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Integrative RNA-seq and ATAC-seq analysis unveils antioxidant defense mechanisms in salt-tolerant rice variety *Pokkali*

Qiaoyu Yang[1,2], Yutong Zheng[1] and Xitao Li[1*]

Abstract

Background Salt stress is one of the most significant environmental challenges, severely impacting rice growth and yield. While different rice varieties exhibit varying levels of tolerance to salinity, *Pokkali*, a traditional salt-tolerant variety, stands out for its ability to thrive in saline conditions. Understanding the molecular and physiological mechanisms that underpin this tolerance is essential for breeding and developing rice varieties with enhanced resilience to salt stress.

Methods In this study, we selected the salt-tolerant rice variety *Pokkali* and the salt-sensitive variety IR29 for a controlled saline stress experiment. Plants were subjected to a 150 mM NaCl treatment for 7 days, after which leaf samples were collected from both varieties. Antioxidant physiological parameters were measured, and RNA-seq and ATAC-seq analyses were conducted to explore gene expression and chromatin accessibility. Key genes identified through sequencing were validated using RT-qPCR.

Results Under salt stress, *Pokkali* demonstrated strong tolerance and a higher antioxidant capacity compared to IR29, as evidenced by increased survival rates and fresh weight. *Pokkali* also showed elevated activity of antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase, along with reduced accumulation of hydrogen peroxide. Transcriptomic and ATAC-seq analyses revealed that *Pokkali*'s upregulated genes were significantly enriched in pathways related to redox homeostasis. These genes were also involved in metabolic processes such as glycan biosynthesis, amino acid metabolism, carbohydrate metabolism, and energy production. Furthermore, ATAC-seq analysis indicated increased chromatin accessibility in the promoter regions of key antioxidant genes under salt stress in *Pokkali*, reflecting enhanced transcriptional activity. Four key antioxidant-related genes—*MnSOD1*, *OsAPx7*, *OsGR1*, and *Osppc3*—were identified and validated by qPCR, showing significant upregulation in *Pokkali*. ATAC-seq data further supported that these genes had increased promoter accessibility under salt stress, aligning with the RNA-seq findings.

Conclusion This study underscores the critical role of antioxidant defense mechanisms in conferring salt tolerance in *Pokkali*. The identification of key genes involved in redox regulation provides valuable insights into the molecular basis of salt tolerance, offering potential targets for the genetic improvement of salt-sensitive rice varieties through breeding programs.

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Keywords Transcriptome analysis, Salt stress, Antioxidant mechanism, ATAC-seq, Rice

Background

Soil salinization is a severe global issue. According to data from the Food and Agriculture Organization's Land and Plant Nutrition Management Service, at least 10% of the world's land is affected by salinization [1]. Soil salinization negatively impacts crop production processes in various ways. As one of the most important staple crops for humans, rice is highly affected by soil salinization, which can lead to reduced yields [2, 3]. The effect of salt stress on rice yield is mainly through reducing key factors such as plant height, thousand-grain weight, and spikelet number, ultimately leading to lower yields [4]. Therefore, understanding the molecular mechanisms of rice salt tolerance and identifying key salt-tolerant genes can accelerate the breeding of salt-tolerant rice varieties. *Pokkali*, one of the earliest salt-tolerant rice varieties, has been widely used in studies of salt-tolerant genotypes in rice [5, 6]. IR29 is a high-yielding indica variety that is sensitive to salt and is commonly used as a model in rice salt tolerance experiments [7]. Comparative analyses of these two rice varieties, can further elucidate salt tolerance mechanisms at the molecular level and provide valuable insights for improving rice survival and yield in saline soils.

Rice is highly sensitive to salinity, with excessive sodium (Na+) ions disrupting cellular homeostasis and leading to ion toxicity, osmotic stress, and oxidative damage [8–10]. Mechanisms of salt tolerance in rice involve complex physiological, biochemical, and molecular responses, including ion homeostasis, osmotic regulation, and detoxification of reactive oxygen species (ROS) [11]. Salt-tolerant varieties, such as *Pokkali*, exhibit superior ion compartmentalization via Na+/H + exchangers like OsNHX1 in the vacuole and better Na + exclusion at the root level through SOS1 transporters, reducing toxic Na + accumulation in shoots [12, 13]. The production of Osmo protectants (proline, glycine betaine) and antioxidants (superoxide dismutase, catalase) also plays a significant role in protecting cellular structures under saline conditions [14, 15].

Gene transcription regulation is one of the critical processes controlling gene expression. Transcriptome sequencing has greatly facilitated in-depth studies of molecular signaling pathways related to rice's response to salt stress and the identification of candidate salt-tolerant genes [16, 17]. While this approach has provided valuable insights into the genetic basis of salt tolerance, it is limited in its ability to capture regulatory elements and chromatin dynamics that play a crucial role in gene expression regulation. Currently, the integration of RNA-seq with Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq) has emerged as a more advanced and comprehensive method for studying complex traits like salt tolerance [18]. ATAC-seq allows for the identification of open chromatin regions, revealing potential regulatory elements such as promoters and enhancers, thus providing a deeper understanding of how gene expression is controlled in response to environmental stress. Despite its potential, the application of ATAC-seq in combination with RNA-seq to study salt tolerance in rice remains relatively underexplored. In this study, we will adapt this integrative approach to advance our understanding of the epigenetic and transcriptional mechanisms underlying salt tolerance, offering new avenues for the genetic improvement of rice varieties.

Methods

Plant materials and salt-stress treatment

IR29, a salt-sensitive variety, and *Pokkali*, a salt-tolerant variety, were used for the study. Plump seeds were selected and soaked in water at 50 °C for 1.5 h, followed by 24 h in a 30 °C incubator. The seeds were then transferred onto germinating paper, kept moist, and incubated at 30 °C for 3 days. After germination, seeds with similar vigor were planted in a 96-well black hydroponic box containing ddH2O. For NaCl treatment, 15-day-old plants were exposed to 150 mM NaCl for 7 days. All plants were grown in a plant growth chamber (Conviron atc26) with a 16 h light/8 h dark cycle at 30 °C during the day and 22 °C at night. Leaves from both varieties were collected before and after NaCl treatment for transcriptome and physiological analyses. A total of 16 samples (four bio-logical replicates per condition) were frozen in liquid nitrogen and stored at – 80 °C.

