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Speed Breeding for the Development of Salt-Tolerant and Early-Maturing Basmati Rice Genotypes via CRISPR/Cas9-Mediated Genome Editing of Stress-Responsive Regulators

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Abstract

Basmati rice, a premium aromatic variety central to the export economies of India and Pakistan (valued at US$14.17 billion and US$3.96 billion in 2024, respectively), faces severe productivity threats from soil salinity, which affects 20% of irrigated lands globally and causes annual losses of US$27.2 billion. Traditional breeding's slow cycles (6–10 years) hinder rapid adaptation, necessitating innovative approaches. This review explores the integration of CRISPR/Cas9-mediated genome editing targeting stress-responsive transcription factors (e.g., NAC, MYB, WRKY families) and negative regulators (e.g., OsARR1, OsARM1) to enhance ion homeostasis, osmotic adjustment, and early maturity with speed breeding (SB) protocols that compress generation times to 4–6 cycles per year via extended photoperiods (22h light/2h dark) and controlled environments. Case studies demonstrate successful introgression of Saltol QTL and editing of OsBADH2 for aroma retention, yielding salt-tolerant lines with EC thresholds up to 8–12 dS/m and 10–15% earlier maturity. Socio-economic analyses highlight potential yield gains of 20–30% in salinized regions, while regulatory frameworks in South Asia emphasize biosafety. This synergistic strategy accelerates climate-resilient Basmati development, ensuring food security and economic stability amid escalating environmental pressures.

Keywords Basmati Rice, Salinity Tolerance, CRISPR/Cas9, Genome Editing, Speed Breeding, Transcription Factors, Saltol QTL, OsBADH2, NAC/MYB/WRKY, Early Maturity, Climate-Smart Agriculture, Food Security

1. Introduction

The global agricultural sector is currently navigating through a period of profound transition, dictated by the dual pressures of a burgeoning human population and the accelerating degradation of arable land due to climate change (Hussain et al., 2022). Rice (Oryza sativa L.) remains at the

epicenter of global food security, providing the primary caloric intake for over half of the world's population (Haque et al., 2018). Within the diverse germplasm of rice, Basmati genotypes represent a unique and economically vital segment, prized globally for their exquisite aroma, extra-long slender grains, and superior cooking qualities (Zafar et al., 2025). Cultivated predominantly in the specific agro-climatic regions of the Indo-Gangetic plains, Basmati rice is not merely a food crop but a high-value commodity that anchors the export economies of India and Pakistan (TradeInt, 2025). However, the continued productivity of Basmati genotypes is severely threatened by soil salinity, a stressor that is compounded by traditional breeding cycles that are too slow to keep pace with environmental shifts (Sindhushree et al., 2025).

The emergence of integrated biotechnological strategies, specifically the combination of CRISPR/Cas9-mediated genome editing and Speed Breeding (SB), offers a revolutionary pathway to develop climate-smart Basmati varieties (IRRI, 2024). By precisely targeting stress-responsive regulators such as transcription factors in the NAC, MYB, and WRKY families and optimizing the rate of generation turnover, researchers can now achieve genetic gains in a fraction of the time required by conventional methods (Beyer, 2025). This report provides an exhaustive analysis of the mechanisms, technologies, and socio-economic imperatives driving the development of salt-tolerant and early-maturing Basmati rice (Kalubarme et al., 2026).

