

Pan-genomic analysis of alternative polyadenylation reveals a 3'UTR remodeling regulatory layer controlling salt tolerance in rice

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ABSTRACT

Alternative polyadenylation (APA) generates mRNA isoforms with variable 3'UTR lengths, yet its genetic basis and functional significance at the population level remain unexplored in plants. Here, we present the population-level APA landscape and the APA quantitative trait loci (3'aQTL) map in rice, through profiling of over 200 rice accessions under normal and salt-stress conditions. We identified 19,938 and 22,251 APA events under normal and salt-stress conditions, respectively, and demonstrate that salt stress globally induces

3'UTR shortening via proximal polyA site selection, which can release miRNA-mediated repression on stress-response genes. We further mapped 1,634 and 1,809 *cis*-3'aQTLs under normal and salt-stress conditions, respectively, which were markedly enriched in 3'UTRs and transcription termination sites, in contrast to expression QTLs that were enriched in promoter regions. This APA genetic framework reveals a post-transcriptional regulatory layer distinct from transcriptional control. Functional validation of two 3'aQTL-associated genes, *OsMyb21* and *OsRNS3*, through CRISPR knockout and 3'UTR deletion confirmed their roles in salt tolerance via APA-mediated regulation. Our findings provide a community resource for understanding post-transcriptional regulation of stress responses and offer genetic targets for breeding salt-tolerant rice varieties.

Keywords: 3' untranslated region (3'UTR), alternative polyadenylation, pan-genome, rice, salt tolerance

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INTRODUCTION

The precise regulation of mature messenger RNA (mRNA) biogenesis is crucial for organism survival, and this regulation encompasses a series of sequential steps: RNA polymerase II (Pol II)-mediated transcription of DNA into nascent mRNA, followed by coordinated RNA processing events including 5' end capping, intron–exon splicing, and 3' end polyadenylation (Tian and Manley, 2017). Among these processes, polyadenylation represents a critical two-step enzymatic reaction involving site-specific cleavage of the nascent transcript and subsequent addition of a polyA tail, which ensures mRNA stability and nuclear export (Mitschka and Mayr, 2022; Lin and Li, 2023). Notably, alternative polyadenylation occurs in approximately 70% of eukaryotic genes through selective utilization of multiple polyA sites, predominantly within 3'UTRs (Wu et al., 2011; Derti et al., 2012; Fu et al., 2016). This mechanism generates transcript isoforms with variable 3'UTR lengths while maintaining identical protein-coding sequences (Tian and Manley, 2017; Mitschka and Mayr, 2022; Lin and Li, 2023). Since the biological significance of APA stems from the enrichment of *cis*-regulatory elements in 3'UTRs, including microRNA (miRNA) binding sites and RNA-binding protein (RBP) motifs that govern mRNA stability, translational efficiency, and subcellular localization, APA has been considered a new paradigm of post-transcriptional gene regulation (Mayr, 2017; Tian and Manley, 2017; Mitschka and Mayr, 2022). Although plant studies have established stress-responsive APA patterns under various abiotic conditions (de Lorenzo et al., 2017; Cao et al., 2019; Chakrabarti et al., 2020; Ma et al., 2022; Wang et al., 2023), the genetic and molecular mechanisms underlying APA-mediated salt stress adaptation remain poorly characterized in plants.

In mammalian and yeast systems, APA regulation is orchestrated by a conserved polyadenylation complex containing Cleavage and Polyadenylation Specificity Factor (CPSF), Cleavage Stimulation Factor (CstF), and Cleavage Factors I/II (CFI/CFII) (Mitschka and Mayr, 2022). Genetic perturbations of these factors generally induce global 3' UTR shortening and/or lengthening, causing developmental abnormalities and oncogenesis (Shi et al., 2009; Chan et al., 2014; Masamha et al., 2014; Xia et al., 2014). Plant orthologs of mammalian polyadenylation components, including CPSF30, CstF77, FIP1, and FLOWERING LOCUS Y (FY), have been functionally characterized through mutant analyses. Many studies have unveiled that mutations of these factors give rise to striking phenotypic outcomes, highlighting the critical role of APA in plant development. For example, FY, the Arabidopsis homolog of the essential yeast RNA 3' processing factor Pfs2p, can interact with FCA to control floral transition through the use of different polyadenylation sites within the *FCA* pre-mRNA for the downregulation of the floral repressor FLC, and *fy* loss-of-function mutants exhibit defects in 3' end processing and late flowering in Arabidopsis (Simpson et al., 2003). CstF77 and CstF64 are two homologs

of the CstF RNA 3' processing complex, and loss-of-function of *CstF77* in the *cstf77-2* mutant causes female gametophytic lethality, while mutation of *CstF64* in the *cstf64-2* mutant leads to reduced organ size, pale leaves, and sterility (Liu et al., 2010). Interestingly, CstF77 and CstF64 function independently to promote 3' processing at the proximal site in the *FLC* antisense transcript to trigger chromatin silencing of the *FLC* sense strand, regulating the onset of flowering (Liu et al., 2010). In Arabidopsis, another well-studied polyadenylation factor is CPSF30, one member of the CPSF family, serving as the central component of the plant polyadenylation machinery. Recent studies verified that the longer isoform CPSF30-L mainly recognizes m⁶A-modified far-upstream elements to control polyadenylation site choice, and the defective mutants of CPSF30-L lengthen the 3'UTR, accelerating mRNA degradation and resulting in dysregulated nitrate metabolism, ABA hypersensitivity, and late flowering in Arabidopsis (Hou et al., 2021; Song et al., 2021). Collectively, the polyadenylation factors play critical roles in ensuring the accuracy of polyadenylation site selection, affecting plant growth and development. However, the molecular mechanism underlying how polyadenylation factors and APA respond to environmental cues is rarely understood.

Traditional approaches for identifying polyA sites and quantifying APA usage rely on specialized 3' end profiling methods such as polyA tag sequencing (PAT-seq) (Sun et al., 2012; Harrison et al., 2015; Fu et al., 2016), which are constrained by small sample sizes and specialized library preparation requirements. By contrast, RNA-seq has become a routine tool for transcriptome analysis and can simultaneously yield multiple molecular phenotypes from the same dataset, including gene expression levels and alternative splicing patterns (Wei et al., 2024; Zhang et al., 2024). Importantly, bioinformatics algorithms such as DaPars enable genome-wide APA identification from conventional RNA-seq data by analyzing read coverage asymmetries across the 3'UTR (Xia et al., 2014), facilitating large-scale population studies of this regulatory mechanism that were previously impractical. In plants, genome-wide association studies (GWAS) have identified thousands of trait-associated variants, yet the majority reside in non-coding regions whose functional characterization remains challenging. Molecular quantitative trait locus (QTL) analyses, including expression QTLs (eQTLs) and splicing QTLs, have partially addressed this gap by linking non-coding variants to transcript-level phenotypes, revealing how *cis*-regulatory variation influences gene expression and alternative splicing at the population scale (Wei et al., 2024). Since APA might provide a new paradigm of post-transcriptional gene regulation, identifying the APA landscape at the population level and mining alternative polyadenylation quantitative trait loci (3'aQTLs) are not only crucial for deciphering the genetic basis of APA in regulating gene function and plant stress responses, but also provide important genetic resources for breeding stress-resistant crops in the future.

Since plants are sessile organisms anchored in place throughout their growth cycles, they frequently encounter abiotic stresses such as drought, salinity, and extreme temperatures during development. In plants, salt stress severely constrains growth and development through mechanisms like osmotic imbalance and ion toxicity, which generally disrupt plant growth and reduce crop yields (Yang and Guo, 2018; Zhang et al., 2022). Rice, serving as a staple crop for half the global population, holds significant application value for utilizing saline soils worldwide to address the global food crisis by improving its salt tolerance. Salt tolerance of rice is a complex quantitative trait controlled by multiple genes. So far, several key genes such as *OsHKT1;5 (SKC1)* (Ren et al., 2005), *DST* (Huang et al., 2009), *OsRR22* (Takagi et al., 2015), and *RST1* (Deng et al., 2022) have been identified as being involved in rice salt tolerance through molecular biology approaches. Recently, the integration of rice pan-genome and multi-omics technologies has ushered in a new phase for mining salt-tolerant genes. In our previous study, integrated GWAS and eQTL analyses identified the *STG5* gene, demonstrating its role in significantly enhancing salt tolerance by coordinating the regulation of multiple members of the *HKT* family (Wei et al., 2024). The favorable haplotype of *STG5* boosts yield by 28.6% under salt stress conditions, substantially improving rice salt tolerance (Wei et al., 2024). However, although many salt-tolerance genes have been functionally characterized, whether and how APA-mediated RNA regulation contributes to rice salt tolerance remains largely underexplored, and population-level APA resources that could accelerate the mining of APA-regulated salt-tolerance genes are still lacking. Since the leaf is the primary organ integrating long-distance salt-stress signaling and the major site of the photosynthesis- and yield-related processes most affected by salinity, we prioritized investigating the important role of APA regulation in the leaf in the response to salt stress.

In this study, we constructed and characterized the population-level APA landscape in rice leaves under normal and salt conditions by using DaPars v.2 (Li et al., 2021) to analyze the transcriptome data from our previous rice pan-genomic population (Shang et al., 2022). In total, we discovered 18,475 genes with dynamic APA events under salt conditions and demonstrated that salt treatment significantly promotes the usage of proximal polyA sites, thereby leading to 3'UTR shortening, resulting in the loss of miRNA binding sites and upregulation of gene expression, possibly due to the upregulation of *OsCPSF30* under salt treatment conditions. Moreover, we conducted 3'aQTL analyses and identified 1,437 genes within *cis*-acting 3'aQTLs in leaves under normal conditions, and 1,599 genes under salt conditions. In contrast to eQTLs, which were significantly enriched in promoter regions, these 3'aQTLs were significantly enriched in 3'UTRs and transcription termination sites. Interestingly, we identified and demonstrated that two 3'aQTL genes, *OsMyb21* and *OsRNS3*, are involved in salt tolerance through dynamic APA in rice. Our findings not only provide insight into the genetic basis of APA in regulating gene

function and plant stress responses, but also provide important genetic resources for breeding stress-resistant crops in the future.

RESULTS

Population-level APA landscape of rice leaves under normal and salt conditions

To characterize the genetic basis of APA and its contribution to salt-stress responses at the population level, we analyzed leaf transcriptomes from our previously described rice super pan-genome population (Shang et al., 2022; Wei et al., 2024), comprising 250 accessions under normal conditions and 219 accessions under salt stress. This population encompasses diverse genetic backgrounds, including Asian cultivated rice (*O. sativa indica*, *Osi*; *O. sativa japonica*, *Osj*), *O. sativa aus* (*aus*), wild rice (*O. rufipogon*, *Or*), African cultivated rice (*O. glaberrima*, *Og*), and African wild rice (*O. barthii*, *Ob*), providing broad representation of rice genetic diversity. The 250 accessions under normal conditions contain 139 *Osi*, 63 *Osj*, 27 *Or*, 10 *Og*, 7 *Ob*, and 4 *aus*, and all 219 salt-treated accessions (139 *Osi*, 63 *Osj*, 10 *Og*, 3 *Ob*, and 4 *aus*) are fully included within these 250 accessions (Figure S1A). Using the DaPars v.2 algorithm (Li et al., 2021), which infers alternative polyadenylation from standard RNA-seq data by modeling read coverage changes near 3'UTRs, we identified proximal and distal polyA sites and quantified APA events using the Percentage of Distal polyA site Usage Index (PDUI). PDUI values range from 0 (exclusive proximal site usage, short 3'UTR) to 1 (exclusive distal site usage, long 3'UTR), enabling quantitative comparison of APA status across accessions and conditions (Figure 1A).