Determination of the antioxidant enzyme activity

A 50 mM phosphate buffer (pH 7.8) supplemented with 2% PVP and 1 mM EDTA was used to homogenize rice leaves. After centrifuging the homogenate for 30 min at 4 °C at 15,000× g, the supernatant was utilized for enzyme activity tests. The NBT method [19] was used to quantify the activity of superoxide dismutase (SOD), with one unit being defined as the amount of enzyme that causes 50% inhibition of NBT reduction at 560 nm. After H_2O_2 breakdown, a reduction in absorbance at 240 nm (E = 39.4 mM – 1 cm – 1) over a 1-minute period was used to measure catalase (CAT) activity [20]. Tracking guaiacol oxidation at 470 nm (E = 26.6 mM – 1 cm – 1) was used to determine the peroxidase (POD) activity [21]. At 25 °C, all enzyme activities were measured.

RNA sequencing

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the 16 samples in accordance with the manufacturer's instructions. The NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, USA) was utilized to generate the cDNA sequencing libraries in accordance with the manufacturer's instructions. Index codes were incorporated to assign sequences to individual samples. Shenzhen Lian Chuan Technologies Co., Ltd. sequenced all libraries on an Illumina HiSeq X-ten platform (Shenzhen, China).

Transcriptome data analysis

Raw sequencing data in FASTQ format were processed using internal Perl scripts. Initially, low-quality reads, adapter sequences, and poly-N reads were removed to produce clean data (clean reads). Quality assessment of the clean data was performed by calculating the Q20, Q30 scores, GC content, and sequence duplication level. "clean data" were defined as follows: Q20 ≥ 95%, Q30 ≥ 85%, GC content within the typical range for rice (approximately 45%), and minimal sequence duplication. These thresholds ensured that the data met high-quality standards for downstream analyses. The reference genome used for mapping the clean reads was the Nipponbare rice cultivar reference genome (http://rice.plantbiology.msu.edu).

The clean reads from each sample were aligned to the reference genome using HISAT2 (version 2.2.1), a highly efficient alignment tool that enables the mapping of reads to a genome. Default parameters were used for HISAT2, except for the inclusion of additional flags to improve mapping specificity.

Read counts of each gene were calculated using FeatureCounts (version 2.0.1) from the Subread package, with gene annotations based on the latest rice reference genome annotation available. Differential expression analysis of the four experimental groups was carried out using DESeq2 (version 1.32.0). Differentially expressed genes (DEGs) were identified using a false discovery rate (FDR) threshold of < 0.05 and a log2 fold change ≥ 1 (upregulated) or ≤ -1 (downregulated). The conditions used for comparison were IR29-control vs. Pokkali-control, IR29-salt stress vs. Pokkali-salt stress, IR29-control vs. IR29-salt stress, and Pokkali-control vs. Pokkali-salt stress. IR29-control was used as the reference sample for comparing differential expressions of genes (DEGs).

The functional relevance of DEGs was carried out using the KEGG and GO databases. KEGG enrichment was performed using KOBAS 2.0 (version 2.1.1), with a p-value threshold of < 0.05 for statistical significance. For Gene Ontology (GO) analysis, DEGs were annotated using the ClusterProfiler R package (version 4.2.2) with a p-value threshold of < 0.05 for statistical significance.

Assay for transposase-accessible chromatin sequencing (ATAC-seq)

Approximately 700 mg of leaf tissue from each sample was homogenized in 1 mL of ice-cold lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% NP-40, and protease inhibitors) using a glass dounce homogenizer. The homogenate was filtered through a 40 µm cell strainer, followed by centrifugation at 500× g for 10 min at 4 °C. The nuclear pellet was resuspended in 50 µL of 1× Tn5 reaction buffer (10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10% dimethyl sulfoxide [DMSO]). Nuclei were subjected to transposition by adding 2.5 µL of Tn5 transposase, followed by incubation at 37 °C for 30 min with gentle mixing. The reaction was stopped by adding EDTA-containing buffer, and DNA was purified using the MinElute PCR Purification Kit.

Following transposition, DNA was amplified using a limited-cycle PCR (12–14 cycles) with Nextera index primers to generate sequencing libraries. Libraries were cleaned using AMPure XP beads and quantified with a Qubit fluorometer. Library size distribution was assessed on an Agilent Bioanalyzer. Paired-end sequencing (50 bp long reads) was performed on an Illumina HiSeq 2500 platform.

Raw sequencing reads were processed using Trim Galore (version 0.6.6) for removal of adaptor sequences and low-quality reads. The trimmed reads were then aligned to the Oryza sativa genome (IRGSP-1.0) using Bowtie2 (version 2.5.1).

Chromatin accessibility peaks were identified using MACS2 (version 2.2.7.1), with an FDR threshold of 0.05. Promoter regions were defined as regions ≤ 1 kb upstream of the transcription start site. Differential peak analysis was conducted using the DiffBind R package (version 3.0.3), comparing Pokkali vs. IR29 samples under salt stress conditions.

Motif enrichment analysis of the upregulated chromatin accessibility in the promoter regions of rice was performed using the HOMER software suite (version 4.11). The analysis focused on the promoter regions defined by a fixed window (e.g., -1 kb to + 500 bp relative to the transcription start site). The default HOMER motif database was used to identify transcription factor binding motifs, with a background set of random genomic sequences for comparison. Motifs were considered significantly enriched if the p-value was < 0.05 (Fisher's exact test), and a minimum of five occurrences of a motif within the input set was required. The size of the sequence surrounding each peak was set to 200–500 bp, depending on the specific experiment design, and motifs were mapped using the rice (Oryza sativa) genome reference. We focused on de novo motif discovery in the upregulated chromatin accessibility in the promoter regions,

specifically examining motifs that were significantly enriched in Pokkali compared to IR29.

Real-time quantitative reverse transcription PCR (RT-qPCR) verification

Four important DEGs were chosen for RT-qPCR from the transcriptome data in order to confirm the accuracy of the RNA sequencing results. The rice gene OsUBQ10 was used as a quantitative internal control and the RT-qPCR primers sequences are listed in the supplementary Table 1. Using a 20 µl reaction volume containing 10 µl SYBR Green Master Mix, 0.4 µl upstream primer, 0.4 µl downstream primer, 1 µl template DNA, and 8.2 µl sterile ultra-pure water, the RT-qPCR experiments were performed using a Bio-Rad CFX96 RT-qPCR apparatus. To ascertain variations in gene expression, the $2-\Delta\Delta Ct$ quantitative technique was employed to examine the RT-qPCR data.

Statistical analysis

Every experiment was conducted three times or more. The data was presented as mean ± SE. The student's t test was used to compare all means for the independent sample. The confidence coefficient was always set at 0.05.