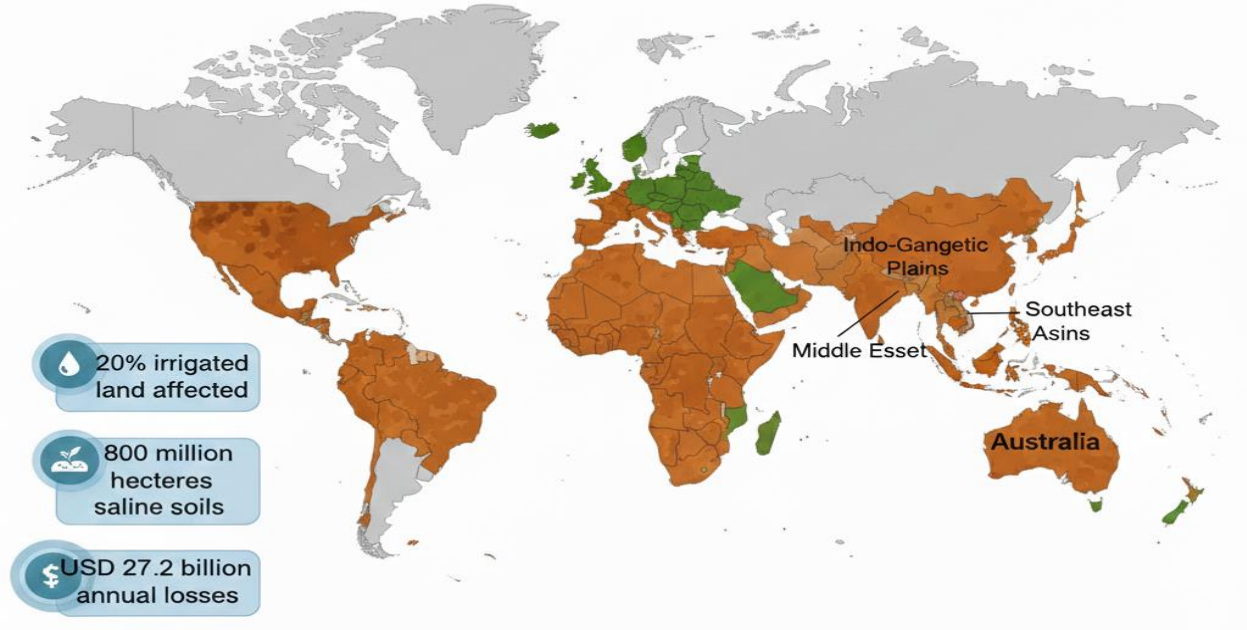
2. The Socio-Economic Landscape and the Challenge of Salinity

The economic importance of Basmati rice is underscored by its dominance in the global aromatic rice market. In the 2024-25 fiscal year, the global rice export market reached a record value of US 39.1 billion, with India and Pakistan emerging as the leading contributors to the premium segment (Shahidullah et al., 2025). India’s rice exports alone were valued at US 14.17 billion, while Pakistan’s exports reached US 3.96 billion, with Basmati rice serving as the flagship product for both nations (USDA, 2024).

2.1. The Growing Threat of Soil Salinization

Soil salinity is a pervasive abiotic stress that currently affects approximately 20% of the world’s irrigated land and over 800 million hectares globally. This salinized area is expanding at an annual rate of 1% to 2% due to factors such as rising temperatures, high evaporation rates, and poor drainage systems in irrigated lowlands (Gebrehiwot, 2018). For rice, which is notoriously sensitive to salt stress, the impact is devastating. Agricultural economists estimate that salt-induced soil deterioration results in an annual loss of USD 27.2 billion in irrigated agriculture (Sackey et al., 2015). In Pakistan and India, where Basmati cultivation is concentrated, salinity has already reduced yields on many farms, particularly in coastal and poorly managed irrigated regions (Ahmed, 2021). Soil salinization is a rapidly expanding environmental constraint affecting rice production worldwide. The global distribution of salinity-affected agricultural lands and their economic implications for rice cultivation are illustrated in figure 1.

Figure 1: Global Impact on Soil Salinity on Rice Production



2.2. Physiological Vulnerability of Basmati Genotypes

Basmati rice is exceptionally vulnerable to salinity during two critical growth stages: the seedling stage and the reproductive phase. Field studies have demonstrated that even moderate salinity levels, such as a seasonal average of 1.9 dS/m, can significantly reduce grain yields, challenging earlier guidelines that set the threshold at 3.0 dS/m (Grattan et al., 2002). When exposed to high concentrations of Na+ and Cl- ions, Basmati plants exhibit a range of detrimental symptoms, including stunted growth, leaf chlorosis, reduced tiller density, and increased floret sterility (Qureshi, 2024).

Table 1: Economic and Agricultural Indicators of the Global Rice Sector

Economic and Agricultural Indicators	Value / Impact Description	Source
Global Rice Export Value (2024)	US 39.1 Billion	(TradeInt, 2025)
Annual Increase in Saline Land	1% to 2%	(Hussain et al., 2022)
Global Economic Loss (Salinity)	USD 27.2 Billion	(Hussain et al., 2022)
Rice Yield Threshold for Salinity	1.9 dS/m to 3.0 dS/m	(Grattan et al., 2002)
Yield Reduction per 1 dS/m increase	12% (above threshold)	(Sindhushree et al., 2025)

The physiological damage is rooted in ionic toxicity and osmotic stress. The accumulation of Na+ in the cytoplasm disrupts essential metabolic processes, while the low osmotic potential of saline soils restricts water uptake, leading to a state of physiological drought (Mansour, 2023). In Basmati germplasm, there is significant variation in response; for instance, genotypes like Bas. 370 have shown a superior ability to exclude Na+ from their shoots, maintaining over 50% of their control yield even under high salinity, whereas other lines like PK 49626 are highly susceptible (Mahmood, 2009).