This analysis yielded 19,938 APA events across 16,338 genes under normal conditions and 22,251 APA events across 18,475 genes under salt stress at population scale, with 19,223 events shared between conditions in 219 paired cultivated rice accessions (Figure 1B; Tables S1–S3). The high overlap between conditions indicates that the majority of APA events are constitutively present. The larger number of salt-specific APA events suggests that the stress condition might induce genome-wide APA reprogramming. Genomic distribution analysis revealed that APA events were non-randomly distributed across chromosomes, with substantial enrichment toward chromosome ends and depletion in centromeric and pericentromeric regions (Figure 1C). Per-individual PDUI scoring revealed significantly more APA events under salt stress compared to normal conditions (Figure 1D), indicating that salt treatment promotes APA occurrence in rice. However, analysis of APA-induced 3'UTR length distribution changes showed no obvious distribution difference between conditions; most lengths remained under 1 kb (Figure 1E). Principal component analysis (PCA) of the 19,223 overlapping APA events revealed clear separation between salt-stress and normal-condition samples (Figure 1F). Heatmap analysis further confirmed these differences (Figure S1B), suggesting that salt stress

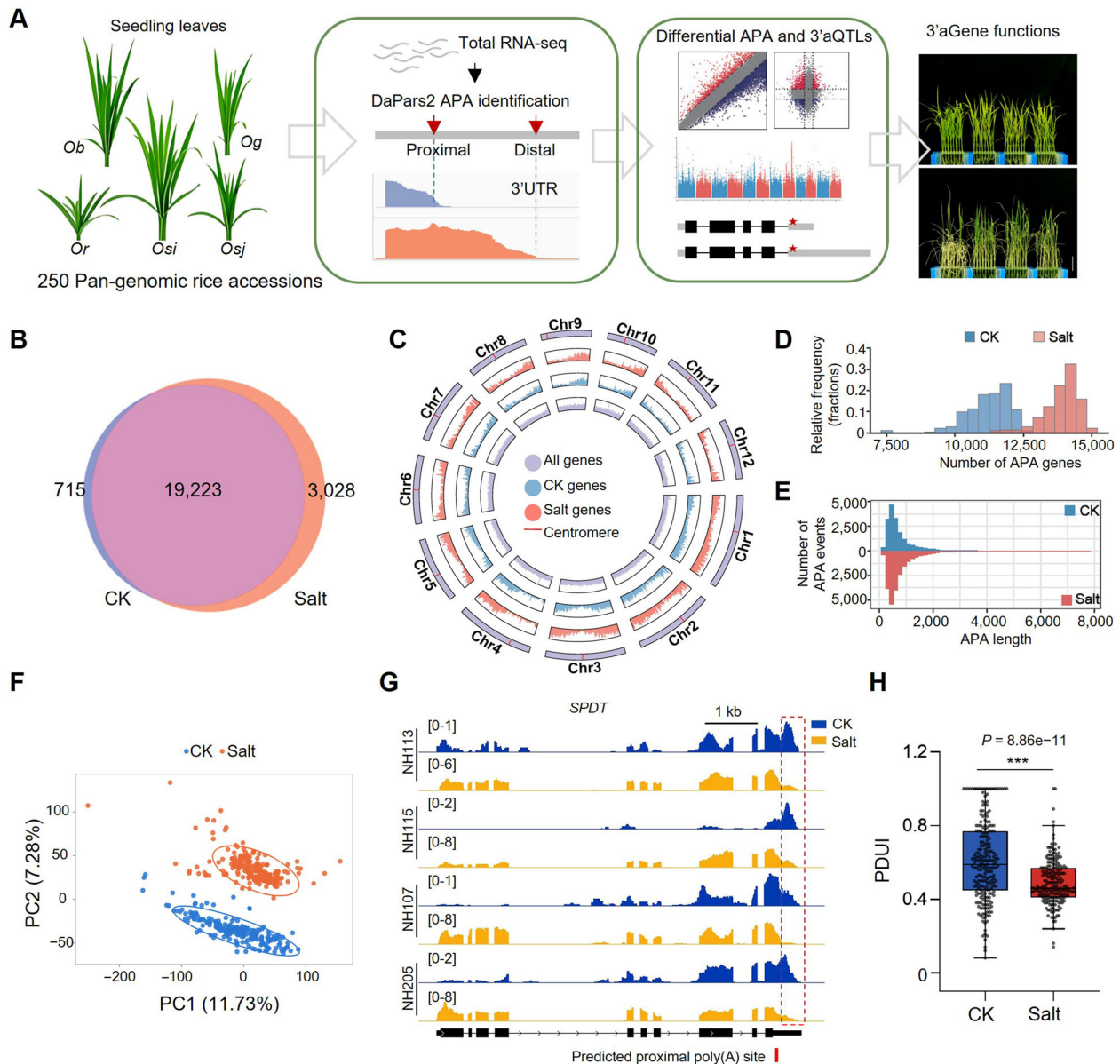


Figure 1. Identification of dynamic 3'UTR APA events in rice population under normal and salt conditions

(A) Workflow of the entire study. **(B)** Number of APA events in the rice population under normal (CK) and salt conditions. **(C)** Distribution of APA events across chromosomes. **(D)** Frequency distribution of APA in each individual plant. **(E)** Length distribution of APA events in each rice population. **(F)** Principal component analysis (PCA) of Percentage of Distal polyA site Usage Index (PDUI) values for overlapping APA events across 219 rice accessions under control and salt-stress conditions. Ellipses denote 95% confidence regions for each group. **(G)** IGV plot showing dynamic 3'UTR APA of the *SPDT* gene in rice under normal and salt conditions. **(H)** Quantification and differential comparison of APA for *SPDT* in the rice population under normal and salt conditions. The box plot shows the PDI distribution, with each dot representing an individual plant. The center line indicates the median value, the box indicates the interquartile range (IQR; 25th–75th percentile), and the whiskers extend to 1.5× the IQR. Statistical significance was determined using Student's *t*-test with ****P* < 0.001.

induces coordinated APA reprogramming at the population level. As a representative example, the *SULTR*-like *Phosphorus Distribution Transporter* (*SPDT*) gene, which was selected for its clear APA dynamics and previous characterization in rice (Fang et al., 2025), exhibited a substantially shortened 3'UTR under salt stress, consistent with a significant decrease in its PDI score (Figure 1G, H). IGV visualization confirmed the shift from distal to proximal polyA site usage under salt treatment

(Figure 1G). Collectively, these data establish the population-level APA atlas in rice and demonstrate that salt stress induces measurable and coordinated changes in APA usage across genetically diverse rice accessions.

3'UTR changes among rice subspecies

To compare differences in 3'UTR changes across rice subspecies, we performed differential APA analysis. For each

APA gene, we calculated the difference in average PDUI values between groups ($\Delta\text{PDUI} = \text{MeanPDUI}_{G1} - \text{MeanPDUI}_{G2}$) and determined significance using a permutation test ($P < 0.01$, $|\Delta\text{PDUI}| > 0.1$). Our normal-condition rice population contained rice subspecies, including Asian wild rice, cultivated *japonica* and *indica*, African wild rice, and African cultivated rice, enabling comparisons related to domestication and differentiation. Generally, PCA analysis of PDUI values from 250 rice accessions revealed that different subspecies were not clearly distinguished; nevertheless, the heatmap indicated that partial APA differences still existed among the subspecies (Figure S2A, B). The differential APA analysis revealed that, relative to wild rice, both *indica* and *japonica* predominantly exhibited significant 3'UTR lengthening events (*Osi*: $n = 1,024$; *Osj*: $n = 964$) over shortening events (*Osi*: $n = 508$; *Osj*: $n = 227$) (Figure S2C, D). For the cultivated rice subgroups, *indica* rice showed more shortening than elongation compared to *japonica* (shortening: $n = 801$; elongation: $n = 372$) (Figure S2E). Unfortunately, comparisons between African wild and African cultivated rice yielded many genes with $|\Delta\text{PDUI}| > 0.1$ but $P > 0.01$ (Figure S2F). Taken together, these results reveal divergent 3'UTR length dynamics between Asian rice subspecies, with cultivated rice showing preferential lengthening relative to wild types while *indica* exhibits increased shortening compared to *japonica*.

Salt treatment significantly promotes 3'UTR shortening in rice leaves

Next, to investigate whether environmental stress stimuli affect 3'UTR length changes by regulating APA, we performed differential APA analysis in the paired 219 rice accessions under normal and salt conditions. Our analysis revealed significant salt-induced APA alterations in 3,975 gene transcripts ($|\Delta\text{PDUI}| > 0.1$, $P < 0.01$): 2,957 gene transcripts exhibited decreased PDUI values (indicating preferential proximal polyA site usage), while 1,018 showed increased PDUI values (Figure 2A; Table S4). The predominance of genes with 3'UTRs shortening (2.9-fold more than those with 3'UTRs lengthening) indicates salt-triggered selection for proximal polyA site usage. As a result, sequence analysis showed that lost 3'UTR fragments were predominantly 200–500 bp (51.37%), while newly gained 3'UTR fragments clustered within 100–400 bp (62.57%), suggesting large-scale APA-mediated 3'UTR remodeling in rice leaves upon salt stress (Figure 2B).

Given the enrichment of *cis*-regulatory elements in 3'UTRs, such as miRNA and RNA-binding protein (RBP) motifs, we examined the functional consequences of this APA-mediated 3'UTR remodeling with salt treatment. miRNA target prediction revealed that 41.97% (1,241/2,957) of 3'UTR-shortened gene transcripts lost at least one miRNA-binding site, whereas 30.43% of transcripts with extended 3'UTRs gained at least one miRNA-binding site under salt treatment (Figure 2C; Table S5). Since miRNA-mediated mRNA cleavage is a key post-transcriptional regulatory

mechanism, we speculated that APA-mediated 3'UTR shortening and lengthening may play important roles in gene expression regulation. To test this, we analyzed the correlation between APA (PDUI) and expression levels for each gene under normal and salt conditions. Although most correlation (*R*) values clustered around zero, we observed 2,225 genes with positive correlations and 1,863 with negative correlations under normal conditions (Figure S3A, B). Under salt stress conditions, 127 genes showed positive correlations and 178 showed negative correlations (Figure S3C). These results indicate that the relationship between APA and gene expression was gene-specific in our dataset. Therefore, we focused on analyzing expression changes in genes exhibiting significant APA alterations. Notably, differential expression analysis identified 1,439 APA-affected genes with significant transcriptional changes under salt conditions compared to normal conditions, and 606 of the 3'UTR-shortened genes were markedly upregulated (Figure 2D, E; Table S6), suggesting that APA-mediated 3'UTR shortening predominantly facilitates adaptive transcriptional reprogramming during salt stress responses.

To further investigate the changes in salt stress response and expression levels mediated by APA at the individual gene level, we conducted a case study using the salt-tolerant gene *OsDREB2A* (Mallikarjuna et al., 2011). We found that under salt treatment, *OsDREB2A* exhibited a significant shift toward proximal polyA site usage, a marked decrease in PDUI, and a significant increase in gene expression compared to the normal condition (Figure 2F–H). Within the shortened 3'UTR region, we predicted the existence of a binding site for miR2100-5p (Figure 2F). To investigate the potential functions of APA in *OsDREB2A*, we generated dual-luciferase (LUC) reporters containing either the full-length or truncated *OsDREB2A* 3'UTR and co-expressed them with the miR2100-5p precursor in tobacco cells (Figure 2I). Compared to controls, transcripts with the full-length 3'UTR exhibited significantly reduced LUC mRNA levels, diminished luciferase activity, and impaired protein translation efficiency (Figure 2J–L). This result demonstrated that the 3'UTR sequence of *OsDREB2A* containing the miR2100-5p binding site negatively regulates gene expression and protein abundance, while salt-induced APA-mediated 3'UTR shortening releases this miRNA-dependent suppression, likely enhancing gene expression at the post-transcriptional level. Collectively, these findings demonstrate that salt stress can orchestrate gene regulatory networks through APA-mediated 3'UTR remodeling, dynamically rewiring post-transcriptional control via *cis*-element reorganization.