Results

Phenotypes of *Pokkali* and *IR29* before and under salt stress for 7 days

To investigate the regulatory mechanisms in rice under salt stress conditions, we selected a salt-tolerant variety, *Pokkali*, and a salt-sensitive variety, IR29, for an experiment simulating a saline-alkali environment. The seeds of both varieties were germinated for three days, followed by hydroponic cultivation for 15 days, during which we recorded the fresh weight and survival rate of the two rice varieties. Subsequently, they were treated with 150 mM saline for seven days, after which we recorded their fresh weight and survival rate again. The results showed that salt stress inhibited the growth of both rice seedlings. As the stress duration increased, the leaves of both varieties gradually curled, with yellowing at the leaf tips, and some stems drooping or even lodging (Fig. 1A). The salt-sensitive variety IR29 showed more severe damage than *Pokkali* after seven days, with a greater reduction in fresh weight (Fig. 1B) and lower survival rates (Fig. 1C).

Analysis of physiological parameters under salt stress

Measuring hydrogen peroxide (H_2O_2) levels and antioxidant enzyme activity is crucial to understanding how plants respond to salt stress. Plants mitigate oxidative stress through a network of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which neutralize H_2O_2 and prevent cellular damage. Under basal conditions, the salinity-tolerant variety *Pokkali* and the salt-sensitive variety *IR29* exhibited distinct differences in ROS levels and antioxidant enzyme activities. *Pokkali* had lower H_2O_2 levels than *IR29*, indicating more efficient basal ROS detoxification (Fig. 2A). This was supported by higher activities of SOD, POD, and CAT in *Pokkali*, reflecting a stronger innate antioxidant defense (Fig. 2B and D). In contrast, *IR29* exhibited higher H_2O_2 levels and lower enzyme activities, suggesting a less active antioxidant system under normal conditions (Fig. 2B and D).

After 7 days of 150 mM NaCl treatment, both varieties showed increased H_2O_2 levels, but *IR29* accumulated significantly more H_2O_2, indicating a weaker ability to manage oxidative stress. While *Pokkali* also showed increased H_2O_2, the fold increase was higher in *Pokkali* (~3-fold) compared to *IR29* (~2-fold), suggesting a more pronounced oxidative stress response. Despite this, *Pokkali* efficiently detoxified ROS through marked increases in SOD, POD, and CAT activities (Fig. 2B and D), with SOD converting superoxide radicals into H_2O_2 and POD and CAT breaking down the excess H_2O_2. In contrast, *IR29* exhibited a more modest increase in antioxidant enzyme activity, which led to inadequate ROS scavenging and higher oxidative damage. These results suggest that although *IR29* starts with higher baseline H_2O_2 levels, *Pokkali*'s enhanced antioxidant system, reflected in its stronger response to salt stress, enables it to better tolerate salt stress compared to *IR29*.

Differential expressed genes identification and enrichment analysis by RNA-seq

To investigate the underlying mechanisms contributing to Pokkali's strong salt tolerance, we performed transcriptome sequencing on the leaves of Pokkali and IR29 before and after 7 days of salt treatment. Quality control of the sequencing data was conducted on 12 samples, yielding a total of 91.33 Gb of clean data. The valid data for each sample ranged from 6.78 to 7.46 Gb, with an average GC content of 45.24%, and the percentage of Q30 bases exceeded 95.17% across all samples (Table S2). Principal component analysis (PCA) was performed to assess the similarity of the samples, resulting in a score plot (Fig. 3A). The analysis showed that samples within each group (Pokkali-control, Pokkali-salt stress, IR29-control, and IR29-salt stress) clustered closely together, indicating high reproducibility within replicates. The first principal component (PC1) explained 52% of the variance, while the second principal component (PC2) accounted for 9.7% of the variance. Clear separation was observed between the two cultivars under both control and salt-stress conditions. We conducted four comparisons: (1) salt stress/control in IR29, (2) salt stress/control in Pokkali, (3) Pokkali/IR29 under control conditions, and (4) Pokkali/IR29 under salt stress conditions. The

