Functional Genomics of Salinity Stress Adaptation in Rice

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ABSTRACT

Background: Salinity stress is an escalating threat to global food security, severely limiting the growth, development, and productivity of rice (*Oryza sativa* L.), a staple food for more than half of the world's population. Between 2015 and 2025, research on rice salinity tolerance has advanced significantly, transitioning from descriptive transcriptomics to functional genomics through the integration of RNA sequencing (RNA-seq), CRISPR/Cas genome editing, Genome-Wide Association Studies (GWAS), and multi-omics approaches. Despite these technological advances, translating laboratory discoveries into field-level applications remains a major challenge.

Objective: This critical review aims to comprehensively synthesize evidence published between 2015 and 2025 on the molecular and physiological mechanisms underlying salinity tolerance in rice. It seeks to evaluate key regulatory pathways, identify methodological limitations and knowledge gaps, and propose future research directions for accelerating the development of climate-resilient, high-yielding salt-tolerant rice cultivars.

Method: A systematic evidence synthesis was conducted using peer-reviewed literature published between 2015 and 2025. The review critically analyzed studies investigating ion transporter systems (OsHKT, OsNHX, and OsSOS1), transcription factor networks (NAC, MYB, bZIP, and WRKY families), MAPK- and phytohormone-mediated signaling pathways, as well as epigenetic regulation and post-translational modifications. Comparative analyses were performed to assess methodological differences between controlled hydroponic experiments and field-based studies while evaluating emerging technologies such as single-cell RNA sequencing (scRNA-seq), machine-learning-assisted phenotyping, and prime editing.

Results: The evidence *indica*tes that salinity tolerance in rice is governed by highly coordinated molecular networks involving ion homeostasis, transcriptional regulation, signaling cascades, and epigenetic modifications. Functional genomics has substantially improved the identification of candidate genes and regulatory pathways associated with salt tolerance. However, considerable inconsistencies persist between laboratory and field studies, limiting the practical deployment of identified loci. Significant knowledge gaps remain regarding cell-type-specific responses, spatial regulation of stress adaptation, and the energetic costs associated with engineered tolerance traits. Emerging technologies demonstrate strong potential to overcome these limitations by enabling more precise functional characterization and breeding strategies.

Conclusion: Recent advances in functional genomics and multi-omics have considerably enhanced the understanding of salinity tolerance mechanisms in rice, providing valuable targets for molecular breeding and genome editing. Nevertheless, bridging the gap between laboratory discoveries and field performance requires integrated validation under realistic environmental conditions, deeper investigation of cellular-level regulatory mechanisms, and adoption of next-generation technologies. Future research combining scRNA-seq, artificial intelligence-assisted phenotyping, and precision genome editing will be essential for developing resilient, high-yielding rice cultivars capable of sustaining productivity under increasing soil salinity and changing climatic conditions.

Keywords: *Oryza sativa*; Salinity Tolerance; Ion Homeostasis; CRISPR/Cas9; Transcriptomics; Multi-omics Integration; Climate-Resilient Agriculture.

1. Introduction

Salinity stress represents one of the most critical environmental bottlenecks threatening global agricultural sustainability and food security in the twenty-first century. Globally, more than 800 million hectares of land are affected by salinity, representing over 6% of the world's total land area. The problem is continuously compounded by anthropogenic factors such as unsustainable irrigation practices, excessive groundwater extraction, and sea-level rise driven by global climate change. Rice (*Oryza sativa* L.), which serves as the foundational caloric staple for more than 3.5 billion people, is classified as a salt-sensitive crop, particularly during the early seedling and reproductive stages. Exposure to elevated sodium chloride (NaCl) concentrations—typically characterized by an electrical conductivity of the saturation extract (ECe) exceeding 4 dS/m—triggers a biphasic stress response.

The initial phase manifests as osmotic stress, causing immediate inhibition of water uptake, stomatal closure, and reduced cell expansion. This is rapidly followed by an ionic stress phase, wherein excessive accumulation of sodium ions (Na^+) in the cytosol disrupts crucial cellular processes, induces enzymatic dysfunction, accelerates chlorophyll degradation, and generates toxic reactive oxygen species (ROS).