The polyadenylation factor OsCPSF30 responds to salt stress and regulates proximal and distal polyA site selection

Eukaryotic mRNA polyadenylation is orchestrated by conserved core factors, including CPSF, CstF, and CFI/CFII complexes. To elucidate the molecular mechanisms of salt-regulated APA dynamics in rice, we analyzed expression

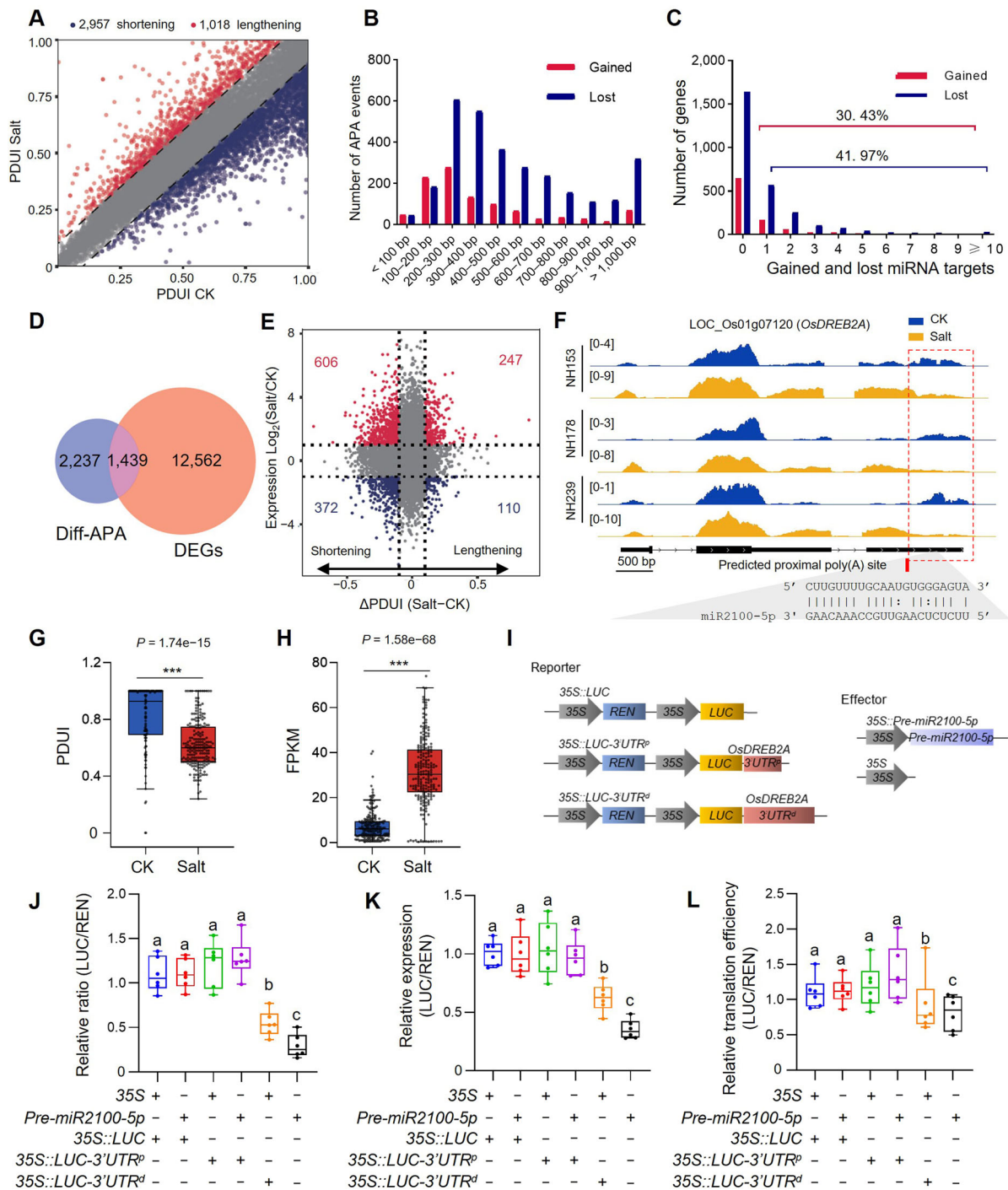


Figure 2. Differential APA analysis under normal and salt conditions

(A) Identification of differential APA events in the rice population under control and salt conditions. Statistical analysis used a permutation test ($n = 10,000$; significance threshold: $|\Delta\text{PDI}| > 0.1$, $P < 0.01$). (B) Length distribution of gained or lost 3'UTR sequences in APA events. (C) Frequency of miRNA target site gain and loss in 3'UTR-shortening APA events. (D) Venn analysis between differential APA genes (left) and differentially expressed genes (right, $P < 0.01$, absolute fold change > 2) under salt stress compared with normal conditions. (E) Correlation distribution between the PDI difference and the expression fold change in APA genes under salt stress. (F) IGV visualization of *OsDREB2A* APA dynamics in different rice cultivars under control and salt conditions. The red box highlights the truncated 3'UTR region; the red rectangle marks the proximal polyA site; and the gray triangle indicates the miR2100-5p binding motif. (G, H) Comparative analysis of *OsDREB2A* PDI values (G) and FPKM expression abundance (H) under control and salt conditions. Statistical significance was determined using Student's *t*-test with $***P < 0.001$. (I) Schematic of the dual-luciferase (LUC) reporter system with full-length (3'UTR^d) or truncated (3'UTR^p) *OsDREB2A* 3'UTR. (J–L) Quantification of LUC luciferase activity (J), transcript abundance (K), and translational efficiency (L). Statistical significance was determined by one-way ANOVA followed by a multiple comparison test; different letters denote significant differences with $P < 0.05$.

patterns of 25 polyadenylation factor homologs between rice populations under normal and salt conditions. Differential expression revealed significant salt-induced upregulation in most factors, including *OsCPSF30* (LOC_Os01g32750), the rice ortholog of Arabidopsis *CPSF30* (Figure 3A). Given that Arabidopsis *CPSF30* directs polyA site selection via m⁶A recognition, with deficiency causing 3'UTR processing defects, reduced mRNA stability, and pleiotropic phenotypes (Hou et al., 2021; Song et al., 2021), and although direct evidence for such an m⁶A-dependent mechanism in rice is currently lacking, we characterized *OsCPSF30*'s functional conservation. qPCR analysis showed its constitutive expression peaking in mature leaves and nodes (Figure 3B). Using CRISPR/Cas9-mediated gene editing, we generated two *OsCPSF30* knockout lines (*oscpsf30#1/2*) and observed that both *oscpsf30* mutants exhibited severe developmental

defects, including reduced height, narrow leaves, and impaired panicle fertility (Figure 3C). To directly determine whether *OsCPSF30* regulates APA processing in rice, we performed leaf transcriptome sequencing of wild-type and *oscpsf30#1* mutant plants. Transcriptome-wide APA analysis identified 2,183 genes with significantly increased PDI (preferential distal polyA site usage/3'UTR lengthening) versus 531 with decreased PDI in *oscpsf30* mutant compared to the wild-type (Figure 3D; Table S7). This result suggests that *OsCPSF30* plays a positive regulatory role in APA processing and in promoting the usage of proximal polyA sites. Notably, PDI-upregulated genes showed higher mRNA abundance (335 vs. 202 genes) (Figure 3E; Table S8), suggesting that *OsCPSF30* could modulate transcript stability through polyA site selection. These results establish *OsCPSF30* as a critical regulator of APA dynamics and

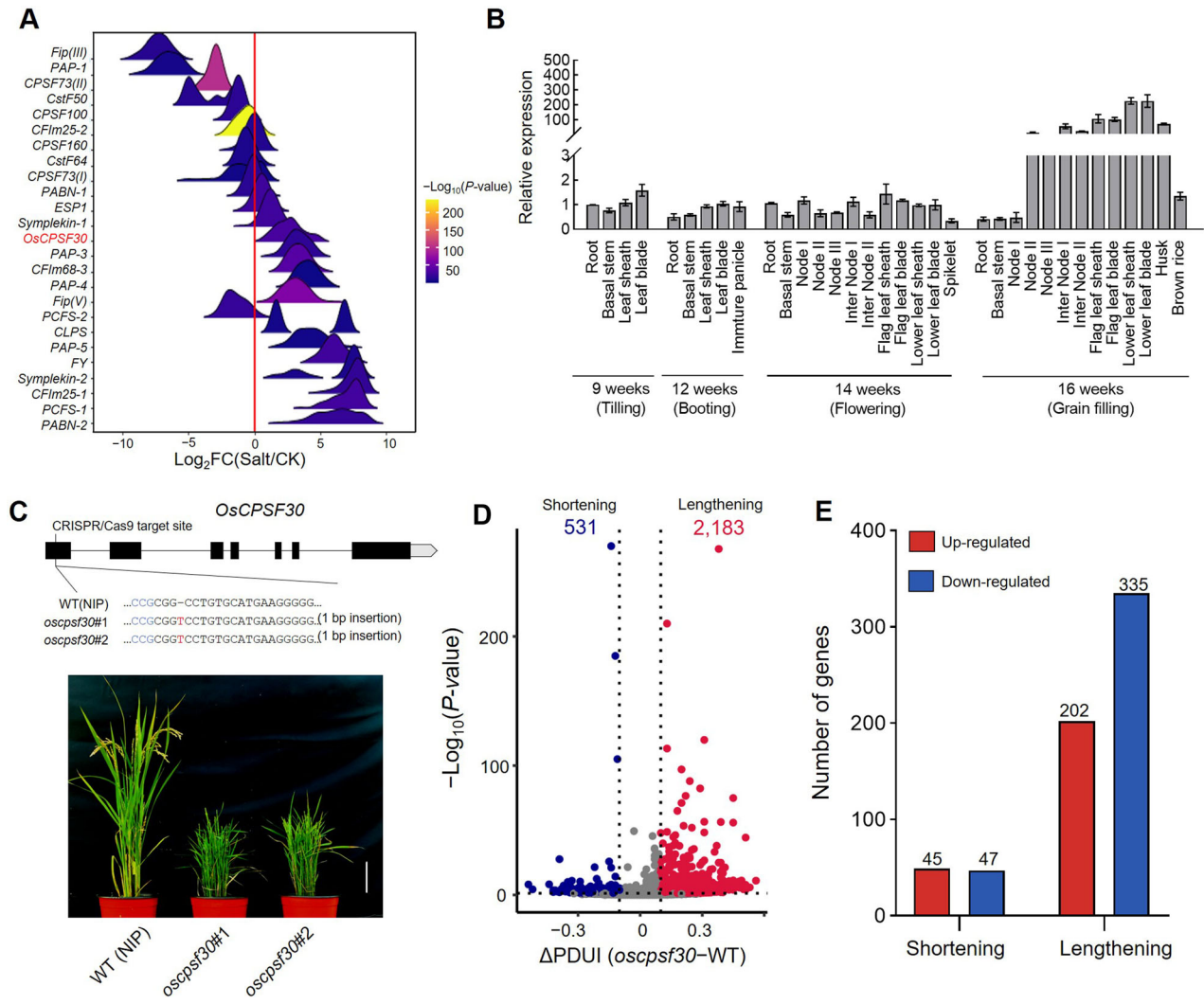


Figure 3. *OsCPSF30* mediates proximal and distal polyA site selection

(A) Fold change distribution of 25 polyA processing factor homologs under salt stress across the rice population. (B) Tissue-specific expression of *OsCPSF30* determined by qPCR analysis. (C) Genotypic validation and mature-stage phenotypic characterization of CRISPR/Cas9-derived *oscpsf30* mutants. Scale bar, 5 cm. (D) Differential APA landscape in *oscpsf30* mutant leaves. Significance threshold: $|\Delta PDI| > 0.1$, $P < 0.01$. (E) Number of differentially expressed APA-dysregulated genes in *oscpsf30* mutants.

development. Collectively, salt-induced upregulation of polyadenylation factors like *OsCPSF30* may promote proximal polyA site selection to shorten 3'UTRs, potentially reconfiguring mRNA metabolism to enhance stress adaptation.