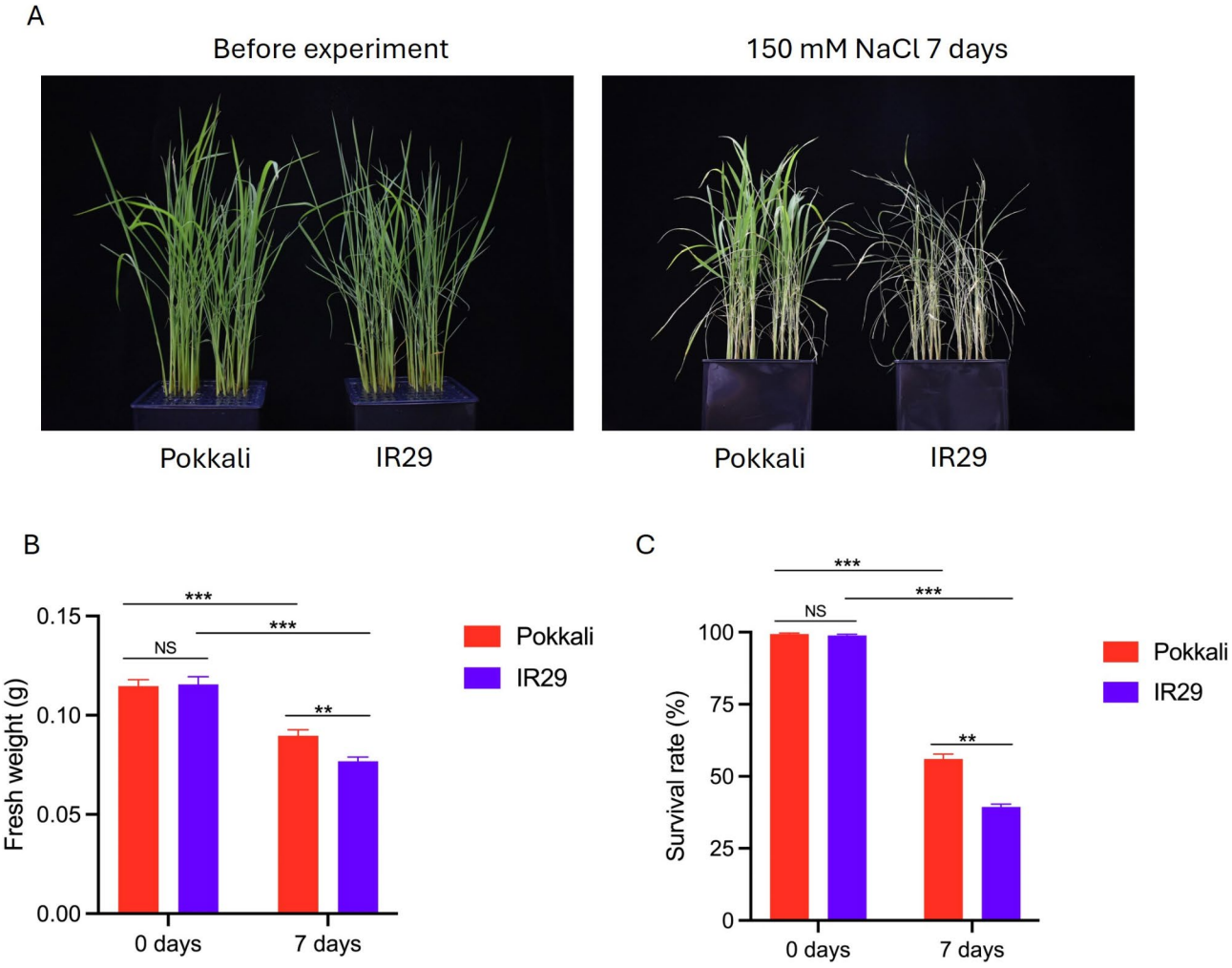

Fig. 1 Phenotypes of *Pokkali* and *IR29* before and under salt stress for 7 days. 15-day-old *Pokkali* and *IR29* plants were exposed to 150 mM NaCl for 7 days. (**A**) the representative images of two variety before and after NaCl treatment for 7 days. (**B**) the fresh weight of two variety before and after treatment. (**C**) the survival rate of two variety before and after treatment. $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

number of upregulated and downregulated differentially expressed genes (DEGs) in each comparison is depicted in the volcano plots (Fig. 3B-E). In comparison 1 (IR29 salt stress vs. control), Gene Ontology (GO) analysis was primarily enriched in defense responses to fungi, immune responses, and organelle localization. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis highlighted pathways related to plant-pathogen interactions and glycan degradation (Fig. 3B). In comparison 2 (Pokkali salt stress vs. control), GO analysis was enriched in processes related to lignin catabolism and phenylpropanoid metabolism, while KEGG pathways were enriched in plant-pathogen interactions and phenylpropanoid biosynthesis (Fig. 3C). In comparison 3 (Pokkali vs. IR29 under control conditions), GO analysis was mainly enriched in responses to acidic chemicals and water, while KEGG analysis was enriched in diterpenoid biosynthesis (Fig. 3D). In comparison 4 (Pokkali vs. IR29 under salt stress conditions), GO analysis was primarily enriched in responses to chemicals and catabolic processes, while KEGG pathways were mostly enriched in phenylpropanoid biosynthesis (Fig. 3E).

Analysis of key degs reveals metabolic and redox pathways contributing to salt tolerance in pokkali and IR29

Under untreated conditions, 680 differentially expressed genes (DEGs) were identified between Pokkali and IR29, while 3,051 DEGs were identified after 7 days of salt treatment, with 1,279 overlapping DEGs between the two conditions (Fig. 4A). These 1,279 DEGs were selected for further analysis. KEGG enrichment revealed 11 significantly enriched pathways, with the top five being phenylpropanoid biosynthesis, oxidative phosphorylation, photosynthesis, pentose phosphate pathway, and peroxisome (Fig. 4B). GO analysis of the DEGs indicated that key biological processes involved include secondary

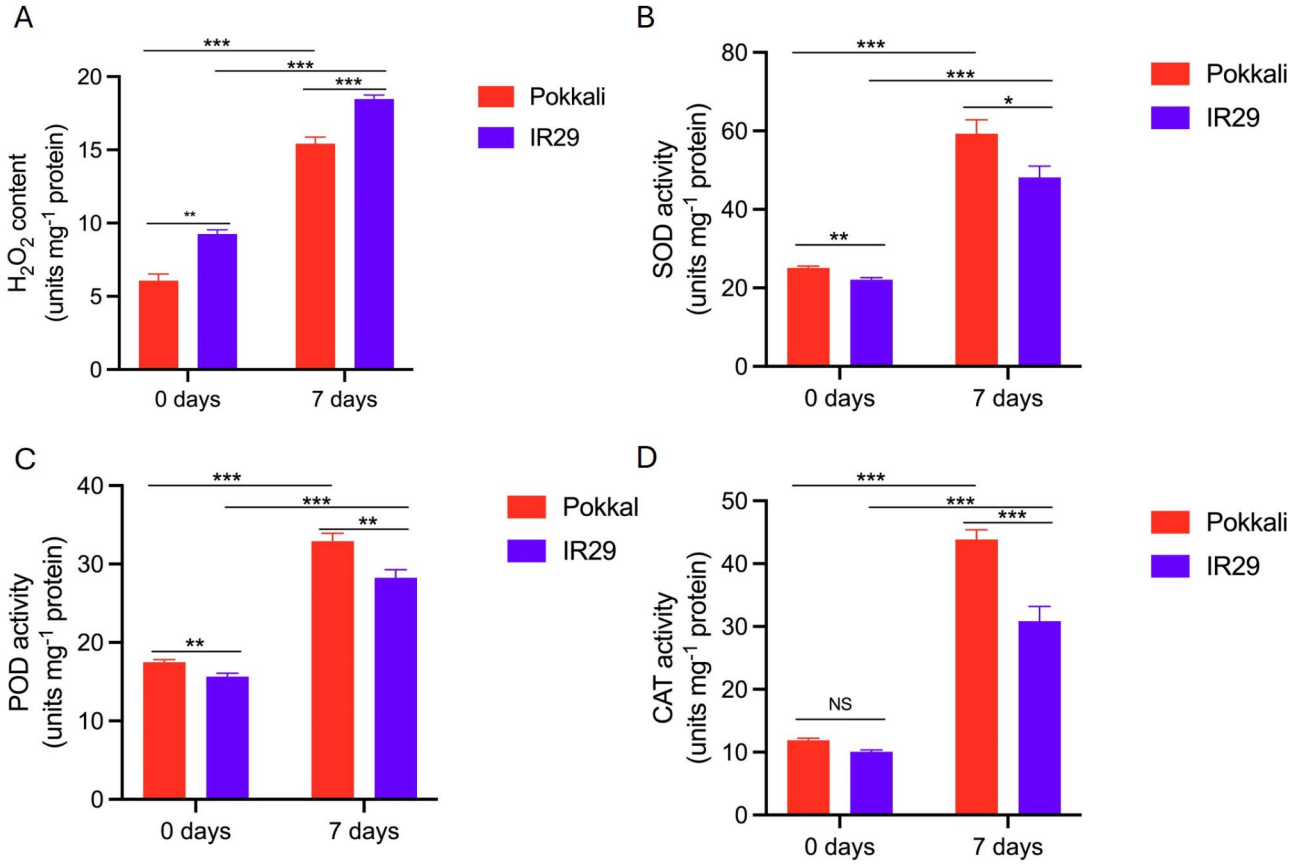

Fig. 2 Analysis of physiological parameters under salt stress. 15-day-old *Pokkali* and *IR29* plants were exposed to 150 mM NaCl for 7 days. (**A**) The content of H2O2 in two variety before and after treatment. (**B**) the SOD activity, (**C**) POD activity and (**D**) CAT activity in two variety before and after treatment. $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

