**) COG functional annotation of differentially expressed genes. (**C**) GO functional enrichment analysis of differentially expressed genes. (**D**) KEGG pathway enrichment analysis of differentially expressed genes.

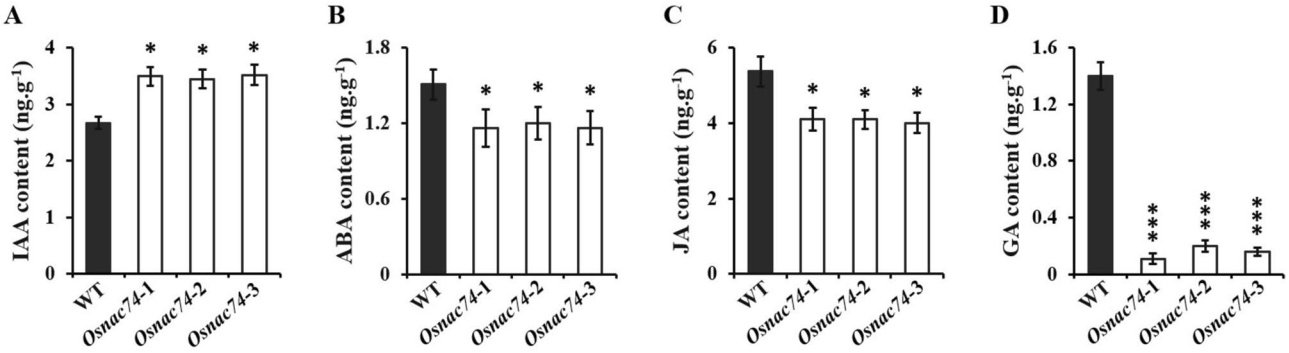

Fig. 5. Hormone content in *OsNAC74* homozygous mutant roots. (**A**) Auxin content. (**B**) Abscisic acid content. (**C**) Jasmonic acid content. (**D**) Gibberellin content. Significant differences determined using a two-tailed *t*-test: $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$. WT: wild type. Error bars represent the standard error of the mean.