Genome-wide identification and molecular characterization of Alternative polyadenylation quantitative trait loci (3'aQTLs) in rice under normal and salt conditions

To systematically investigate the genetic regulation basis of APA, we performed GWAS analysis using PDUI values as molecular traits, leveraging our previous rice pan-genome SNP dataset. Under normal and salt-stress conditions, we identified 1,634 and 1,809 *cis*-3'aQTLs (variants within ± 10 kb of target genes) (Table S9), respectively, alongside numerous *trans*-3'aQTLs (Figure 4A). Given the potential downstream importance of *trans*-acting variants, we first characterized the *trans*-3'aQTLs in this population. We detected 163,576 and 163,857 *trans*-3'aQTL loci, respectively, with 127,145 loci shared and similar chromosomal distribution between conditions (Figure S4A, B; Tables S10, S11). *Trans*-regulatory hotspots analysis identified the leading hotspot region, Chr8: 22,898,850–23,098,843, which regulated 278 and 182 target genes, representing a promising candidate locus for dissecting *trans*-acting APA regulators (Figure S4C, D). However, given the direct regulatory relevance of *cis*-acting loci, we prioritized *cis*-3'aQTLs (hereafter referred to as 3'aQTLs) for functional validation in our study, and the *trans*-3'aQTL map serves as a community resource for studying *trans*-regulation of APA in the future. Moreover, parallel *cis*-expression quantitative trait loci (eQTL) mapping revealed 9,202 (normal) and 11,006 (salt stress) loci (Table S12). To evaluate the overlap between target genes regulated by 3'aQTLs (3'aGenes) and eQTLs (eGenes) under normal and salt-stress conditions, a four-way Venn analysis was performed. Under normal conditions, 928 genes overlapped between 3'aGenes and eGenes, while under salt-stress treatment, 1,141 genes overlapped between 3'aGenes and eGenes. A core set of 556 genes was shared across all four groups, representing QTLs detected for both traits irrespective of growth conditions (Figure 4B). Comparative analysis also showed substantial overlap between 3'aQTLs and eQTLs but distinct genomic distributions, featuring both shared colocalization hotspots and unique regulatory regions (Figure 4C), indicating potential functional convergence and divergence in post-transcriptional and transcriptional regulation.

To characterize the molecular features of 3'aQTLs, we compared 3'aGenes and eGenes. The results revealed distinct structural patterns: 3'aGenes exhibited significantly longer 3'UTRs, shorter 5'UTRs, and reduced CDS lengths compared to eGenes, with this structural bias enhanced under salt stress (Figure 4D). Moreover, using SnpEff v5.0 to annotate 3'aQTLs and eQTLs under both normal and salt conditions, we found that eQTLs were predominantly localized to promoter regions (upstream gene regions),

whereas 3'aQTLs were markedly enriched in downstream gene regions, particularly within 3'UTRs (Figure 4E). These results aligned with metagene analysis showing that eQTLs were enriched at transcription start sites (TSS), while 3'aQTLs were enriched at transcription end sites (TES) (Figure 4F), suggesting that APA-mediated targeting of 3'UTRs represents a distinct post-transcriptional regulatory mechanism downstream of genes, differing from transcriptional initiation regulation. To characterize the functional difference between 3'aGenes and eGenes under salt conditions, we conducted Gene Ontology (GO) enrichment analysis. This revealed significant overrepresentation of 3'aGenes in amide metabolic process, translation, and small molecule metabolic process pathways, whereas GO terms related to response to stimulus, protein phosphorylation, and programmed cell death were significantly enriched for eGenes (Figure 4G), suggesting that APA, distinct from eQTL in expression regulation, could modulate these core biological processes to influence stress responses. To elucidate the genetic architecture of APA variation regulated by 3'aQTLs, we estimated heritability under control and salt conditions. 3'aQTLs explained 24.90% ($h^2 = 0.2490 \pm 0.19$) of APA variation on average under control conditions versus 15.88% ($h^2 = 0.1588 \pm 0.19$) under salt stress (Figure 4H). Notably, > 50% of APA variation was attributable to 3'aQTLs in 9.75% of APA genes under control conditions compared to 7.92% under salt stress. These results are consistent with environmentally dependent genetic control of APA variation by 3'aQTLs, with salt stress reducing overall heritability while retaining a subset of high-impact loci governing post-transcriptional regulation. Collectively, our findings provide the first systematic characterization of APA quantitative trait loci in plants, establishing 3'UTRs as novel regulatory hubs and creating a genetic framework for identifying salt stress response regulators.

OsMyb21 co-located by 3'aQTL and eQTL positively regulates salt tolerance in rice

To identify key APA-regulated genes functionally important for salt-stress response, we integrated differential expression, differential APA, and 3'aQTL/eQTL colocalization analyses across control and salt-stress conditions. This identified 36 overlapping candidate genes (Figure S5; Tables S6, S9), among which we prioritized the *Myb* family transcription factor gene LOC_Os11g45740 (*OsMyb21*), previously reported to negatively regulate bacterial blight resistance in rice (Yang et al., 2021). Notably, under both normal and salt conditions, *OsMyb21* was associated with significant and co-localized 3'aQTL ($P = 2.38 \times 10^{-31}$ salt stress) and eQTL ($P = 9.13 \times 10^{-15}$ salt stress) signals at its genomic locus (Figure 5A, B). Consistent with these genetic associations, we found that salt stress significantly decreased PDUI values while upregulating expression across rice accessions (Figure 5C, D), indicating coordinated APA-transcriptional regulation in response to salinity. Intriguingly, haplotype analysis of the lead SNP (snp11_27670323) for

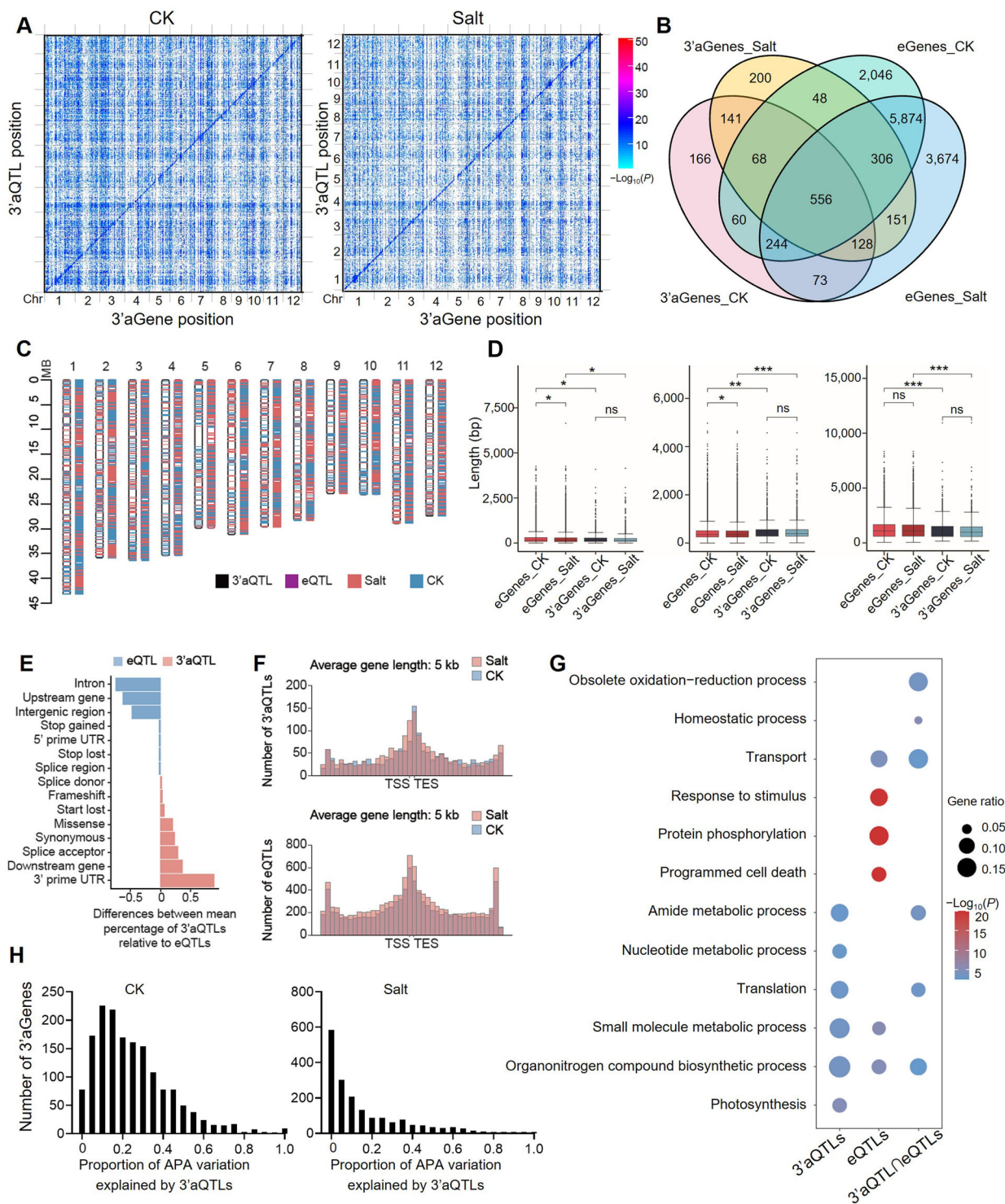


Figure 4. Mapping and molecular characteristics of 3'UTR alternative polyadenylation quantitative trait loci (3'aQTLs)

(A) Identification of *cis*- and *trans*-3'aQTLs in the rice population grown under normal and salt conditions. (B) Overlap analysis between target genes regulated by *cis*-3'aQTLs and *cis*-eQTLs under normal and salt conditions. (C) Genome-wide distribution patterns of 3'aQTLs and eQTLs. (D) Sequence length comparisons of 5'UTR, 3'UTR, and coding sequence (CDS) between 3'aGenes and eGenes under normal and salt conditions. Statistical significance was determined by one-way ANOVA followed by a multiple comparison test with *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant. (E) Differences in mean percentages between 3'aQTLs and eQTLs for significant variant annotations. Red bars indicate annotations enriched for 3'aQTLs, whereas blue bars represent annotations enriched for eQTLs, both with an $FDR \leq 0.01$. (F) Relative distance distribution of 3'aQTLs (upper) and eQTLs (lower) to their associated genes. TES, transcription end site. TSS, transcription start site. (G) Functional enrichment of 3'aGenes under salt conditions. (H) Heritability estimates (h^2) of 3'aGenes under normal (left) and salt (right) conditions.