metabolism, oxidative stress response, respiratory electron transport, phenylpropanoid metabolism, and lignin catabolism (Fig. 4C). These findings highlight significant differences in metabolic and redox processes between Pokkali and IR29 under salt stress, suggesting that altered metabolism and antioxidant capacity contribute to the distinct salt tolerance of these varieties. Additionally, metabolic network analysis using the iPath database revealed dense involvement in carbohydrate, amino acid, and energy metabolism pathways (Fig. 4D), underscoring their role in the differential salt stress response in rice.

Annotation of peaks from ATAC-seq datasets and motif enrichment in promoters

To fully understand the molecular mechanisms underlying the differences between the salt-tolerant *Pokkali* and salt-sensitive *IR29* rice varieties, RNA-seq alone is not sufficient. While RNA-seq provides critical insights into gene expression profiles, it does not reveal the regulatory mechanisms driving these expression changes. ATAC-seq allows for the identification of open chromatin regions, offering insights into transcription factor binding sites and epigenetic regulatory elements that control gene expression. By integrating RNA-seq with ATAC-seq, we can obtain a more comprehensive understanding of how chromatin accessibility influences transcriptional responses to salt stress, helping to identify key regulatory pathways that differentiate *Pokkali* and *IR29* at the molecular level.

We performed ATAC-seq on the leaves of the two groups after salt treatment, and the results showed that the salt-tolerant variety *Pokkali* had a higher number of accessible chromatin regions (Fig. 5A). Specifically, we identified 19,707 unique accessible chromatin regions in *Pokkali* and 11,574 accessible chromatin regions in *IR29* (Fig. 5B). We hypothesize that these unique accessible chromatin regions in *Pokkali* lead to the increased expression of key genes, thereby contributing to *Pokkali*'s enhanced salt tolerance. Subsequently, we performed motif enrichment analysis on the 19,707 accessible chromatin regions to identify transcription factor (TF) binding motifs. The top 5 enriched motifs and their corresponding transcription factors, including OsWRKY54, AtSPT4-2, CbWRKY27, AhWRKY75, and GmNACO6, are shown in Fig. 5C.

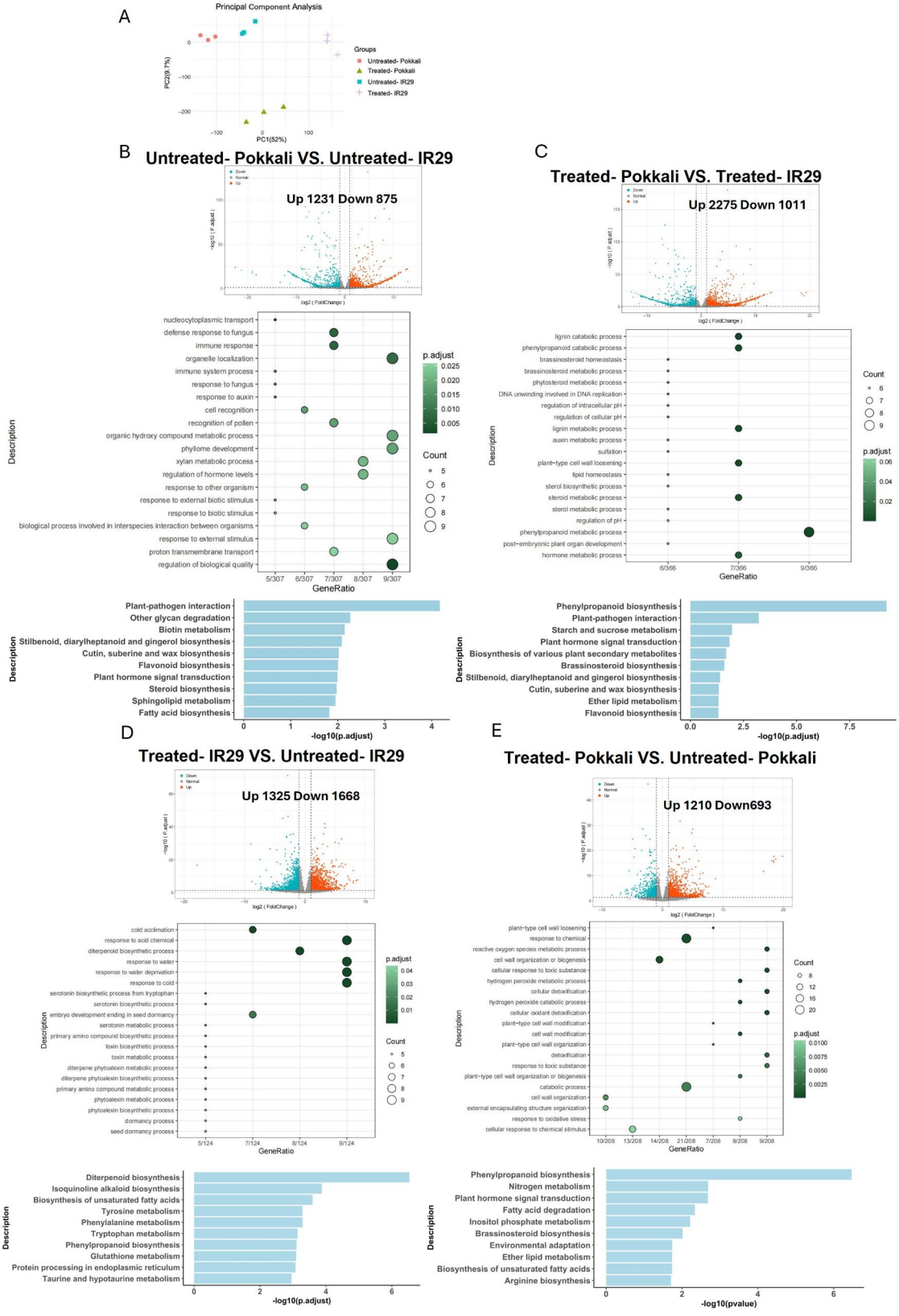

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Fig. 3 Differential expressed genes identification and enrichment analysis by RNA-seq.15-day-old *Pokkali* and *IR29* plants were exposed to 150 mM NaCl for 7 days, the leaves from two variety before and after treatment were collected for RNA-seq. (**A**) Principal component analysis (PCA) of RNA-seq data from Pokkali and IR29 under control and salt stress conditions (**B-E**) Volcano plots depicting the upregulated and downregulated differentially expressed genes (DEGs) in four comparisons: (**B**) IR29 under salt stress vs. control, (**C**) Pokkali under salt stress vs. control, (**D**) Pokkali vs. IR29 under control conditions, (**E**) Pokkali vs. IR29 under salt stress conditions