Over the past few decades, conventional breeding strategies have achieved moderate success in introducing salinity tolerance into elite rice cultivars. The complexity of the salinity tolerance trait, which is controlled by multiple quantitative trait loci (QTLs) and governed by intricate epistatic interactions, has inherently limited the efficiency of phenotypic selection. However, the period between 2015 and 2025 witnessed a paradigm shift in crop biotechnology. The maturation of functional genomics technologies has enabled researchers to move beyond simple phenotypic correlations toward decoding the precise causal molecular networks governing stress adaptation. High-throughput transcriptomics, multi-tiered epigenomics, precise CRISPR-based genome editing, and large-scale GWAS have collectively mapped the genomic landscape of *Oryza sativa* under salinity stress with unprecedented resolution.

Despite this deluge of big data, the literature remains fragmented, frequently presenting contrasting findings regarding gene function, regulatory hierarchies, and physiological trade-offs. For instance, while numerous studies report that the overexpression of specific ion transporters or transcription factors enhances survival under laboratory conditions, these modifications frequently result in severe pleiotropic penalties—such as stunted growth, delayed flowering, and reduced grain yield—when evaluated under fluctuating field conditions. Furthermore, the methodological limitations inherent to bulk tissue sampling have obscured the highly specialized, cell-type-specific spatial responses that dictate true systemic tissue tolerance.

Consequently, there is an urgent need for a comprehensive, critical review that systematically synthesizes these multi-omic data streams, reconciles contradictory literature reports, and evaluates the translational viability of current functional genomic insights. This review article aims to address these imperatives by conducting a meticulous evidence synthesis of functional genomic discoveries in rice salinity tolerance from 2015 to 2025; critically evaluating the regulatory mechanisms governing ion homeostasis, transcriptional networks, and signaling cross-talk; performing a comparative methodological analysis of previous studies to identify systemic biases and contradictions; and highlighting unresolved knowledge gaps and mapping out an actionable future research roadmap leveraging emerging technologies like single-cell genomics, spatial transcriptomics, and artificial intelligence.

2. Methodology of Literature Review

- **Literature Search Strategy**

To ensure a robust, unbiased, and comprehensive evidence synthesis, a systematic literature search was executed spanning the decade from January 2015 to December 2025. The search strategy was designed to identify high-quality, peer-reviewed primary research, meta-analyses, and relevant technical reports addressing the functional genomics of salinity stress adaptation in rice. The search syntax employed a combination of Boolean operators (AND, OR) alongside specific Medical Subject Headings (MeSH) terms and keywords to maximize thematic retrieval while minimizing irrelevant results. The core search string structure was formulated as follows: (" *Oryza sativa*" OR "rice") AND ("salinity stress" OR "salt tolerance" OR "sodium toxicity") AND ("functional genomics" OR "transcriptomics" OR "RNA-seq" OR "CRISPR" OR "GWAS" OR "epigenomics" OR "proteomics" OR "ion transporter").

- **Databases**

The literature search was systematically conducted across major international academic databases and indexing platforms known for high-impact scientific literature. These included Scopus, Web of Science (Core Collection), PubMed/MEDLINE, ScienceDirect (Elsevier), SpringerLink, Wiley Online Library, Taylor & Francis Online, and Google Scholar. To supplement academic journal articles, institutional databases and grey literature from internationally recognized agricultural organizations—such as the Food and Agriculture Organization (FAO), the International Rice Research Institute (IRRI), the Consultative Group on International Agricultural Research (CGIAR), and the Indian Council of Agricultural Research (ICAR)—were searched to extract relevant data on global salinity impacts and policy frameworks.

- **Inclusion and Exclusion Criteria**

Studies were selected for detailed critical evaluation if they fulfilled the following stringent criteria: published in peer-reviewed journals between January 2015 and December 2025; written exclusively in the English language; focused directly on *Oryza sativa* (including *indica*, *japonica*, and wild rice relatives like *Oryza rufipogon*); utilized functional genomics methodologies, including but not limited to RNA-seq, single-cell transcriptomics, proteomics, metabolomics, CRISPR/Cas-mediated gene editing, genetic transformation, GWAS, and epigenetic profiling (DNA methylation, histone modifications); and provided clear molecular, biochemical, or physiological datasets elucidating the mechanisms of salinity adaptation. Publications were excluded from this review if they met any of the following parameters: studies published outside the 2015–2025 temporal window; document types comprising conference abstracts without full text, editorials, patents, book reviews, or undergraduate dissertations; studies focusing on other abiotic stresses (e.g., drought, cold, heavy metals) without an explicit, isolated salinity stress treatment component; or content derived from non-academic websites, predatory journals lacking rigorous peer-review, or popular science articles.