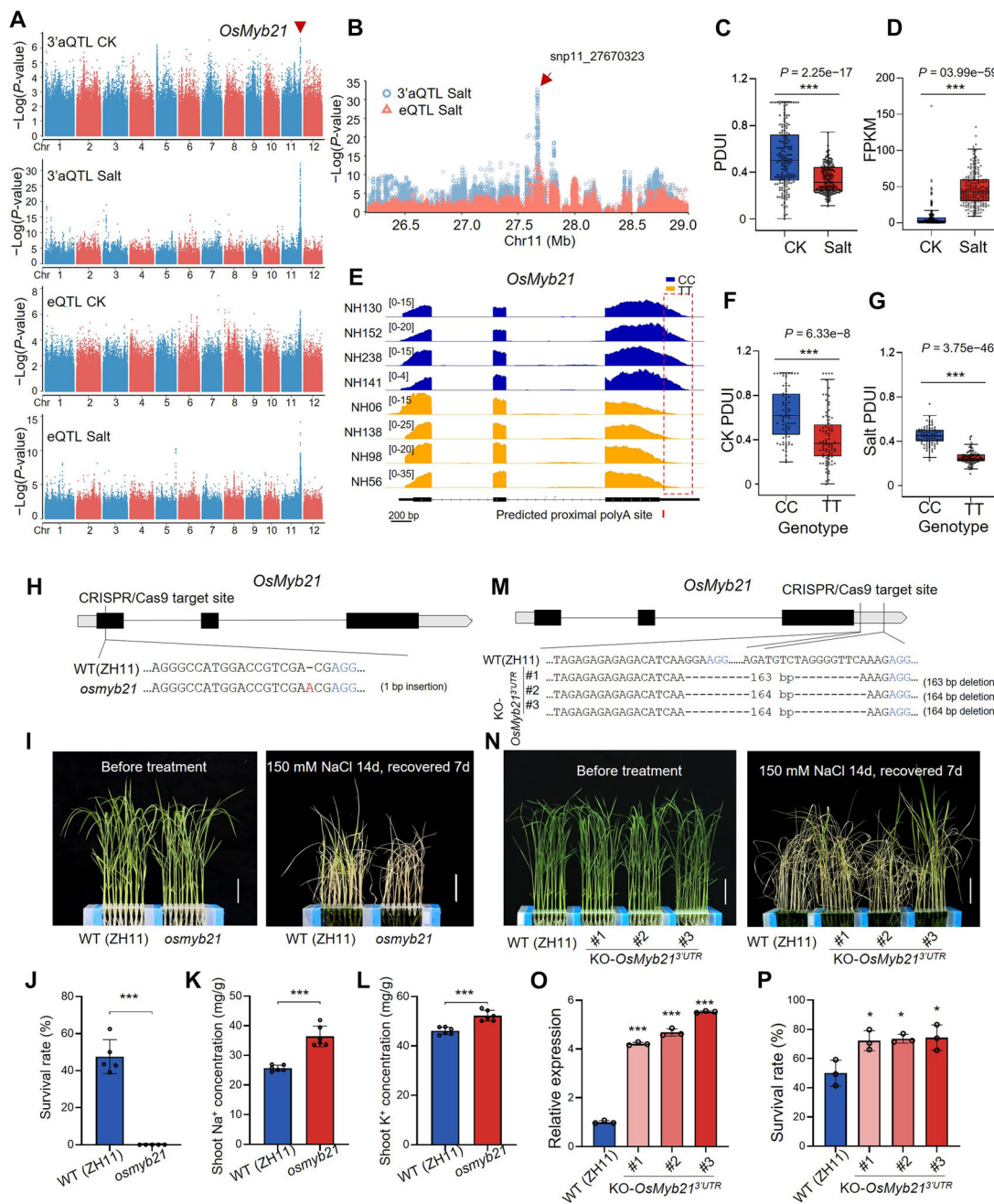


Figure 5. Functional analysis of the 3'aQTL/eQTL gene *OsMyb21*

(A) Manhattan plots showing *cis*-3' aQTL and eQTL signals associated with APA and expression of *OsMyb21* under normal (CK) and salt (Salt) conditions. **(B)** Co-localization signals of 3' aQTL and eQTL for *OsMyb21* under salt conditions. The red arrow indicates the lead SNP for 3' aQTL. **(C, D)** Comparison of *OsMyb21* PDU values **(C)** and FPKM **(D)** in the rice population under normal and salt conditions. **(E)** IGV plot showing genotype-specific APA differences in proximal and distal polyA site usage for *OsMyb21* under salt conditions. The red rectangle indicates the predicted proximal polyA site. CC and TT represent the two genotypes of the lead SNP. **(F, G)** Comparison of APA PDU values between the two genotypes under normal **(F)** and salt **(G)** conditions. **(H)** Schematic diagram of CRISPR/Cas9 target sites in the *OsMyb21* gene. The black boxes indicate exons. The mutation sequence indicated in red is shown below the gene model, and the protospacer adjacent motif (PAM) is shown in blue. **(I)** Phenotypes of wild-type (WT) ZH11 and *osmyb21* mutant plants grown before (left) and after (right) salt treatment. Scale bar, 5 cm. **(J–L)** Survival rate **(J)** ($n = 5$ biological replicates, 24 individual plants per biological replicate), shoot Na⁺ concentration **(K)** ($n = 6$), and K⁺ concentration **(L)** ($n = 6$) of WT and *osmyb21* plants after salt treatment. **(M)** Schematic diagram showing a large sequence deletion in the 3' UTR of *OsMyb21* (KO-*OsMyb21*^{3'UTR}) generated using CRISPR/Cas9 gene editing. **(N)** Phenotypes of WT and KO-*OsMyb21*^{3'UTR} plants grown before (left) and after (right) salt treatment. Scale bar, 5 cm. **(O)** Relative expression of *OsMyb21* in leaves of WT and KO-*OsMyb21*^{3'UTR} plants ($n = 3$). *UBQ* (LOC_Os03g13170) was used as an internal reference. **(P)** Survival rate of WT and KO-*OsMyb21*^{3'UTR} plants ($n = 3$ biological replicates, 24 individual plants per biological replicate). The box plot shows the distribution of values, with each dot representing an individual plant. The center line indicates the median value, the box indicates the interquartile range (IQR; 25th–75th percentiles), and the whiskers extend to 1.5× the IQR. The data in the bar chart are presented as means ± SD. *** $P < 0.001$; * $P < 0.05$; ns, not significant, using Student's *t*-test.

3'aQTL of *OsMyb21* revealed that rice accessions carrying the CC haplotype preferentially utilized distal polyA sites, whereas TT haplotype accessions favored proximal polyA usage, and this haplotype-dependent divergence was further amplified under salt stress (Figure 5F, G).

To investigate the biological function of *OsMyb21* in rice salt-stress response, we generated transgenic knockout *osmyb21* plants using CRISPR/Cas9 gene editing (Figure 5H). Phenotypic analysis at the seedling stage revealed no difference between *osmyb21* mutants and wild-type controls under normal conditions (Figure 5I). However, under salt stress, *osmyb21* mutants exhibited salt-sensitive phenotypes with significantly reduced survival rates (Figure 5I, J). Quantitative growth phenotyping analyses demonstrated that *osmyb21* mutants exhibited pleiotropic defects under salt stress. Specifically, shoot fresh weight (SFW) and root fresh weight (RFW) were significantly reduced by 46.03% and 39.06%, respectively, compared to wild-type. Similarly, shoot dry weight (SDW) and root dry weight (RDW) decreased by 22.03% and 46.32% in *osmyb21* mutants (Figure S6A–D). Ion concentration analysis further demonstrated that *osmyb21* shoots accumulated 41.58% higher Na^+ and 13.28% higher K^+ concentrations under salt stress, leading to a marked elevation in the shoot Na^+/K^+ ratio (Figures 5K, L, S6E). In roots, *osmyb21* mutants showed 40.55% lower Na^+ and 51.74% diminished K^+ concentrations compared to WT; however, the root Na^+/K^+ ratio remained significantly higher in mutants (Figure S6F–H). These results indicate that *osmyb21* loss-of-function dysregulates ion homeostasis by promoting excessive Na^+ and K^+ uptake during salt stress, ultimately driving salt-sensitive phenotypes. A field test at the maturity stage revealed that *osmyb21* mutants maintained comparable plant height and panicle grain number compared to wild-type, but significantly reduced grain length, grain width, and 1,000-grain weight while increasing tiller number, ultimately resulting in no significant difference in yield per plant (Figure S7). These findings suggest that *OsMyb21* also influences key agronomic traits. To further investigate the role of APA in *OsMyb21* salt-stress responses, we generated transgenic lines with large-fragment knockout of the *Osmyb21* 3'UTR (KO-*OsMyb21*^{3'UTR}) via CRISPR/Cas9 editing (Figure 5M). Sanger sequencing confirmed 163 bp (line#1) and 164 bp (lines#2 and #3) deletions in the *Osmyb21* 3'UTR of these lines, which were utilized to mimic proximal polyA site usage. Interestingly, qPCR analysis revealed significantly elevated *OsMyb21* expression in knockout lines, while phenotypic assessment demonstrated that all 3'UTR-knockout lines exhibited enhanced salt tolerance with significantly increased survival rates under salt stress (Figure 5N–P). Collectively, these results establish that APA-mediated regulation through the *OsMyb21* 3'UTR functions as a molecular switch that fine-tunes salt-stress responses by controlling transcript abundance and ion homeostasis in rice.

***OsRNS3*, a 3'aQTL-specific gene, negatively regulates salt tolerance in rice**

To identify APA-specifically regulated genes functionally distinct from eQTL-mediated transcriptional control in rice

salt-stress responses, we performed exclusion analysis between 3'aGenes and eGenes. This identified 141 genes exhibiting 3'aQTL associations under both normal and salt conditions, without corresponding eQTL signals (Figure 4B), representing an APA-exclusive regulatory mechanism. Among these 141 genes, only 41 exhibited significantly altered expression under salt stress (Figure S8; Tables S6, S9), from which we prioritized a T2 family ribonuclease gene, *OsRNS3*. Under both normal and salt conditions, *OsRNS3* was associated with significant 3'aQTL signals at its genomic locus ($P = 1.07 \times 10^{-10}$ normal; $P = 6.97 \times 10^{-17}$ salt), whereas no significant eQTL signals were observed (Figure 6A). Additionally, salt treatment significantly induced *OsRNS3* upregulation while concurrently reducing its transcript PDI values (Figure 6B, C), indicating that salinity promotes proximal polyA site selection and 3'UTR shortening in *OsRNS3*. Notably, the 3'aQTL lead SNP of *OsRNS3* (snp8_21052525) under salt conditions is embedded within its 3'UTR, precisely overlapping the binding motif of small RNA miR2905. Across rice accessions under both normal and salt conditions, the CC genotype of this lead SNP consistently exhibited significantly higher PDI values than the TT genotype, resulting in preferential usage of distal polyA sites and longer 3'UTRs (Figure 6D–G). These findings suggest a miRNA-mediated regulation of APA dynamics for *OsRNS3*. To directly test whether the *OsRNS3* 3'UTR, including its miR2905 binding site, suppresses gene expression at the post-transcriptional level, we performed a dual-luciferase reporter assay in which the full-length *OsRNS3* 3'UTR or its 3'UTR-deleted counterpart was fused downstream of the LUC reporter and co-expressed with a miR2905 precursor (Figure S9A). The full-length 3'UTR significantly reduced luciferase activity and translational efficiency without altering mRNA abundance, and co-expression of the miR2905 precursor further potentiated this suppression (Figure S9B–D). These results suggest that the *OsRNS3* 3'UTR mediates miR2905-dependent translational suppression, supporting the model that salt-induced 3'UTR shortening relieves this miRNA-mediated repression.

Expression pattern analysis of *OsRNS3* through qPCR revealed its constitutive expression across tissues, with preferential but not exclusive expression in roots; importantly, *OsRNS3* is detectably expressed in leaves, the tissue in which the 3'aQTL was mapped (Figure S10). To further investigate the biological functions of *OsRNS3* and its alternative polyadenylation in rice salt-stress responses, we generated transgenic knockout lines (*osrns3*) using CRISPR/Cas9 gene editing (Figure 6H). Phenotypic analysis at the seedling stage revealed no discernible differences between *osrns3* mutants and wild-type under normal conditions. However, under salt stress, mutants exhibited enhanced salt tolerance with significantly higher survival rates and increased fresh and dry weights in both shoots and roots compared to wild-type (Figures 6I, J, S11A–D). To elucidate the physiological mechanism, we quantified ion concentrations in tissues with salt treatment and found that *osrns3* mutants showed significantly reduced Na^+ and K^+