3. Molecular Orchestration of Salt Tolerance in Rice

The development of salt-tolerant Basmati requires a deep understanding of the molecular pathways that rice uses to mitigate ionic and osmotic stress. These pathways are primarily centered on ion transport, osmotic adjustment, and the regulation of oxidative damage (Ullah et al., 2022).

3.1. Ionic Homeostasis and the HKT Transporter Family

The maintenance of a favorable Na+/K+ ratio in the cytoplasm is the cornerstone of salinity tolerance. Rice employs several ion transporters to prevent Na+ from reaching toxic levels in photosynthetic tissues (Chakraborty et al., 2018). The High-affinity Potassium Transporter (HKT) gene family, particularly OsHKT1;5, is a critical regulator in this process. OsHKT1;5 is localized in the xylem parenchyma cells and functions by unloading Na+ from the xylem sap, thereby preventing its translocation to the shoots (Khan et al., 2020).

Complementing HKT transporters is the Salt Overly Sensitive (SOS) signaling pathway. The SOS pathway consists of three main components: SOS1 (a plasma membrane Na+/H+ antiporter), SOS2 (a protein kinase), and SOS3 (a calcium-binding protein) (Sen, 2021). When salt stress triggers a calcium spike, the SOS3-SOS2 complex activates SOS1, leading to the efflux of Na+ from the cytoplasm into the apoplast. Furthermore, vacuolar Na+/H+ antiporters such as NHX1 facilitate the sequestration of Na+ into vacuoles, which not only detoxifies the cytoplasm but also aids in osmotic adjustment (El Mahi et al., 2019).

3.2. Osmotic Adjustment and Secondary Metabolites

To maintain water uptake under saline conditions, rice plants synthesize and accumulate compatible osmolytes such as proline, glycine betaine, and soluble sugars. These compounds lower the cellular osmotic potential without interfering with normal metabolic functions (Chakraborty & Wylie, 2025). The accumulation of these metabolites is regulated by complex signaling networks involving phytohormones like abscisic acid (ABA) and jasmonic acid (JA) (Li et al., 2025). Transcription factors, particularly those from the bZIP and MADS-box families, have been identified as key drivers of these osmotic adjustments (Liu et al., 2026).

3.3. ROS Scavenging and Oxidative Stress Management

Salinity stress inevitably leads to the overproduction of reactive oxygen species (ROS), including superoxide (O2.-), hydrogen peroxide (H2O2), and hydroxyl radicals (.OH). If not neutralized, ROS cause extensive damage to lipids, proteins, and DNA. Rice plants employ a robust antioxidant defense system involving enzymes such as superoxide dismutase (SOD), catalase (CAT), and various peroxidases to scavenge these toxic molecules (Fujita et al., 2020). The regulation of these antioxidant enzymes is often controlled by stress-responsive transcription factors, making them ideal targets for genetic engineering (Sachdev et al., 2021).

4. Genetic Architecture of Flowering Time and Maturation

Early maturation is a highly desirable trait for Basmati rice, as it allows for efficient water management and enables the crop to escape terminal heat or cold stresses . The timing of flowering, or "heading date," in rice is controlled by a complex network of quantitative trait loci (QTLs) that integrate environmental signals like photoperiod and temperature (Wei et al., 2010).

4.1. The Core Regulatory Network: Hd1, Ghd7, and DTH8

Three major genes Heading date 1 (Hd1), Grain number, plant height, and heading date 7 (Ghd7), and Days to heading 8 (DTH8) form the core of the photoperiodic flowering pathway in rice (Xue et al., 2008).

- **Ghd7:** Acts as a powerful floral repressor under long-day (LD) conditions. It suppresses the expression of Early heading date 1 (Ehd1), which is a key activator of the florigens Hd3a and RFT1 (Song et al., 2024).
- **Hd1:** An ortholog of the Arabidopsis CONSTANS gene, Hd1 can either promote or repress flowering depending on the day length and the presence of Ghd7 (Takagi et al., 2023).
- **DTH8:** Encodes a HAP3 subunit of the heme-activated protein complex and suppresses flowering under LD conditions while also influencing plant height and grain number (Sim et al., 2019).

4.2. Fine-Tuning Maturity via CRISPR/Cas9

Traditional breeding for early maturity often involves the introduction of loss-of-function alleles for these genes, which can lead to excessively early flowering and significant yield penalties. However, CRISPR/Cas9 technology allows for the precise "fine-tuning" of these traits (Gatica-Arias et al., 2025). By targeting the cis-regulatory regions (promoters) of Hd1, Ghd7, and DTH8, researchers have successfully created a continuum of flowering times in elite varieties (Pinto et al., 2023). This multiplex promoter-targeting (HMP) strategy enables the development of genotypes that head just early enough to escape seasonal stresses while maximizing their photosynthetic window (Monfort et al., 2025).