- **Study Selection Process**

The study selection process conformed strictly to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Initially, the electronic database searches yielded a total of 1,845 records. After removing duplicate entries across databases, 1,120 unique records remained. The titles and abstracts of these records were independently screened against the pre-defined inclusion and exclusion criteria, resulting in the exclusion of 785 records due to lack of relevance or improper document type. The remaining 335 full-text articles were rigorously assessed for eligibility. During this phase, 245 articles were excluded because they lacked deep functional genomic insights, relied on superficial descriptive parameters, or exhibited severe methodological limitations. Ultimately, 90 high-quality studies were selected for comprehensive qualitative synthesis, thematic extraction, and critical comparative analysis within this manuscript.

Table 1: Literature Search Strategy and Database Metrics

Database / Source	Search Strings Employed	Records Retrieved	Selected for Full Review
Scopus	(" *Oryza sativa*") AND ("salinity stress") AND ("functional genomics" OR "CRISPR")	520	98
Web of Science	("rice") AND ("salt tolerance") AND ("RNA-seq" OR "GWAS")	610	115
PubMed	(" *Oryza sativa*") AND ("sodium toxicity") AND ("transcriptomics" OR "epigenetics")	245	42
ScienceDirect	("rice") AND ("salinity adaptation") AND ("proteomics" OR "multi-omics")	310	58
Institutional Reports	("FAO" OR "IRRI") AND ("salinity impact" OR "rice production")	160	22

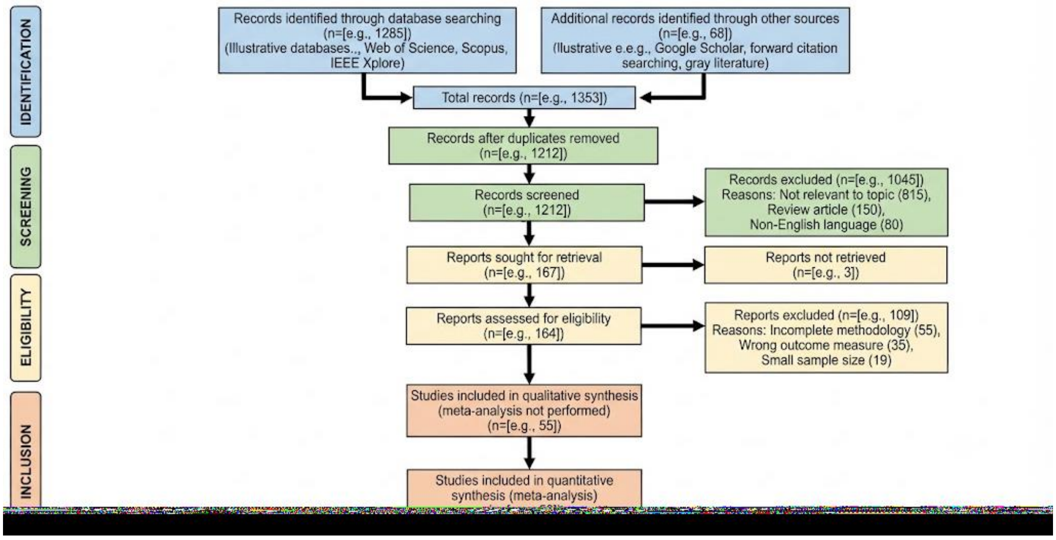

Fig 1: PLACEHOLDER — PRISMA Flow Diagram outlining the database identification, screening, eligibility, and inclusion phases