Quantitative real-time PCR validation of key DEGs with differentially accessible promoter regions

Under salt stimulation conditions, ATAC sequencing identified 386 upregulated genes, while RNA sequencing identified 271 upregulated genes, with 41 overlapping genes (Fig. 6A). To further identify the core genes, we constructed a PPI network of the 41 overlapping genes using STRING, and the data was then imported into Cytoscape's CytoHubba plugin to identify the top 13 hub genes (Fig. 6B). The top four most strongly connected PPI nodes were selected as hub genes: *MnSOD1*, *OsAPx7*, *OsGR1* and *OsPPC3* (Fig. 6C). To verify the reliability of the sequencing results, we performed qPCR validation on the leaves. The results showed that these four genes were highly expressed at both the mRNA level and promoter level in *Pokkali* (Fig. 6D and G). The genes *MnSOD1*, *OsAPx7*, *OsGR1*, and *Osppc3* play significant roles in conferring salt tolerance in rice through various antioxidative and metabolic mechanisms. Together, these genes work in coordination to reduce oxidative stress and maintain cellular homeostasis, contributing to the enhanced salt tolerance observed in specific rice varieties like *Pokkali*.

Discussion

Our findings highlight the critical role of the antioxidant defense system in mitigating oxidative stress induced by salt in rice. Specifically, the salt-tolerant variety Pokkali exhibited significantly higher enzymatic activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) compared to the salt-sensitive IR29. This enhanced enzymatic activity in Pokkali efficiently converts superoxide radicals into less toxic products like hydrogen peroxide (H_2O_2), which is then detoxified into water and oxygen, thus preventing ROS-induced cellular damage [22–24]. The upregulation of these antioxidant enzymes in Pokkali suggests a robust defense mechanism, which likely contributes to its superior salt tolerance. In contrast, the lower antioxidant activity observed in IR29 results in an accumulation of ROS, exacerbating oxidative damage and impairing its ability to cope with salt stress [25].

ATAC-seq analysis revealed significant changes in chromatin accessibility in Pokkali, particularly in the promoter regions. A total of 19,707 unique accessible chromatin regions in Pokklai and 11,574 unique accessible chromatin regions in IR29 were identified, indicating dynamic regulation of gene expression in response to salt stress. The unique accessible chromatin regions in Pokklai likely correspond to enhanced transcriptional activity of genes involved in salt tolerance. These findings support the hypothesis that chromatin remodeling plays a key role in stress-responsive gene expression [26]. Motif analysis of the upregulated promoters identified key transcription factors, such as OsWRKY54, AtSPT4-2, CbWRKY27, AhWRKY75, and GmNACO6, known to regulate stress response pathways in plants [27–30]. Notably, the enrichment of WRKY transcription factors in Pokkali suggests a prominent role for WRKY-mediated signaling in its defense against salt stress [5]. Future studies focusing on the functional validation of these transcription factors could provide valuable targets for genetic engineering to improve salt tolerance in rice and other crops.

Additionally, our study identified the upregulation of OsMnSOD1, a mitochondrial MnSOD family member, in Pokkali under salt stress. OsMnSOD1 is known to detoxify superoxide radicals (O_2^-), reducing H_2O_2 accumulation [31]. This upregulation aligns with previous studies demonstrating that MnSOD overexpression enhances ROS scavenging, suggesting that its high expression in Pokkali contributes to its enhanced oxidative stress tolerance. Our RT-PCR analysis also revealed the upregulation of OsAPx7, a chloroplast-localized ascorbate peroxidase (APX) involved in the ascorbate-glutathione cycle [32]. APX plays a critical role in H_2O_2 detoxification, and its increased expression in Pokkali may further strengthen the plant's ability to manage oxidative stress. Knockout studies of OsAPx7 mutants have shown that H_2O_2 accumulation is detrimental under salt stress, reinforcing the importance of APX in mitigating oxidative damage [33].

We also observed the upregulation of OsGR1, a glyoxylate reductase involved in aldehyde detoxification, in Pokkali under salt stress. This enzyme helps neutralize harmful aldehydes that accumulate under stress and impair plant growth [34]. Previous studies in other species have suggested a protective role for GR1 under stress conditions, and our results suggest a similar function in rice during salt stress [35]. Finally, the upregulation of Osppc3, a phosphoenolpyruvate carboxylase gene, in Pokkali suggests its potential involvement in salt tolerance. Although its role in salt stress has not been previously reported, its upregulation implies a possible contribution to the metabolic adjustments needed for growth under saline conditions [36].