Table 2: Genetic Targets and Phenotypic Effects of CRISPR Editing

Target Gene	Primary Regulatory Role	Effect of CRISPR-Mediated Inactivation	Reference
Ghd7	Floral repressor (LD); increases plant height	Accelerated flowering; reduced height	(Xue et al., 2008)
Hd1	Photoperiod sensor; floral promoter/repressor	Quantitative variation in heading date	(Li et al., 2025)
DTH8	Floral repressor (LD); influences panicle size	Shorter plant height; earlier heading	(Wei et al., 2010)
OsRR22	Negative regulator of salt tolerance	Significantly improved salt tolerance	(Mansi & Danai, 2026; Sindhushree et al., 2025)
OsNAC113	Stress-responsive regulator	Enhanced water retention under salinity	(Tianjin Academy, 2025)

5. CRISPR/Cas9: A Precision Tool for Basmati Improvement

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system has emerged as the most potent tool for genome editing in crops (Alamillo et al., 2023). Its simplicity, high efficiency, and ability to target multiple genes simultaneously make it particularly suitable for improving complex traits like salt tolerance in Basmati rice (Qin et al., 2020).

5.1. Mechanism of Action

The CRISPR/Cas9 system operates as an RNA-guided endonuclease. It consists of the Cas9 protein and a single-guide RNA (sgRNA) that contains a 20-nucleotide sequence complementary to the target genomic site (Chang, 2022). The Cas9-sgRNA complex binds to the target DNA next to a protospacer adjacent motif (PAM) and generates a double-stranded break (DSB) (Nawaz et al., 2019). These DSBs are repaired by the cell's internal mechanisms, primarily through non-homologous end joining (NHEJ), which frequently introduces small insertions or deletions (indels)

that disrupt the target gene's function (Mansi & Danai, 2026). CRISPR/Cas9 enables precise manipulation of stress-responsive genes in rice through RNA-guided genome editing. The major steps involved in CRISPR-mediated gene modification in Basmati rice are presented in figure 2.

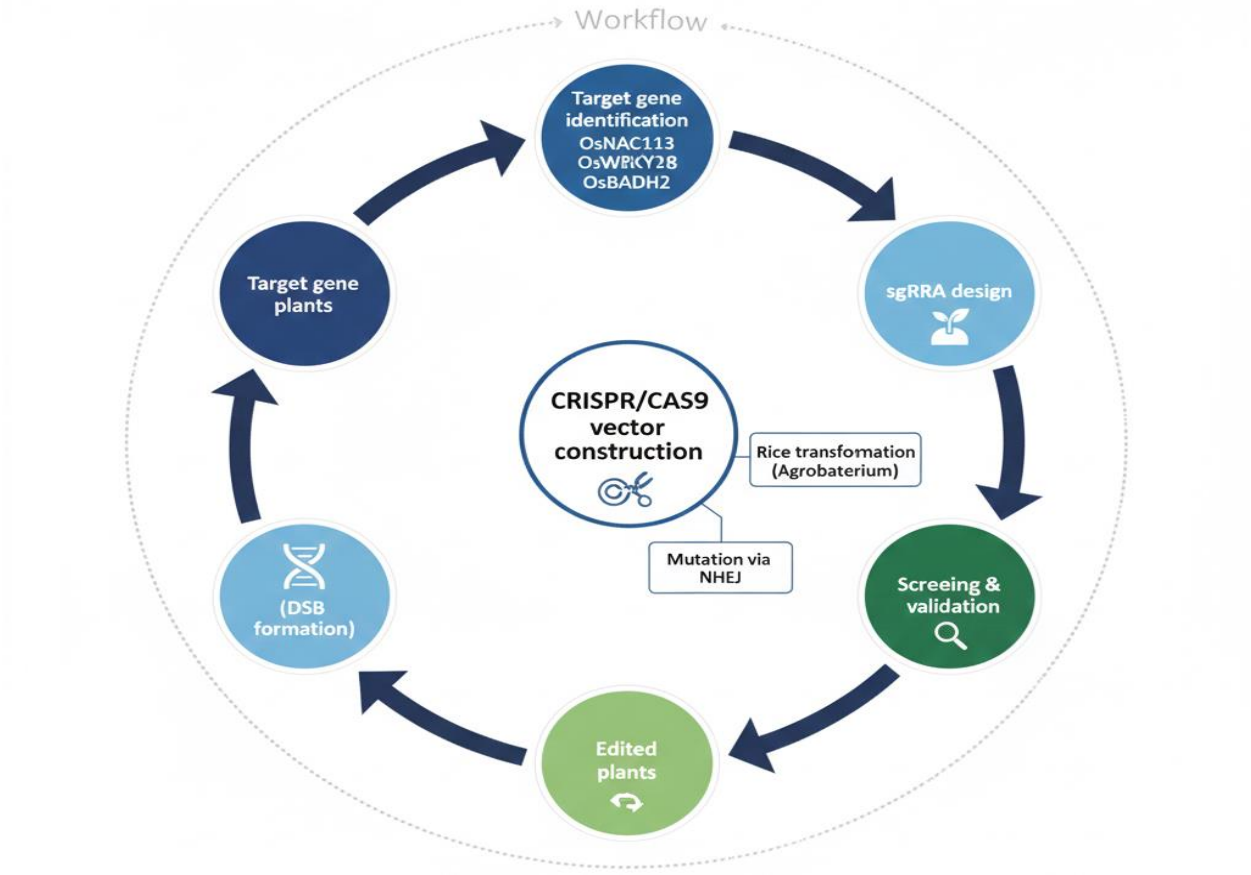
5.2. Multiplex Editing and tRNA-Processing