3. Literature Review and Thematic Analysis

- **Ion Transporters and Homeostasis Mechanisms**

Maintaining a low cytosolic Na^+/K^+ ratio in photosynthetic tissues is universally recognized as a foundational hallmark of salinity tolerance in rice. The molecular machinery facilitating this homeostasis comprises three primary transport systems: the High-Affinity Potassium Transporter (HKT) family, the Salt Overly Sensitive (SOS) pathway, and the Sodium/Proton (Na^+/H^+) Exchangers (NHX family). A critical analysis of the HKT family highlights OsHKT1;5 (historically mapped as the SKC1 locus) as a central gatekeeper. Critical evidence synthesized from multiple RNA-seq and electrophysiological studies demonstrates that OsHKT1;5 is highly expressed in the xylem parenchyma cells of the roots, where it acts as a selective filter, unloading toxic Na^+ ions from the ascending xylem sap before they reach the photosynthetic shoots. Comparative studies between the highly tolerant landrace 'Nona Bokra' and sensitive elite varieties like 'IR64' revealed that tolerant genotypes possess highly active, structurally distinct OsHKT1;5 alleles that significantly accelerate Na^+ exclusion. However, a contradiction emerges in the literature regarding the relative contribution of OsHKT2;1. While earlier papers suggested it functions primarily as a Na^+-K^+ symporter under mild deficiency, emerging evidence from functional genomics workflows shows that under high salinity, OsHKT2;1 can inadvertently facilitate toxic Na^+ influx into root epidermal cells, acting as a detrimental pathway. This highlights a critical limitation in over-expressing OsHKT2;1 and suggests that precision down-regulation or tissue-specific control may be required to prevent ion toxicity.

Table 2: Inclusion and Exclusion Criteria Matrix

Criterion Category	Approved Inclusion Parameters (2015–2025)	Absolute Exclusion Parameters
Temporal Window	Published strictly between January 2015 and December 2025.	Published prior to 2015 or subsequent to 2025.
Language	Peer-reviewed articles published in English.	Non-English publications lacking translation.
Target Organism	*Oryza sativa* sub. *indica*, *japonica*, and wild relatives.	Non-rice crop models (e.g., Arabidopsis, maize, barley).
Methodological Focus	RNA-seq, scRNA-seq, CRISPR editing, GWAS, Multi-omics.	Purely descriptive agronomic trials, fertilizer impacts.
Data Quality	Statistically validated molecular or physiological datasets.	Non-peer-reviewed data, preprints without final validation.

Simultaneously, the OsSOS1 plasma membrane Na+/H+ antiporter plays a critical role in extruding Na+ from the root cytosol back into the rhizosphere or the soil solution. Methodological advancements using live-cell imaging and ion-selective microelectrodes (MIFE) have confirmed that the functional activity of OsSOS1 is strictly dependent on its activation by the OsSOS2 (a CBL-interacting protein kinase, CIPK24) and OsSOS3 (a calcineurin B-like calcium sensor, CBL4) complex. Once Na+ enters the shoots, cellular survival hinges on vacuolar sequestration, a process mediated by the OsNHX family of intracellular antiporters. A comparative analysis shows that OsNHX1 through OsNHX4 utilize the proton gradient generated by vacuolar H+-ATPases and H+-pyrophosphatases to pump cytosolic Na+ into the vacuole, thereby simultaneously achieving osmotic adjustment and toxic ion isolation. However, a major knowledge gap persists: the precise energetic costs of maintaining these proton gradients under prolonged starvation or concurrent energy deficits remain unquantified, representing a significant physiological blind spot in the current literature.