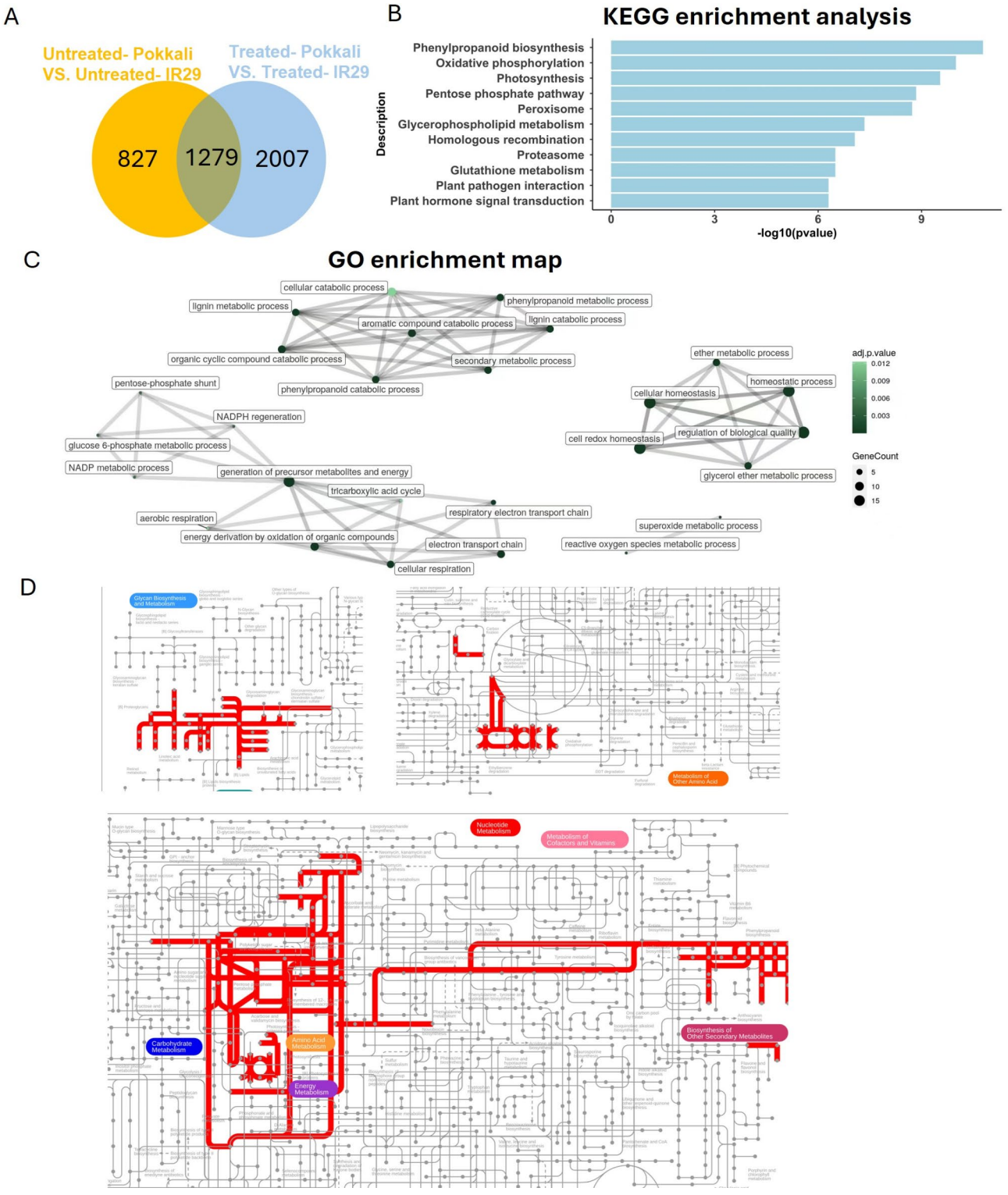

Fig. 4 Analysis of Differentially Expressed Genes (DEGs) and Enrichment Pathways Under Salt Stress in Pokkali and IR29. (**A**) Venn diagram illustrating the number of differentially expressed genes (DEGs) identified between Pokkali and IR29 under control and salt stress conditions. A total of 1,279 overlapping DEGs were selected for further analysis. (**B**) KEGG enrichment analysis of the 1,279 overlapping DEGs. (**C**) GO enrichment analysis of the 1,279 DEGs (**D**) Metabolic network analysis of the DEGs using the iPath database, highlighting the involvement of carbohydrate, amino acid, and energy metabolism pathways

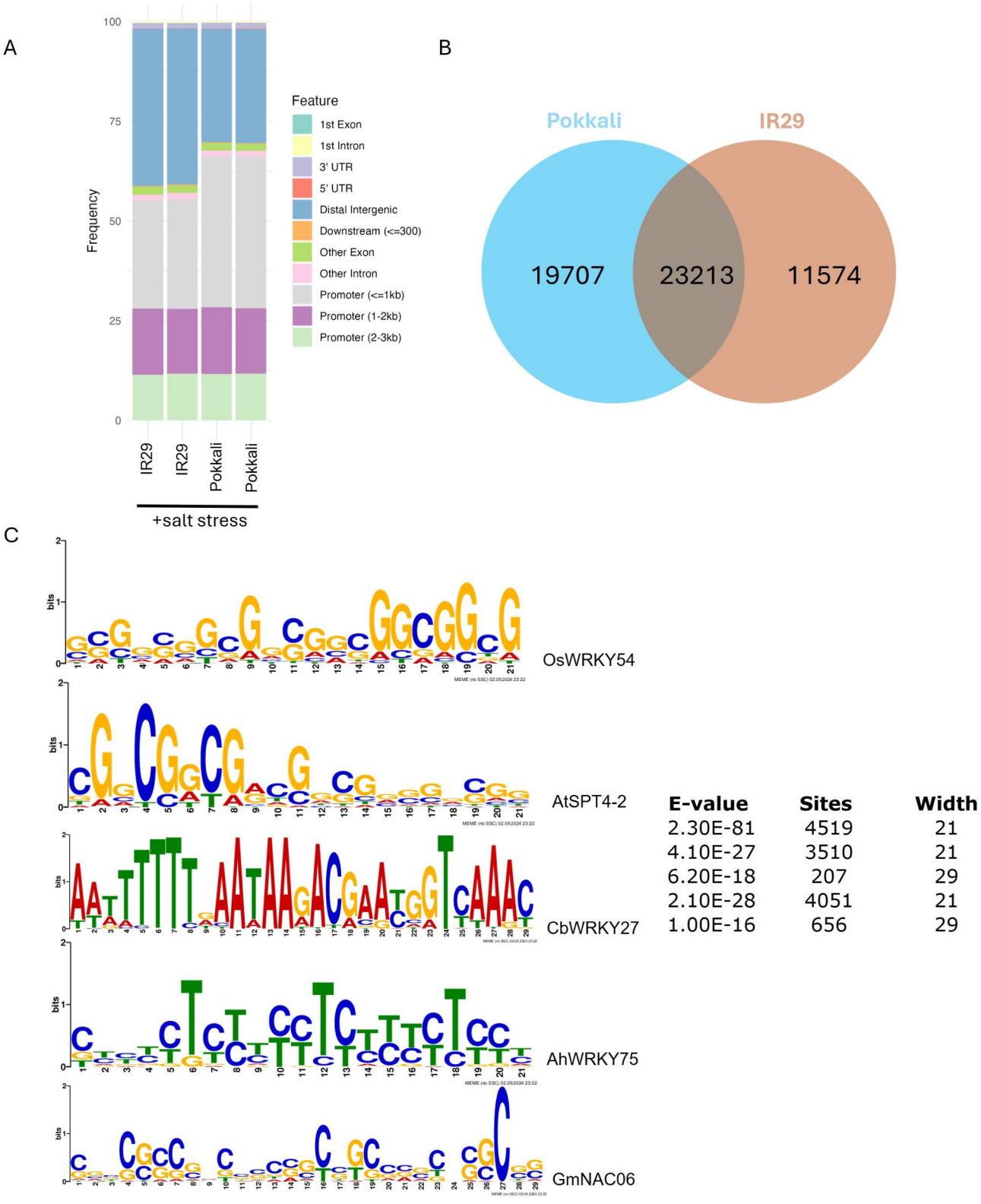

Fig. 5 Annotation of peaks from ATAC-seq datasets and motif enrichment in promoters. The leaves from two varieties after NaCl treatment were collected for ATAC-seq. (**A**) the ratio of different genomic region was identified in two varieties. (**B**) the unique peaks were identified between two varieties. (**C**) the top five motifs prediction based one the unique peaks and the targeted transcription factors of these five motifs