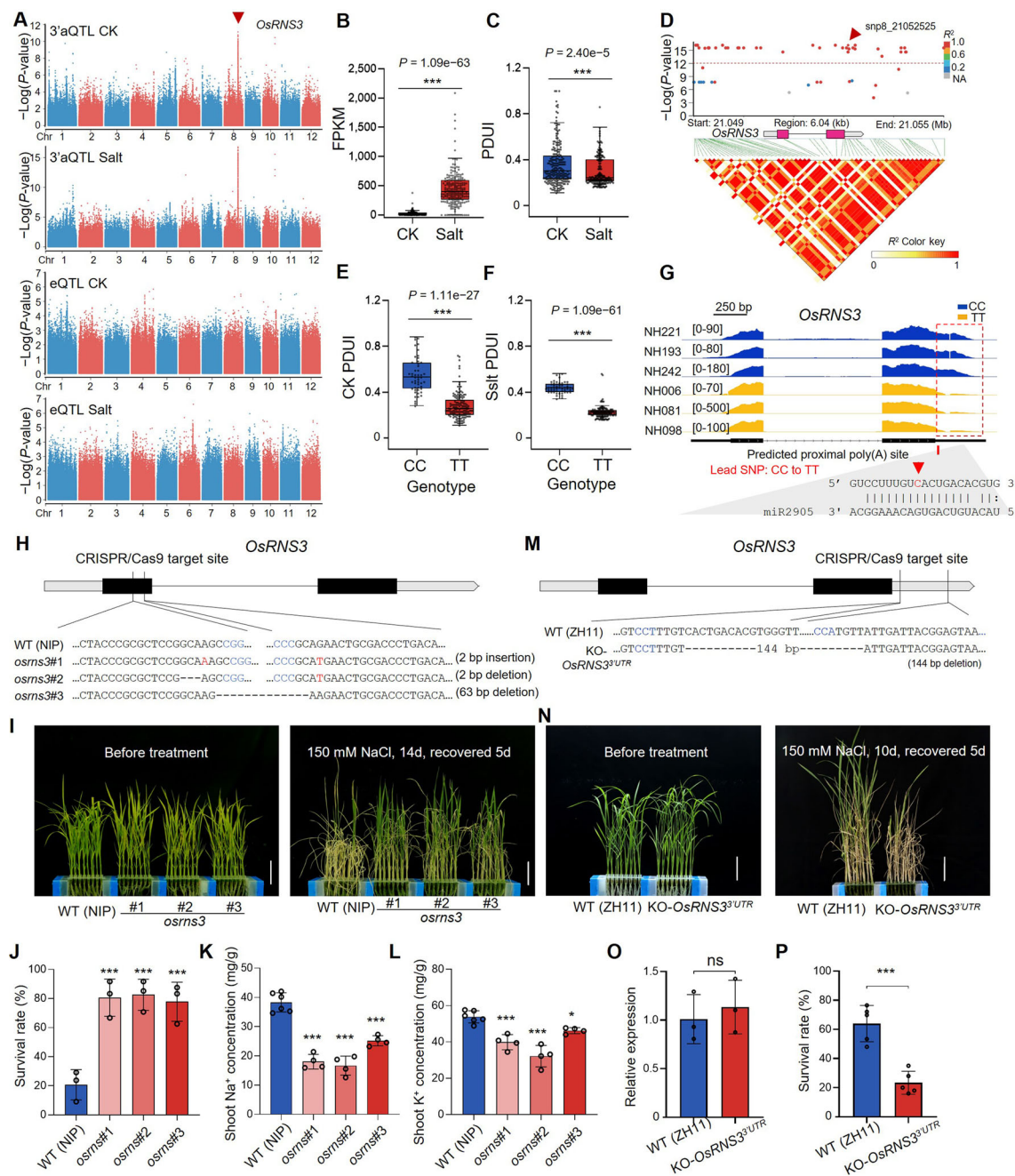


Figure 6. Functional analysis of the 3'aQTL-specific gene *OsRNS3*

(A) Manhattan plots showing *cis*-3'aQTL and eQTL signals associated with APA and expression of *OsRNS3* under normal (CK) and salt (Salt) conditions. **(B, C)** Comparison of *OsRNS3* FPKM (**B**) and PDI values (**C**) in leaves in the rice population under normal and salt conditions. **(D)** Local Manhattan plot showing *cis*-3'aQTL under salt conditions and a linkage disequilibrium heatmap for the region surrounding *OsRNS3*. The red arrow indicates the lead SNP snp8_21052525. **(E, F)** Comparison of APA PDI values between the two genotypes of the lead SNP in the population under normal (**E**) and salt (**F**) conditions. **(G)** IGV plot showing genotype-specific APA differences in proximal and distal polyA site usage for *OsRNS3* under salt conditions. The red rectangle indicates the predicted proximal polyA site. CC and TT represent the two genotypes of the lead SNP. **(H)** Schematic diagram of CRISPR/Cas9 target sites in the *OsRNS3* gene. The black boxes indicate exons. Mutation sequences are shown below the gene model, and the protospacer adjacent motif (PAM) is shown in blue. **(I)** Phenotypes of wild-type (WT) Nipponbare and *osrms3* mutant plants grown before (left) and after (right) salt treatment. Scale bar, 5 cm. **(J–L)** Survival rate (**J**) ($n=3$ biological replicates, 24 individual plants per biological replicate), shoot Na^+ concentration (**K**) ($n=4$), and K^+ concentration (**L**) ($n=4$) of WT and *osrms3* mutant plants after salt treatment. **(M)** Schematic diagram showing a large sequence deletion in the 3' UTR of *OsRNS3* (KO-*OsRNS3*^{3'UTR}) generated using CRISPR/Cas9 gene editing. **(N)** Phenotypes of WT and KO-*OsRNS3*^{3'UTR} plants grown before (left) and after (right) salt treatment. Scale bar, 5 cm. **(O)** Relative expression of *OsRNS3* in leaves of WT and KO-*OsRNS3*^{3'UTR} plants ($n=3$). *UBQ* (LOC_Os03g13170) was used as an internal reference. **(P)** Survival rate of WT and KO-*OsRNS3*^{3'UTR} plants ($n=3$ biological replicates, 24 individual plants per biological replicate). The box plot shows the distribution of values, with each dot representing an individual plant. The center line indicates the median value, the box indicates the interquartile range (IQR; 25th–75th percentiles), and the whiskers extend to 1.5× the IQR. The data in the bar chart are presented as means $\pm$ SD. *** $P < 0.001$; * $P < 0.05$; ns, not significant, using Student's *t*-test.

concentrations and lower Na^+/K^+ ratios in shoots, whereas in roots, Na^+ slightly increased, K^+ significantly increased, and Na^+/K^+ ratios significantly decreased versus wild-type (Figures 6K, L, S11E–H), demonstrating that loss-of-function of *OsRNS3* could inhibit Na^+/K^+ uptake and translocation, thereby promoting salinity tolerance. During the reproductive stage, no significant differences were observed between *osrns3* mutant and wild-type plants in critical agronomic traits, including plant architecture, panicle morphology, grain characteristics, or yield per plant (Figure S12). Moreover, to investigate the functional role of APA-mediated 3'UTR remodeling of *OsRNS3* in salt-stress responses, we generated transgenic lines with a 144 bp deletion in the 3'UTR of *OsRNS3* (KO-*OsRNS3*^{3'UTR}) via CRISPR/Cas9 gene editing (Figure 6M), thereby mimicking preferential proximal polyA site usage in *OsRNS3*. Salt treatment assay revealed that the KO-*OsRNS3*^{3'UTR} 3'UTR-deficient lines exhibited pronounced salt-sensitive phenotypes, with survival rates significantly reduced compared to wild-type controls (Figure 6N–P). This indicates that the 3'UTR sequence of *OsRNS3* plays a negative role in salinity tolerance. Notably, *OsRNS3* expression levels showed no significant difference between 3'UTR-deficient lines and wild-type (Figure 6O). These results suggest that *OsRNS3* likely modulates salinity tolerance through APA-mediated remodeling of the 3'UTR, perhaps regulating translational efficiency by the miRNA binding site in the 3'UTR.

DISCUSSION

Traditional identification of loci important for agricultural traits has primarily focused on mining coding-region genes; however, GWAS have revealed that the vast majority of salt tolerance-associated variations are located in noncoding regions, whose functional characterization has long been constrained. Prior population studies have partially accounted for such variation through eQTLs (Liu et al., 2020; Liang et al., 2022; Wei et al., 2024) and sQTLs (Chen et al., 2018; Zhang et al., 2024), yet a substantial number of noncoding variations remain mechanistically unexplained, urging a move beyond the transcription-centric paradigm. In humans, genome-wide mapping has shown that 3'aQTLs are largely independent of eQTLs and contribute substantially to complex-trait heritability (Mittleman et al., 2020; Li et al., 2021). APA offers such an alternative: As a post-transcriptional mechanism through the dynamic selection of 3'UTR polyadenylation sites (Mitschka and Mayr, 2022), APA participates in stress responses in *Arabidopsis thaliana* and wheat (Ma et al., 2022, 2023). Unlike conventional promoter-based regulation, APA might fine-tune gene function through dynamic reconfiguration of downstream noncoding regions; however, its genetic basis and functional consequences at the population level have remained largely unexplored in plants. In this study, we constructed the first population-scale dynamic APA landscape in rice leaves under normal and

salt-stressed conditions. By integrating this landscape with pan-genomic variation, we established a comprehensive 3'aQTL map, thereby providing a community resource for understanding how noncoding variation shapes post-transcriptional gene regulation in leaves during environmental adaptation. Nevertheless, extending such population-level APA and 3'aQTL analyses to roots under salt-stressed conditions will be important, as key salt-tolerance determinants such as *OsHKT1;5/SKC1* act predominantly in roots.

Our results reveal APA as a regulatory layer distinct from, and complementary to, transcriptional control. Under salt stress, proximal polyA site usage was globally promoted, leading to widespread 3'UTR shortening that enables rapid post-transcriptional reprogramming through *cis*-element reorganization without altering core protein-coding sequences, as exemplified by the loss of the miR2100-5p binding site in the shortened 3'UTR of *OsDREB2A* (Figure 2). This view is reinforced by the genetic architecture of 3'aQTLs: The 1,634 and 1,809 *cis*-3'aQTLs identified under normal and salt conditions, respectively, were markedly enriched in 3'UTRs and transcription termination sites, whereas eQTLs were enriched at promoters and transcription start sites (Figure 4), mirroring the largely independent 3'aQTL–eQTL architecture reported in human tissues (Li et al., 2021). 3'aQTLs therefore act through 3'UTR reconfiguration rather than promoter-proximal control, and the concurrent upregulation of core polyadenylation factors such as *OsCPSF30* may reinforce this process by promoting proximal polyA site selection (Figure 3). Such a 3'UTR-based regulatory system enables plants to rapidly reestablish cellular homeostasis upon stress without altering core protein-coding sequences, underscoring the role of APA as a conserved, independently operating regulatory layer.

To assess the functional significance of this APA-mediated regulation, we validated two 3'aQTL genes, *OsMyb21* and *OsRNS3*, and established that APA acts as a molecular switch controlling salt-stress responses. For *OsMyb21*, salt-induced 3'UTR shortening and 3'UTR-knockout lines that mimic proximal polyA site usage resulted in elevated transcript levels and enhanced salt tolerance (Figure 5). For *OsRNS3*, the lead 3'aQTL SNP (snp8_21052525) resides within the 3'UTR and precisely overlaps the miR2905 binding site (Figure 6A–G). A dual-luciferase reporter assay showed that the full-length 3'UTR, which retains the intact miR2905 binding site, suppresses translational efficiency without altering mRNA abundance, and that this suppression is potentiated by miR2905 co-expression (Figure S9). Although *OsRNS3* shows root-preferential expression, it is detectably expressed in leaves, the tissue in which the 3'aQTL was mapped (Figure S10), and its functional validation affected both shoot and root ion concentrations under salt stress, supporting roles in both organs. Together, these results demonstrate that APA-mediated 3'UTR remodeling regulates gene output at both the transcript and translational levels, directly linking natural variation in 3'UTR length to salt-tolerance phenotypes.

The two mutants, *osmyb21* and *osrns3*, displayed opposite salt-tolerance phenotypes that were accompanied by consistent changes in ion homeostasis. Maintaining a low shoot Na^+/K^+ ratio is a central strategy for plant salt tolerance, because Na^+ competes with K^+ for uptake through non-selective cation channels and certain K^+ transporters, and the ionic imbalance that ensues is a major cause of salt-induced growth inhibition (Ma et al., 2026). The concurrent change of both Na^+ and K^+ concentrations in the two mutants therefore reflects a perturbation of ion-uptake/transport homeostasis rather than an independent effect on a single ion. In *osmyb21*, shoot Na^+ and K^+ concentrations both increased and the shoot Na^+/K^+ ratio rose, consistent with enhanced Na^+ and K^+ uptake under salinity, whereas in *osrns3*, shoot Na^+ and K^+ concentrations both decreased and the shoot Na^+/K^+ ratio fell, consistent with reduced Na^+ uptake and translocation (Figures 5, 6, S6 and S11). These observations suggest that APA-mediated regulation of *OsMyb21* and *OsRNS3* impinges on the ion-transport machinery, potentially through HKT, NHX, SOS, and AKT-KUP family members, whose expression and activity are integrated with K^+ -for- Na^+ selectivity under salinity (Ma et al., 2026). Indeed, *OsWRKY53*, a transcription factor identified by GWAS of rice landraces, *trans*-represses *OsHKT1;5* to control root Na^+ flux, and the *OsWRKY53*–*OsMKK10.2* module coordinates defense against ionic stress (Yu et al., 2023). Likewise, the outward-rectifying shaker K^+ channel *OsK5.2* promotes salt tolerance by reducing transpirational Na^+ delivery to shoots while sustaining root xylem K^+ loading, contributing to leaf K^+/Na^+ homeostasis (Zhou et al., 2022). A direct link between alternative 3'UTRs and ion-transport regulation has been established in the halophyte *S. alterniflora*, where the alternative 3'UTR of the high-affinity K^+ transporter *SaHKT1* enhances RNA stability and protein synthesis under salt stress (Wang et al., 2023), supporting the notion that APA-mediated control of ion transporters is a conserved mechanism of salt tolerance.