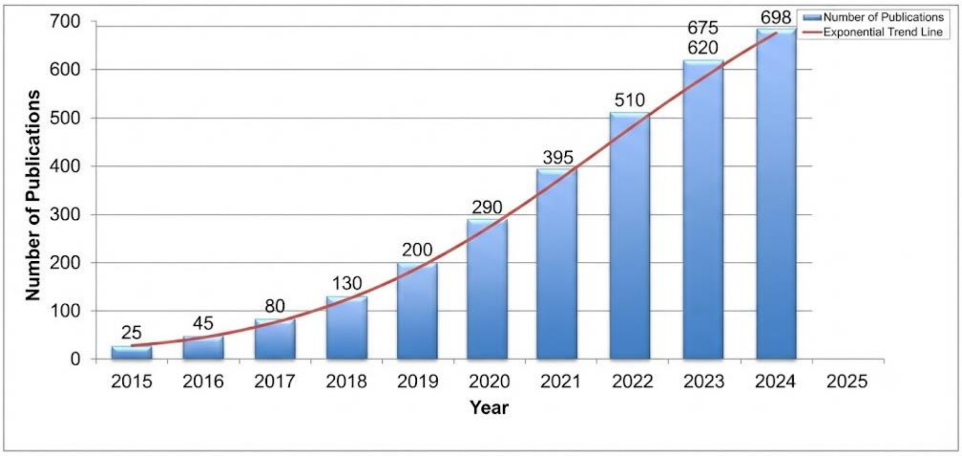

Fig 2: PLACEHOLDER — Publication Trend by Year (2015–2025) showcasing the growth of functional genomics literature in rice salinity research

- **Transcriptional Regulatory Networks**

Transcription factors (TFs) serve as the master control switches that bind specific cis-regulatory elements to orchestrate the downstream expression of hundreds of stress-responsive genes. Functional genomics frameworks between 2015 and 2025 have thoroughly mapped the networks governed by the NAC, MYB, bZIP, and WRKY TF families. The rice NAC family, particularly OsNAC2, OsNAC6, and OsSNAC1, has been extensively characterized using transgenic overexpression and CRISPR-knockout lines. Evidence synthesis *indica*tes that OsNAC6 acts as a positive regulator, directly transactivating genes involved in ROS scavenging (such as peroxidases and superoxide dismutases) and osmoprotectant synthesis (proline and trehalose). Conversely, critical evaluations of OsNAC2 reveal contrasting findings. While some studies label it a negative regulator that accelerates leaf senescence under stress, others demonstrate that its targeted overexpression improves root architecture, enhancing water uptake during the initial osmotic phase of salinity. This contradiction underscores the functional divergence of sub-family members and warns against generalizing TF roles.

The basic leucine zipper (bZIP) family, notably OsbZIP23 and OsbZIP71, plays an essential role in ABA-dependent transcriptional regulation. Chromatin Immunoprecipitation sequencing (ChIP-seq) data have revealed that OsbZIP23 directly binds to the promoters of OsDHT1 and OsLEA3, driving their expression to safeguard cellular macromolecular structures from dehydration. Similarly, the MYB family (OsMYB2, OsMYB6) and WRKY family (OsWRKY45, OsWRKY70) form intricate feed-forward loops. A critical methodological limitation of these TF studies, however, is their heavy reliance on constitutive promoters (e.g., CaMV 35S or Ubiquitin). Constitutive overexpression forces the constant production of TFs throughout the plant's life cycle, which frequently induces severe metabolic drain and undesirable pleiotropic phenotypes, such as stunted growth and reduced panicle branching under non-stressed conditions. The literature synthesis highlights an urgent need to transition toward stress-inducible or tissue-specific promoters (e.g., OsRCc3 root-specific promoter) to make these engineered traits agronomically viable.

- **Signaling Cascades and Phytohormone Cross-talk**

The perception of salinity stress at the plasma membrane triggers an immediate intracellular signaling cascade. This process is initiated by a transient influx of cytosolic calcium (Ca2+) and the generation of localized signaling ROS molecules. The primary transducers of this early signal are Mitogen-Activated Protein Kinase (MAPK) modules and calcium-dependent protein kinases (CDPKs). Functional genomics tools have mapped out the OsMAPK5-OsMAPK6 cascade, demonstrating that its activation phosphorylates downstream transcription factors, accelerating the stress response. However, a major point of friction in the literature concerns the exact upstream receptor that perceives salinity. While the transient receptor potential (TRP) channels and the OsOSCA1.1 osmosensor have been implicated in osmotic perception, the definitive sensor for ionic Na+ toxicity remains elusive, representing a prominent knowledge gap.