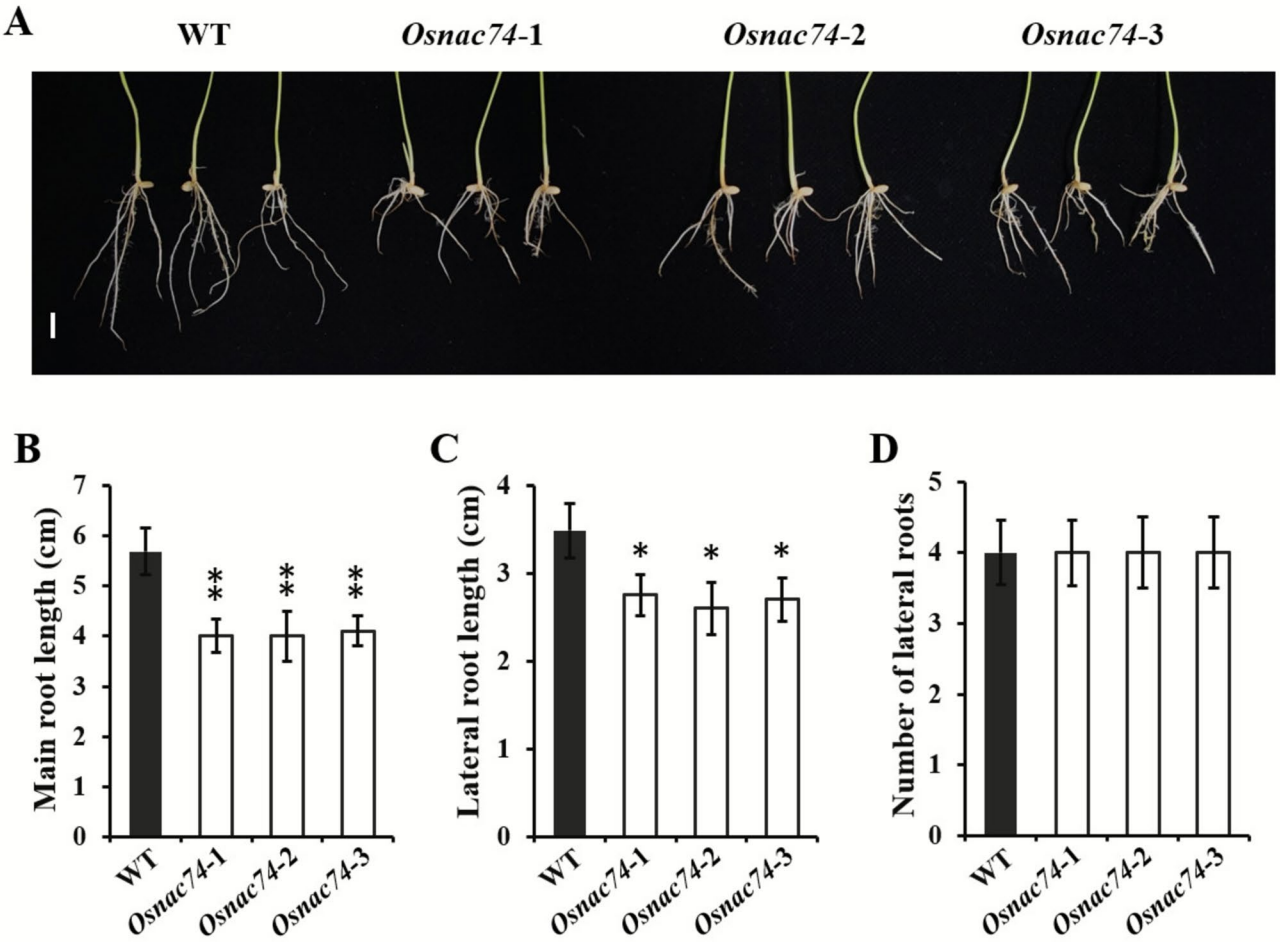

Fig. 6. Root development analysis in *OsNAC74* homozygous mutants. (**A**) Root growth phenotype of *OsNAC74* homozygous mutants. (**B**) Main root length. (**C**) Lateral root length. (**D**) Lateral root number. Significant differences determined using a two-tailed *t*-test: $^{**}P \leq 0.01$, $^{*}P \leq 0.05$. WT: wild type. Bars: 1 cm. Error bars represent the standard error of the mean.

involved in processes such as jasmonic acid-mediated signaling, abscisic acid regulation, and metabolic pathways like plant hormone signaling and the MAPK signaling pathway. These findings suggested that *OsNAC74* may regulate salt tolerance by influencing plant hormones such as jasmonic acid and abscisic acid. We measured the hormone concentrations and the expression levels of plant hormone-related genes in roots to confirm the regulation of hormone content in rice by *OsNAC74*. The results showed that the auxin concentration in the roots of *OsNAC74* homozygous mutants significantly increased after seven days of treatment with 100 mM NaCl, while the concentrations of abscisic acid, jasmonic acid, and gibberellin significantly decreased. Furthermore, the expression levels of plant hormone-related genes corresponded with the changes in root hormone concentrations. Therefore, *OsNAC74* regulates the salt sensitivity of rice seedlings by modulating plant hormone signaling.

Auxin plays an important role in the growth and development of plant roots. As a key hormone, auxin is crucial for regulating root formation, elongation, branching, and responses to environmental stresses[40,41]. The results of this study on the root system of *OsNAC74* homozygous mutants showed that the *OsNAC74* mutation did not affect the number of lateral roots in rice but caused a significant reduction in the length of both the main and lateral roots. Additionally, transcriptome sequencing revealed differentially expressed genes involved in plant hormone signaling pathways, some of which are related to auxin metabolism. Furthermore, the concentration of auxin in the roots of the *OsNAC74* homozygous mutants was significantly higher, and the expression levels of auxin biosynthesis-related genes were notably upregulated. Previous reports indicated that excessive auxin concentration can inhibit plant growth and root development[40]. These findings collectively suggested that *OsNAC74* regulates the growth and development of rice roots by modulating auxin metabolism pathways, ultimately influencing the salt tolerance of rice seedlings.

Conclusion

The transcription factor *OsNAC74* is involved in various plant hormone pathways in rice, regulating seed germination, root growth and development, and seedling salt tolerance. The gibberellin concentration in

OsNAC74 mutants' seeds was significantly reduced, leading to a slower germination rate. *OsNAC74* affects root growth and development by regulating the expression of auxin biosynthesis-related genes and auxin content in rice roots. Additionally, *OsNAC74* participates in jasmonic acid and abscisic acid signaling pathways, thereby reducing the salt tolerance of rice seedlings. Therefore, *OsNAC74* plays a crucial role in regulating rice root growth, development, and salt stress response, providing an important genetic resource for the molecular breeding of new high-quality, multi-resistant rice varieties in the future. Based on existing research, the next step will focus on constructing a rice salt stress response regulatory network with *OsNAC74* as the core through systematic gene interaction screening strategies, and deeply analyzing the molecular mechanism of this gene regulating rice salt tolerance.

Data availability

The datasets generated and/or analysed during the current study are available in the China National Center for Bioinformatics (https://www.cncb.ac.cn/) repository, accession number CRA027253.

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Declarations

Competing interests
The authors declare no competing interests.

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