Beyond the ion-homeostasis phenotypes described above, the regulatory role of *OsMyb21* extends to the coordination of both biotic and abiotic stress responses. *OsMyb21* was previously reported to negatively regulate bacterial blight resistance in rice (Yang et al., 2021), whereas the present study shows that it positively regulates salt tolerance. Notably, we also uncovered its pleiotropic effects on tiller number, grain size, and 1,000-grain weight (Figure S7), further supporting such a broad role. This dual role exemplifies the emerging view of MYB transcription factors as hubs that integrate hormone signaling and diverse stress responses (Wu et al., 2024; Zhang et al., 2025; Htun et al., 2026), coordinating the SA, JA, ET, and ABA pathways whose interplay under combined stresses remains a frontier; other rice R2R3-MYBs, including *OsMYB9* and *OsMYB2*, likewise enhance salt tolerance through distinct downstream targets (Nie et al., 2025). We propose that *OsMyb21* integrates biotic and abiotic signals by regulating distinct target-gene sets, with APA fine-tuning its output in a stress- and tissue-specific manner, a notion further supported

by APA events shared between stress-responsive networks in rice (Ye et al., 2019). Because 3'aQTLs represent directly selectable noncoding variants, these findings promote breeding strategies that combine marker-assisted selection of 3'aQTLs and the enrichment of APA genes within rice agronomic QTLs, underscoring their value for crop improvement.

MATERIALS AND METHODS

Plant materials, growth, and phenotypic measurements

A total of 250 and 219 rice accessions were selected from our previously described super pan-genome population to identify salt tolerance phenotypes at the seedling stage under normal and salt conditions, respectively, and perform leaf transcriptome sequencing (Shang et al., 2022; Wei et al., 2024). All 219 salt-treated accessions are fully included within the 250 control accessions, and all population-level APA comparisons (differential APA and 3'aQTL mapping) were conducted exclusively on these 219 paired accessions. The salt treatment procedure for rice seedlings followed our previous study (Wei et al., 2024). Briefly, germinated seedlings were cultivated in a greenhouse under 12 h light/12 h dark cycles at 30°C, grown in Yoshida's nutrient solution for approximately 16 d. They were then transferred to nutrient solutions containing either 0 mM or 150 mM NaCl for 10 to 14 d (duration adjusted based on salt tolerance phenotypes), followed by recovery in standard nutrient solution for 7 to 14 d. Survival rates, fresh weight, and dry weight were measured using more than six plants per biological replicate. Rice plants were cultivated in field conditions until the pre-heading stage, then transplanted into the greenhouse with intact soil root systems. Subsequently, they received weekly irrigation with 75 mM NaCl solution for approximately 1 month, continuing until reaching the grain filling maturity stage. Field-cultivated plant materials were planted in Shenzhen, China, during the regular rice-growing season.

Mapping of RNA-seq data

The leaf transcriptome data under normal treatment and salt treatment are from our previous studies (Shang et al., 2022; Wei et al., 2024). First, all raw data were cleaned using the Trimmomatic software (Version: v0.39, parameters: ILLUMINACLIP:TruSeq. 3-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36). The clean data were aligned to the Nipponbare reference genome (Msu v7.0) using STAR (Version: 2.5.2b, parameters: --runMode alignReads --readFilesCommand gunzip -c --quantMode Transcriptome SAM GeneCounts --twopassMode Basic --outSAMtype BAM SortedByCoordinate --outSAMstrandField intronMotif --outFilter MultimapNmax 10 --outFilterMultimapScoreRange 1 --sjdb Score 2 --alignIntronMin 20 --alignSJoverhangMin 8 --alignSJD BoverhangMin 1). The bam files were processed using the genemecov module of bedtools (Version: 2.26.0, parameters: -bga -split -trackline) to generate the wig files.

APA identification and differential APA analysis

The Dapars v.2 (Li et al., 2021) software was used to generate APA regions based on the reference genome annotation (MSU v7.0) with the following parameters: read coverage cutoff of 30, minimum number of samples with coverage ≥ 10 , step size of 1 bp, and an FDR threshold of 0.05 for multiple testing correction. All samples were normalized according to sequencing depth. APA events occurring on 12 chromosomes were identified separately and then merged into a population APA matrix. The R package Deducer was used to perform significance tests on the PDUI values identified in the transcriptomes of normal and salt-treated samples (parameters: statistic=c('t','mean'), alternative=c('two-sided'), midp=TRUE, B=100,000). The differential APAs were determined with the threshold P -value < 0.01 and $|\Delta\text{PDUI}| > 0.1$. The differentially expressed genes in rice accessions under normal and salt conditions were identified by the R package DESeq2 with the threshold P -value < 0.01 and absolute fold change > 2 . The miRNA targets of APA-mediated 3'UTR remodeling regions with salt treatment were predicted with the software sPARTA.

Mapping of 3'aQTL

The variant information of the population comes from previous studies (Shang et al., 2022). VcfTools (Version: 0.1.16, parameters: maf 0.05 max-missing 0.9) to perform quality control on the raw variant file. SnpEff (v5.0) was used to annotate the SNPs after quality control. Plink (Version: 1.90b6.21, parameters: --pac 10) was used to estimate the population structure of the samples, and the first five principal components were selected as covariates for candidate QTL analysis. At the same time, the R-PEER package was used to calculate batch effects on the PDUI matrix, and the top 20 factors were selected as covariates for the subsequent QTL analysis. 3'aQTL analysis was performed using the MatrixEQTL package (Version: 2.2); the window was set to 100 kb, with a significance threshold of 1×10^{-6} for both *cis*-3'aQTLs and *trans*-3'aQTLs. The eQTL results were derived from previous studies (Wei et al., 2024), and here we set the threshold consistent with that of the 3'aQTLs. We used the Plink software to calculate linkage disequilibrium (LD) blocks and merged the results of 3'aQTLs based on these blocks, with the most significant association result within each block serving as the significant value for that QTL. The GCTA (Version: 1.94.1, parameters: --reml) software was used to estimate the heritability of the 3'UTR regions of genes.

Plasmid construction

The CRISPR/Cas9 gene editing vectors for *OsCPSF30*, *OsMyb21*, and *OsRNS3* were constructed following established protocols adapted from previous studies (Ma et al., 2015). For the dual-luciferase reporter assay system, the 35S promoter was first amplified by PCR and cloned into the pGreenII 0800-LUC vector to replace the native LUC promoter, generating the control construct

Pan-genomic APA in controlling salt tolerance in rice

designated as 35S::REN-35S::LUC. The 3'UTRs of *OsDREB2A* and *OsRNS3* were amplified and then inserted downstream of the LUC stop codon to test the effects of these 3'UTRs on LUC expression and its protein function. Additionally, synthesized MIR2100-5p and MIR2905 sequences were inserted after the 35S promoter for constructing the effector vector. All primers are listed in Table S13.

Real-time quantitative PCR

Total RNA was extracted from plant tissue using TRIzol reagent (Invitrogen), and cDNA was synthesized using the HiScript® III 1st Strand cDNA Synthesis Kit with gDNA wiper (Vazyme, China). Real-time quantitative PCR (qPCR) was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China). Data analysis used the $2^{-\Delta\Delta C_t}$ method. For each sample, qPCR was performed with at least three biological replicates, and the housekeeping gene *UBQ* (LOC_Os03g13170) was used as an internal control to normalize gene expression levels.

Measurement of Na⁺ and K⁺ concentration

The Na⁺ and K⁺ measurement method referred to the previous study (Wei et al., 2024). Briefly, root and shoot samples were harvested from both wild-type rice and mutants after salt treatment (each biological replicate consisted of three pooled plants), followed by thorough rinsing and oven-drying of the samples. Na⁺ and K⁺ were extracted from the ground samples and measured by flame photometer (AP1402).

Transcriptome sequencing and analysis

Leaves were collected from wild-type and *oscpsf30* mutant plants with three biological replicates. Total RNA was extracted from these samples. RNA-seq libraries were prepared using the NEBNext Ultra™ RNA Library Prep Kit for Illumina (New England Biolabs, USA) following the manufacturer's instructions. RNA-seq libraries were sequenced (paired-end 150-bp) on an Illumina NovaSeq. 6000 platform at Novogene (Beijing, China). After quality control and adapter trimming, clean reads were mapped to the Nipponbare reference genome (Msu7.0) using HISAT2. Gene quantification and differential expression analysis were subsequently performed with Cufflinks. The differentially expressed genes were determined using the threshold P -value < 0.05 and absolute fold change > 1.5 . The GO enrichment analysis was conducted using agriGO v.2.0 and TBtools.

Data availability statement

The RNA-seq raw data for 250 and 219 rice populations grown under normal and salt conditions have been deposited in the NCBI SRA database with BioProject numbers PRJNA692672 and PRJNA1009219, respectively. The raw

sequence data for leaves of wild-type and *oscpsf30* mutant plants reported in this paper have been deposited in the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA029523) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Q.Q., L.S., H.W., Hui.H., and C.S. conceived the study and designed the project. C.S., Y.C., Zhang.W., and W.Z. performed most of the molecular biology experiments. C.S., L.Y., and X.W. performed APA identification and differential APA, DEG, 3'aQTL, and eQTL analyses. Zhi.W., Y.W., and D.C. collected the samples and investigated phenotypes. C.S. and W.Z. wrote the manuscript. Q.Q., L.S., Hao.H., Hui.H., and H.W. revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

- Cao, J., Ye, C., Hao, G., Dabney-Smith, C., Hunt, A.G., and Li, Q.Q. (2019). Root hair single cell type specific profiles of gene expression and alternative polyadenylation under cadmium stress. *Front. Plant Sci.* **10**: 589.
- Chakrabarti, M., de Lorenzo, L., Abdel-Ghany, S.E., Reddy, A.S.N., and Hunt, A.G. (2020). Wide-ranging transcriptome remodelling mediated by alternative polyadenylation in response to abiotic stresses in Sorghum. *Plant J.* **102**: 916–930.
- Chan, S.L., Huppertz, I., Yao, C., Weng, L., Moresco, J.J., Yates, 3rd, J.R., Ule, J., Manley, J.L., and Shi, Y. (2014). CPSF30 and Wdr33 directly bind to AAUAAA in mammalian mRNA 3' processing. *Genes Dev.* **28**: 2370–2380.
- Chen, Q., Han, Y., Liu, H., Wang, X., Sun, J., Zhao, B., Li, W., Tian, J., Liang, Y., Yan, J., et al. (2018). Genome-wide association analyses reveal the importance of alternative splicing in diversifying gene function and regulating phenotypic variation in maize. *Plant Cell* **30**: 1404–1423.
- Deng, P., Jing, W., Cao, C., Sun, M., Chi, W., Zhao, S., Dai, J., Shi, X., Wu, Q., Zhang, B., et al. (2022). Transcriptional repressor RST1 controls salt tolerance and grain yield in rice by regulating gene expression of asparagine synthetase. *Proc. Natl. Acad. Sci. U.S.A.* **119**: e2210338119.
- Derti, A., Garrett-Engle, P., Macisaac, K.D., Stevens, R.C., Sriram, S., Chen, R., Rohl, C.A., Johnson, J.M., and Babak, T. (2012). A quantitative atlas of polyadenylation in five mammals. *Genome Res.* **22**: 1173–1183.
- Fang, S., Zhao, Y., Zhang, X., Yu, F., and Zhang, P. (2025). Molecular mechanism underlying phosphate distribution by SULTR family transporter SPDT in *Oryza sativa*. *Sci. Adv.* **11**: eady3442.
- Fu, H., Yang, D., Su, W., Ma, L., Shen, Y., Ji, G., Ye, X., Wu, X., and Li, Q.Q. (2016). Genome-wide dynamics of alternative polyadenylation in rice. *Genome Res.* **26**: 1753–1760.
- Harrison, P.F., Powell, D.R., Clancy, J.L., Preiss, T., Boag, P.R., Traven, A., Seemann, T., and Beilharz, T.H. (2015). PAT-seq: A method to study the integration of 3'-UTR dynamics with gene expression in the eukaryotic transcriptome. *RNA* **21**: 1502–1510.
- Hou, Y., Sun, J., Wu, B., Gao, Y., Nie, H., Nie, Z., Quan, S., Wang, Y., Cao, X., and Li, S. (2021). CPSF30-L-mediated recognition of mRNA m⁶A modification controls alternative polyadenylation of nitrate signaling-related gene transcripts in Arabidopsis. *Mol. Plant* **14**: 688–699.
- Htun, A.A., Wang, Y., Liu, J., Guo, Z., Zheng, Y., Wang, W., Ma, S., Jia, A., Ren, Y., and Chen, F. (2026). MYB factors: Hubs of plant stress and hormone crosstalk. *Plant Biotechnol. J.* **24**: 5201–5223.
- Huang, X.Y., Chao, D.Y., Gao, J.P., Zhu, M.Z., Shi, M., and Lin, H.X. (2009). A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. *Genes Dev.* **23**: 1805–1817.
- Li, L., Huang, K.L., Gao, Y., Cui, Y., Wang, G., Elrod, N.D., Li, Y., Chen, Y.E., Ji, P., Peng, F., et al. (2021). An atlas of alternative polyadenylation quantitative trait loci contributing to complex trait and disease heritability. *Nat. Genet.* **53**: 994–1005.
- Liang, Z., Myers, Z.A., Petrella, D., Engelhorn, J., Hartwig, T., and Springer, N.M. (2022). Mapping responsive genomic elements to heat stress in a maize diversity panel. *Genome Biol.* **23**: 234.
- Lin, J., and Li, Q.Q. (2023). Coupling epigenetics and RNA polyadenylation: Missing links. *Trends Plant Sci.* **28**: 223–234.
- Liu, F., Marquardt, S., Lister, C., Swiezewski, S., and Dean, C. (2010). Targeted 3' processing of antisense transcripts triggers Arabidopsis FLC chromatin silencing. *Science* **327**: 94–97.
- Liu, S., Li, C., Wang, H., Wang, S., Yang, S., Liu, X., Yan, J., Li, B., Beatty, M., Zastrow-Hayes, G., et al. (2020). Mapping regulatory variants controlling gene expression in drought response and tolerance in maize. *Genome Biol.* **21**: 163.
- de Lorenzo, L., Sorenson, R., Bailey-Serres, J., and Hunt, A.G. (2017). Noncanonical alternative polyadenylation contributes to gene regulation in response to hypoxia. *Plant Cell* **29**: 1262–1277.
- Ma, H., Cai, L., Lin, J., Zhou, K., and Li, Q.Q. (2022). Divergence in the regulation of the salt tolerant response between *Arabidopsis thaliana* and its halophytic relative *Eutrema salsgineum* by mRNA alternative polyadenylation. *Front. Plant Sci.* **13**: 866054.
- Ma, H., Lin, J., Mei, F., Mao, H., and Li, Q.Q. (2023). Differential alternative polyadenylation of homoeologous genes of allohexaploid wheat ABD subgenomes during drought stress response. *Plant J.* **114**: 499–518.
- Ma, L., Li, J., Li, J., Huo, Y., Yang, Y., Jiang, C., and Guo, Y. (2026). Plant salt-tolerance mechanisms: Classic signaling pathways, emerging frontiers, and future perspectives. *Mol. Plant* **19**: 538–570.
- Ma, X., Zhang, Q., Zhu, Q., Liu, W., Chen, Y., Qiu, R., Wang, B., Yang, Z., Li, H., Lin, Y., et al. (2015). A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Mol. Plant* **8**: 1274–1284.