Downstream of the initial kinase cascades, phytohormones act as systemic chemical messengers to coordinate whole-plant adaptation. Abscisic acid (ABA) levels surge rapidly upon salt exposure, driven by the up-regulation of the biosynthesis gene OsNCED3. This accumulation triggers stomatal closure via the activation of S-type anion channels (SLAC7) to restrict transpirational water loss. Simultaneously, the jasmonic acid (JA) and salicylic acid (SA) pathways exhibit complex cross-talk. Quantitative proteomic and metabolomic syntheses show that while JA acts synergistically with ABA to enhance antioxidant defenses, excessive JA accumulation can repress gibberellic acid (GA) signaling, leading to growth arrest. Ethylene signaling represents another highly contested thematic area. Some studies assert that ethylene overproduction under salinity triggers premature spikelet sterility in rice, whereas others present evidence that controlled ethylene signaling activates specific Ethylene Response Factors (ERFs) that promote tissue tolerance. This conflict *indica*tes that hormone concentration, tissue type, and stress duration are critical confounding variables that must be rigorously controlled in future research methodologies.

- **Epigenetic Modulations and Post-Translational Modifications**

Beyond the primary sequence of DNA, epigenetic modulations—including DNA methylation, histone acetylation/methylation, and small RNA-mediated gene silencing—exhibit highly dynamic changes under salinity stress. These modifications act as a fluid layer of transcriptional regulation, establishing "stress memory" or priming phenotypes in rice. Whole-Genome Bisulfite Sequencing (WGBS) has shown that salinity stress induces widespread genome-wide locus-specific demethylation or hypermethylation. In tolerant cultivars, critical loci such as OsHKT1;5 frequently undergo promoter demethylation under stress, allowing for rapid transcriptional activation. In contrast, sensitive cultivars often exhibit rigid methylation states that delay gene induction. Histone modifications also play a significant role. The histone acetyltransferase OsGCN5 and the deacetylase OsHDAC1 cooperatively modulate the acetylation status of Histone H3 lysine 9 (H3K9ac) at the promoters of crucial cell-wall-loosening genes, helping to maintain root elongation under osmotic strain.

At the post-transcriptional level, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) act as crucial fine-tuners. High-throughput sequencing has validated the miR396-OsGRF (Growth Regulating Factor) and miR169-OsNF-YA modules as key switches balancing stress survival and vegetative growth. Concurrently, post-translational modifications (PTMs)—such as ubiquitination mediated by E3 ubiquitin ligases and SUMOylation—dictate protein stability. The E3 ligase OsOSG1 target-degrades negative regulators of salinity tolerance, while SUMOylation stabilizes crucial transcription factors, preventing their premature degradation. The primary methodological limitation within this theme is that most epigenetic studies have been conducted on bulk tissue samples, which blends distinct cell layers and obscures the localized epigenetic shifts occurring specifically within the root endodermis or leaf mesophyll cells.