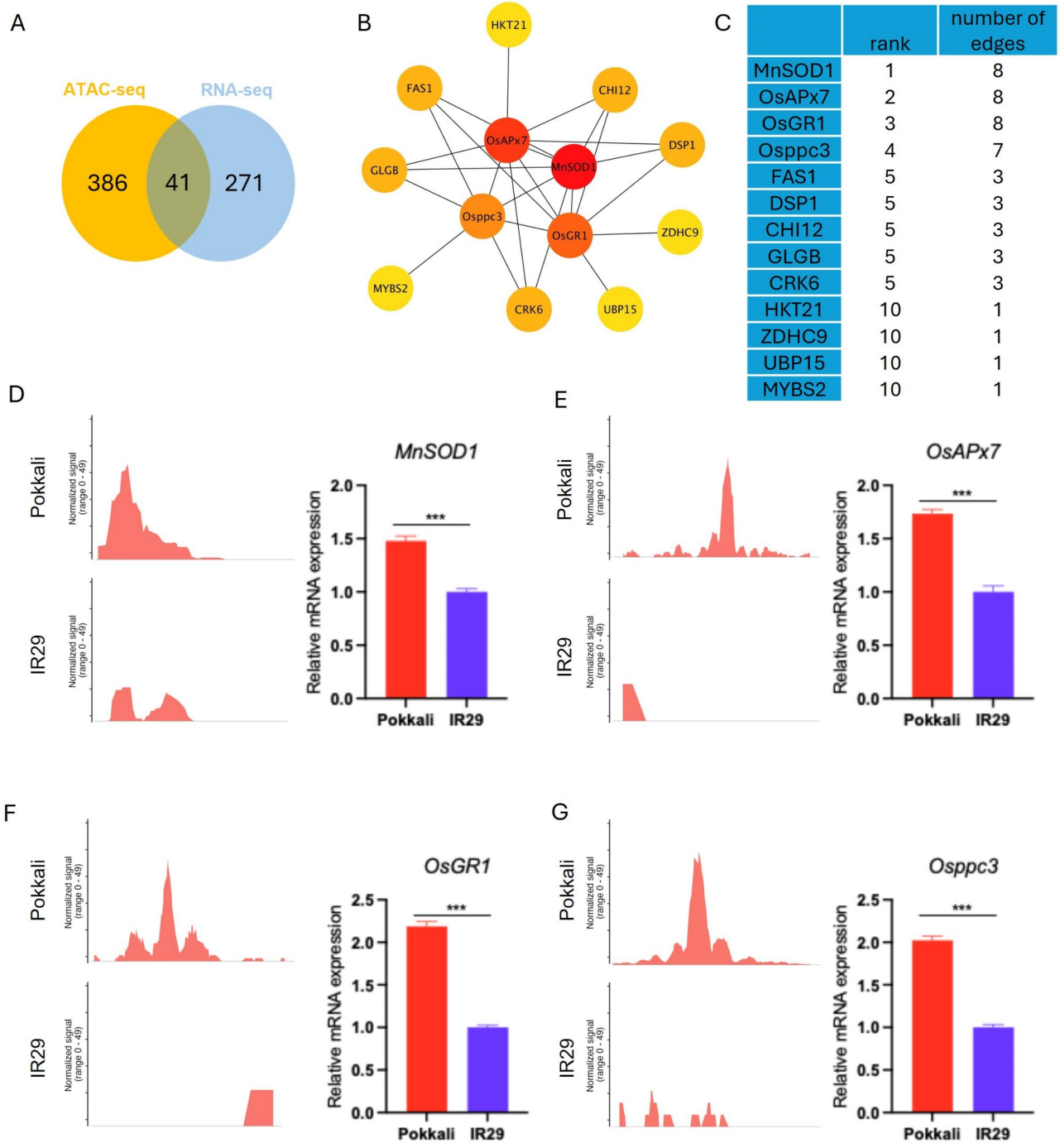

Fig. 6 Quantitative real-time PCR validation of key DEGs with differentially accessible promoter regions. (**A**) Total 41 overlapped up regulated genes between ATAC-seq and RNA-seq. (**B**) STRING analysis the overlapped genes (top 13). (**C**) The rank of number of edges of top 13 hub genes. (D–G) The QPCR validation and genes peaks results from ATAC-seq data. (**D**) *MnSOD1*, (**E**) *OsAPx7*, (**F**) *OsGR1*, and (**G**) *Osppc3*. $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Conclusions

In conclusion, our study highlights the critical role of antioxidative defense mechanisms in enhancing salt tolerance in the rice variety *Pokkali*. The increased activity of key antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in *Pokkali* mitigates oxidative damage by efficiently detoxifying reactive oxygen species (ROS), particularly H_2O_2, under salt stress. Notably, we identified four key genes—*MnSOD1*, *OsAPx7*, *OsGR1*, and *Osppc3*—that are significantly upregulated in Pokkali, contributing to its superior stress response. *OsMnSOD1* plays a vital role in ROS detoxification, *OsAPx7* in chloroplast antioxidant defense, and *OsGR1* in aldehyde de-toxification,

while *Osppc3* is likely involved in energy metabolism, although its specific role in salt tolerance requires further investigation. Together, these findings suggest that the coordinated regulation of these genes underlies *Pokkali*'s enhanced ability to maintain redox homeostasis and cellular integrity, offering valuable insights for breeding salt-tolerant rice varieties.

Abbreviations

ROS	Reactive oxygen species
ATAC-seq	Assay for Transposase-Accessible Chromatin sequencing
DEGs	Differentially expressed genes
SOD	Superoxide dismutase
CAT	Catalase
POD	Peroxidase
qRTPCR	Fluorescent quantitative realtime PCR
H_2O_2	Hydrogen peroxide
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06387-z.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Not applicable.

Author contributions
Q. Y. Conceptualization, Data curation, Investigation, Validation, Writing—original draft. Y. Z. Investigation, Validation. X. L. Conceptualization, Funding acquisition, Project administration, Writing—review and editing. All authors have read and agreed to the published version of 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 PRJNA1177105 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1177105). All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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