- Mallikarjuna, G., Mallikarjuna, K., Reddy, M.K., and Kaul, T. (2011). Expression of OsDREB2A transcription factor confers enhanced dehydration and salt stress tolerance in rice (*Oryza sativa* L.). *Biotechnol. Lett.* **33**: 1689–1697.
- Masamha, C.P., Xia, Z., Yang, J., Albrecht, T.R., Li, M., Shyu, A.B., Li, W., and Wagner, E.J. (2014). CFIm25 links alternative polyadenylation to glioblastoma tumour suppression. *Nature* **510**: 412–416.
- Mayr, C. (2017). Regulation by 3'-untranslated regions. *Annu. Rev. Genet.* **51**: 171–194.
- Mitschka, S., and Mayr, C. (2022). Context-specific regulation and function of mRNA alternative polyadenylation. *Nat. Rev. Mol. Cell Biol.* **23**: 779–796.
- Mittleman, B.E., Pott, S., Warland, S., Zeng, T., Mu, Z., Kaur, M., Gilad, Y., and Li, Y. (2020). Alternative polyadenylation mediates genetic regulation of gene expression. *eLife* **9**: e57492.
- Nie, S., Huang, W., He, C., Wu, B., Duan, H., Ruan, J., Zhao, Q., and Fang, Z. (2025). Transcription factor OsMYB2 triggers amino acid transporter *OsANT1* expression to regulate rice growth and salt tolerance. *Plant Physiol.* **197**: kiae559.
- Ren, Z.H., Gao, J.P., Li, L.G., Cai, X.L., Huang, W., Chao, D.Y., Zhu, M.Z., Wang, Z.Y., Luan, S., and Lin, H.X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nat. Genet.* **37**: 1141–1146.
- Shang, L., Li, X., He, H., Yuan, Q., Song, Y., Wei, Z., Lin, H., Hu, M., Zhao, F., Zhang, C., et al. (2022). A super pan-genomic landscape of rice. *Cell Res.* **32**: 878–896.
- Shi, Y., Di Giammartino, D.C., Taylor, D., Sarkeshik, A., Rice, W.J., Yates, 3rd, J.R., Frank, J., and Manley, J.L. (2009). Molecular architecture of the human pre-mRNA 3' processing complex. *Mol. Cell* **33**: 365–376.
- Simpson, G.G., Dijkwel, P.P., Quesada, V., Henderson, I., and Dean, C. (2003). FY is an RNA 3' end-processing factor that interacts with FCA to control the Arabidopsis floral transition. *Cell* **113**: 777–787.
- Song, P., Yang, J., Wang, C., Lu, Q., Shi, L., Tayier, S., and Jia, G. (2021). Arabidopsis N⁶-methyladenosine reader CPSF30-L recognizes FUE signals to control polyadenylation site choice in liquid-like nuclear bodies. *Mol. Plant* **14**: 571–587.
- Sun, Y., Fu, Y., Li, Y., and Xu, A. (2012). Genome-wide alternative polyadenylation in animals: Insights from high-throughput technologies. *J. Mol. Cell Biol.* **4**: 352–361.
- Takagi, H., Tamiru, M., Abe, A., Yoshida, K., Uemura, A., Yaegashi, H., Obara, T., Oikawa, K., Utsushi, H., Kanzaki, E., et al. (2015). MutMap accelerates breeding of a salt-tolerant rice cultivar. *Nat. Biotechnol.* **33**: 445–449.
- Tian, B., and Manley, J.L. (2017). Alternative polyadenylation of mRNA precursors. *Nat. Rev. Mol. Cell Biol.* **18**: 18–30.
- Wang, T., Ye, W., Zhang, J., Li, H., Zeng, W., Zhu, S., Ji, G., Wu, X., and Ma, L. (2023). Alternative 3'-untranslated regions regulate high-salt tolerance of *Spartina alterniflora*. *Plant Physiol.* **191**: 2570–2587.
- Wei, H., Wang, X., Zhang, Z., Yang, L., Zhang, Q., Li, Y., He, H., Chen, D., Zhang, B., Zheng, C., et al. (2024). Uncovering key salt-tolerant regulators through a combined eQTL and GWAS analysis using the super pan-genome in rice. *Natl. Sci. Rev.* **11**: nwae043.
- Wu, X., Liu, M., Downie, B., Liang, C., Ji, G., Li, Q.Q., and Hunt, A.G. (2011). Genome-wide landscape of polyadenylation in Arabidopsis provides evidence for extensive alternative polyadenylation. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 12533–12538.
- Wu, X., Xia, M., Su, P., Zhang, Y., Tu, L., Zhao, H., Gao, W., Huang, L., and Hu, Y. (2024). MYB transcription factors in plants: A comprehensive review of their discovery, structure, classification, functional diversity and regulatory mechanism. *Int. J. Biol. Macromol.* **282**: 136652.
- Xia, Z., Donehower, L.A., Cooper, T.A., Neilson, J.R., Wheeler, D.A., Wagner, E.J., and Li, W. (2014). Dynamic analyses of alternative polyadenylation from RNA-seq reveal a 3'-UTR landscape across seven tumour types. *Nat. Commun.* **5**: 5274.
- Yang, W., Zhao, J., Zhang, S., Chen, L., Yang, T., Dong, J., Fu, H., Ma, Y., Zhou, L., Wang, J., et al. (2021). Genome-wide association mapping and gene expression analysis reveal the negative role of OsMYB21 in regulating bacterial blight resistance in rice. *Rice (N Y)* **14**: 58.
- Yang, Y., and Guo, Y. (2018). Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytol.* **217**: 523–539.
- Ye, C., Zhou, Q., Wu, X., Ji, G., and Li, Q.Q. (2019). Genome-wide alternative polyadenylation dynamics in response to biotic and abiotic stresses in rice. *Ecotoxicol. Environ. Saf.* **183**: 109485.
- Yu, J., Zhu, C., Xuan, W., An, H., Tian, Y., Wang, B., Chi, W., Chen, G., Ge, Y., Li, J., et al. (2023). Genome-wide association studies identify OsWRKY53 as a key regulator of salt tolerance in rice. *Nat. Commun.* **14**: 3550.
- Zhang, D., Zhou, H., Zhang, Y., Zhao, Y., Zhang, Y., Feng, X., and Lin, H. (2025). Diverse roles of MYB transcription factors in plants. *J. Integr. Plant Biol.* **67**: 539–562.
- Zhang, H., Chen, W., Zhu, D., Zhang, B., Xu, Q., Shi, C., He, H., Dai, X., Li, Y., He, W., et al. (2024). Population-level exploration of alternative splicing and its unique role in controlling agronomic traits of rice. *Plant Cell* **36**: 4372–4387.
- Zhang, H., Zhu, J., Gong, Z., and Zhu, J.K. (2022). Abiotic stress responses in plants. *Nat. Rev. Genet.* **23**: 104–119.
- Zhou, J., Nguyen, T.H., Hmidi, D., Luu, D.T., Sentenac, H., and Very, A.A. (2022). The outward shaker channel OsK5.2 improves plant salt tolerance by contributing to control of both leaf transpiration and K⁺ secretion into xylem sap. *Plant Cell Environ.* **45**: 1734–1748.

SUPPORTING INFORMATION

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Figure S1. Rice accessions of the pan-genome population used in the APA study

Figure S2. APA characteristics of different rice subpopulations under normal conditions

Figure S3. Correlation analysis of gene APA and expression levels

Figure S4. Genome-wide characterization of *trans*-3'aQTLs in rice

Figure S5. Analysis of candidate genes important for APA and expression in response to salt stress

Figure S6. Biomass and ion concentration of WT and *osmyb21* mutant after salt treatment at the seedling stage

Figure S7. Agronomic traits of WT and *osmyb21* mutant at the maturation stage under field conditions

Figure S8. Analysis of 3'aQTL-specific genes important for APA in response to salt stress

Figure S9. The *OsRNS3* 3'UTR suppresses translational efficiency in a dual-luciferase reporter assay

Figure S10. Expression pattern analysis of *OsRNS3* in rice

Figure S11. Biomass and ion concentration of WT and *osrms3* mutants after salt treatment at the seedling stage

Figure S12. Agronomic traits of WT and *osrms3* mutants at the maturation stage under field conditions

Table S1. Identification of APA in leaves of 250 rice accessions under normal conditions

Table S2. Identification of APA in leaves of 219 rice accessions under salt conditions

Table S3. The overlapping APA events in 219 paired rice accessions under normal and salt conditions

Table S4. The differential APA analysis in the 219 rice accessions under normal and salt conditions

Table S5. The miRNA target prediction in APA-mediated 3'UTR remodeling regions with salt treatment

Table S6. Differential gene expression analysis in leaves of rice population under normal and salt conditions

Table S7. Differential APA events in leaves of WT and *oscpsf30* mutant

Table S8. The expression levels (FPKM) of differentially expressed genes in leaves of WT and *oscpsf30* mutant

Table S9. List of *cis*-3'aQTLs in rice populations under normal and salt conditions

Table S10. List of *trans*-3'aQTLs in rice populations under normal conditions

Table S11. List of *trans*-3'aQTLs in rice populations under salt conditions

Table S12. List of *cis*-eQTLs in rice populations under normal and salt conditions

Table S13. A list of primers



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