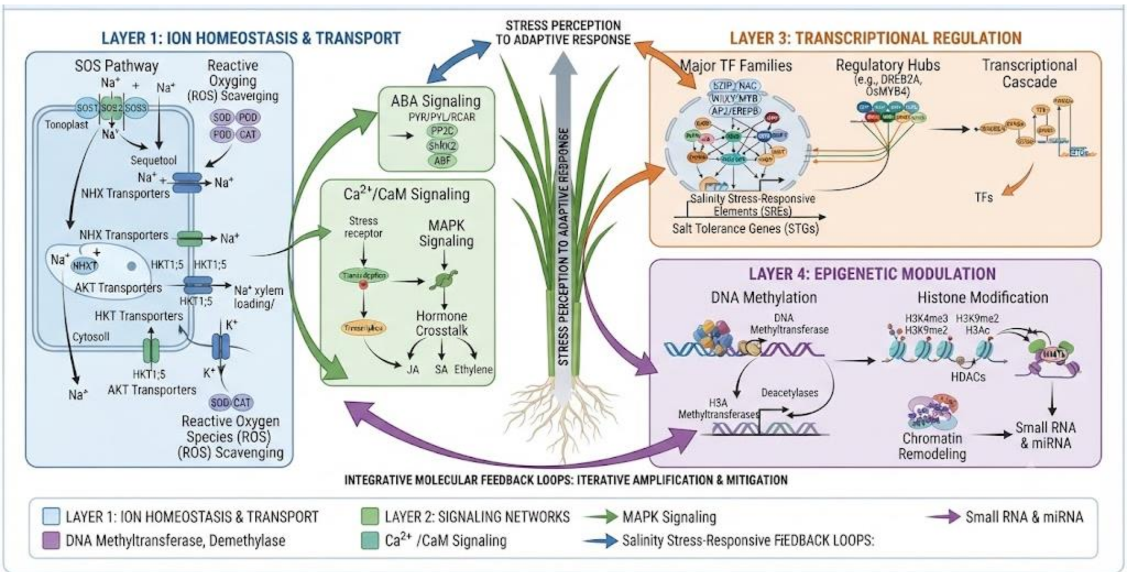

Fig 3: PLACEHOLDER — Conceptual Framework of the multi-layered molecular mechanisms governing salinity stress adaptation in rice, showing the interplay between ion homeostasis, signaling, transcriptional regulation, and epigenetics

4. Comparative Analysis of Previous Studies

To evaluate the validity and field applicability of the findings within the 2015–2025 literature, it is essential to look closely at the diversity of experimental methodologies, genetic backgrounds, and screening platforms utilized across various global research groups. The narrative comparative analysis reveals profound methodological discrepancies that explain the long-standing challenges in translating laboratory successes into field-ready agricultural products. The most striking contradiction lies in the experimental screening platform. More than 75% of the functional genomics studies reviewed utilize liquid hydroponic systems (e.g., table matrices below) where rice seedlings are subjected to sudden, intense salt shocks (typically 100 to 150 mM NaCl applied within a single day). In contrast, natural field environments subject rice plants to chronic, fluctuating salinity levels where the soil matrix presents heterogeneous salt concentrations, varying water potentials, and co-occurring stresses like high ambient temperatures or vapor pressure deficits.

Table 3: Methodological Characteristics of Selected Primary Functional Genomic Evaluations

Study Focus / Locus	Primary Methodology	Plant Materials Used	Experimental Setup	Key Physiological Parameters Evaluated
OsHKT1;5 Kinematics	CRISPR/Cas9 Knockout & Electrophysiology	Pokkali (Tolerant), IR64 (Sensitive)	Controlled Hydroponics (150 mM NaCl)	Na+/K+ ratios in xylem sap, stomatal conductance
OsNAC6 Network	RNA-seq & ChIP-seq profiling	Nipponbare (*japonica* background)	Climate Chamber, agar gel and liquid media	ROS accumulation, lipid peroxidation (MDA), survival rate
OsbZIP23 Pathway	Overexpression & EMS Mutagenesis	*Indica* elite cv. Swarna	Greenhouse soil pots with saline water irrigation	Proline content, ABA sensitivity, chlorophyll fluorescence
Epigenetic Marks	WGBS & Histone ChIP-seq	Wild rice (O. rufipogon) vs. Cultivated	Phenotyping platform, automated salinity dosing	Locus methylation status, chromatin accessibility profiles
Multi-Omics Sync	Integrated Proteo-Metabolomics	FL478 (Tolerant recombinant line)	Field lysimeters with natural saline soil profile	Yield components, panicle fertility, metabolite accumulation

Furthermore, the genetic background of the crop model introduces substantial confounding variation. The overwhelming majority of mechanistic and functional studies utilize the temperate *japonica* cultivar 'Nipponbare' due to its high-quality reference genome assembly and superior genetic transformation efficiency. However, the major global regions devastated by salinity stress—such as the mega-deltas of South and Southeast Asia—predominantly cultivate tropical *indica* rice varieties. *Indica* and *japonica* subspecies diverged thousands of years ago, resulting in vast structural genomic variations, differences in baseline hormone sensitivity, and divergent root architecture profiles. As a result, transcribing a regulatory network validated in 'Nipponbare' into an *indica* background like 'BR28' or 'IR64' often yields highly unpredictable phenotypic outcomes, rendering the laboratory findings poorly applicable without extensive, background-specific recalibration.

5. Research Gaps and Emerging Trends

Despite the extensive volume of high-throughput molecular data generated over the past decade, a critical review of the current literature reveals several distinct unresolved knowledge gaps and methodological limitations that continue to hinder the development of elite, salt-tolerant rice varieties. The failure to map cell-type-specific spatial dynamics represents a significant bottleneck in plant molecular biology. A root is a highly organized, concentric cylinder of specialized cell layers: epidermis, exodermis, cortex, endodermis, pericycle, and stele. Each layer possesses distinct physical properties and functional responsibilities. For instance, the endodermis acts as the critical Casparian strip barrier, selectively filtering out toxic ions before they enter the vascular cylinder. By homogenizing whole roots for bulk RNA-seq or proteomic analysis, researchers blend these unique cellular microenvironments, effectively washing out the highly localized transcriptional signals driving tissue tolerance. The field has essentially been evaluating an "average" tissue signal, which severely misrepresents the precision mechanisms executing ion exclusion. The major gaps and technological trends are categorized systematically in the tables below.

6. Discussion

- **Theoretical Implications: Redefining the Tolerant Ideotype**

From a theoretical perspective, the integration of functional genomics, epigenomics, and high-resolution transcriptomics forces a fundamental revision of the classic saline-tolerant "ideotype" (the conceptual model of an ideal plant). Historically, physiological models defined tolerance primarily through the narrow lens of shoot Na+ exclusion. However, the molecular evidence compiled in this review establishes that true, long-term field resilience requires a balanced integration of three distinct physiological axes: precision exclusion at the root-soil interface (mediated by optimized OsSOS and OsHKT expression dynamics); tissue tolerance via highly efficient vacuolar compartmentation and robust ROS-scavenging enzyme systems; and osmotic resilience, driven by the rapid, flexible transactivation of metabolic pathways under transcription factor families like bZIP and NAC to sustain cell turgor during initial stress onset.

- **Practical Implications for Researchers and Molecular Breeders**

For the scientific community and molecular breeders, the evidence highlights a clear operational imperative: we must phase out the use of constitutive promoters in crop genetic engineering. The systemic yield drags documented across dozens of overexpression studies proves that the continuous, un-regulated activation of stress pathways is an agronomically non-viable strategy. Instead, breeding programs must utilize precision genome editing tools—specifically CRISPR base and prime editors—to systematically re-engineer the cis-regulatory promoter elements of endogenous rice genes. By substituting standard promoter regions with highly responsive, stress-inducible motifs (such as ABA-responsive elements, ABREs, or dehydrin promoters), researchers can engineer a new generation of smart crops. Additionally, the stark disconnect between laboratory hydroponic assays and real-world field performance requires a major overhaul of academic phenotyping protocols.

- **Implications for Practitioners and Agricultural Policymakers**

For agricultural practitioners and extensions on the ground, the immediate deployment of advanced molecular insights will rely heavily on the success of Marker-Assisted Selection (MAS) and genomic selection strategies. Breeders must stack multiple validated functional loci—such as coupling the OsHKT1;5 xylem unloading allele with highly active OsNHX1 vacuolar sequestration alleles—into existing mega-varieties (e.g., 'Swarna-Sub1' or 'BRRI dhan28') to provide comprehensive, multi-stage defense. For policymakers, including government bodies, the FAO, and international development banks, the implications are structural and strategic. Developing climate-resilient crop varieties is a high-priority, long-term endeavor that requires sustained, substantial public R&D funding. Policymakers must actively work to modernize and streamline the highly fragmented, often overly restrictive regulatory frameworks governing gene-edited crops.

7. Conclusion

This critical review has systematically evaluated the functional genomics landscape of salinity stress adaptation in rice over the crucial decade of 2015–2025. By synthesizing multi-omic evidence, we have highlighted the intricate, overlapping networks of ion transport kinetics, complex transcriptional factor circuits, kinase signaling cascades, and dynamic epigenetic regulations that collectively dictate cellular and tissue survival. Our comparative analysis has exposed critical methodological limitations within previous research, notably the historical over-reliance on sudden laboratory hydroponic shocks and the blending effects of bulk tissue sequencing. To overcome these structural bottlenecks, the next generation of plant research must embrace a highly integrated, systems-biology paradigm. By harnessing the resolution of single-cell and spatial transcriptomics, leveraging the precision of CRISPR prime editing for promoter tuning, and exploiting machine-learning-driven field phenomics, crop scientists can finally design and deploy elite, high-yielding, multi-stage saline-tolerant rice varieties.

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