One of the most significant advancements in CRISPR technology is the ability to edit multiple genes concurrently. In rice, this is often achieved by utilizing the endogenous tRNA-processing system (Bindal & Rath. 2025). By stringing together multiple tRNA-sgRNA units in a single transcript, researchers can target several components of a stress-response pathway or combine stress tolerance with yield and quality traits (Ma et al., 2019). For Basmati, this means that salinity tolerance genes, flowering time regulators, and grain quality genes can be modified in a single transformation event (Zafar et al., 2020).

5.3. Case Study: Aroma Preservation and OsBADH2

The distinct aroma of Basmati rice is primarily due to the presence of 2-acetyl-1-pyrroline (2-AP). This trait is controlled by the betaine aldehyde dehydrogenase 2 (OsBADH2) gene on chromosome 8. In non-aromatic rice, a functional BADH2 enzyme prevents the accumulation of 2-AP (Shao et al., 2017). CRISPR/Cas9 has been used to successfully introduce mutations in the BADH2 gene of non-aromatic elite varieties, thereby "aromatizing" them (Shao et al., 2017). When developing salt-tolerant Basmati, it is critical to ensure that the genome editing process does not inadvertently compromise the BADH2 mutation, or to use CRISPR to revive the aroma in high-yielding, salt-tolerant backgrounds (Zafar et al., 2025).

Figure 2: CRISPR/CaS9 Genome Editing Workflow in Basmati Rice



6. Speed Breeding: Accelerating the Breeding Cycle

Traditional rice breeding is a slow process, often requiring 6 to 10 years to release a new variety. Speed Breeding (SB) addresses this bottleneck by manipulating environmental conditions such as light, temperature, and humidity to significantly shorten the plant's generation time (Shikari, 2025).

6.1. The SpeedFlower Protocol

The International Rice Research Institute (IRRI) has developed a groundbreaking SB protocol for rice called "SpeedFlower". This protocol can achieve up to 5 generations of rice per year, compared to the 1-2 generations typical of conventional breeding. The SpeedFlower protocol utilizes a two-stage light strategy (IRRI, 2024).

Table 3: SpeedFlower Protocol Parameters for Accelerated Generation Turnover

Environmental Factor	SpeedFlower Setting	Purpose in Breeding
Photoperiod (Initial)	24-hour Continuous Light	Optimizes growth and biomass (Singh et al., 2024).
Photoperiod (Reproductive)	10-hour Short Day	Induces early flowering in rice (Singh et al., 2024).
Light Spectrum	2R > 1B (Red-to-Blue ratio)	Promotes rapid maturation (Singh et al., 2024).
Light Intensity	800 micromol m-2 s-1	Supports high photosynthetic rates (Singh et al., 2024).
Day/Night Temp	32 C / 30 C	Accelerates metabolic processes (Singh et al., 2024).
Humidity	65%	Prevents transpiration stress (Singh et al., 2024).

The SpeedFlower protocol has been validated across 198 diverse rice genotypes, achieving flowering in as little as 52 to 60 days. This speed is crucial for rapidly advancing CRISPR-edited lines to homozygosity and conducting multi-generational stress testing (Fajardo et al., 2025).

6.2. Reducing Seed Maturity Duration

In addition to accelerating flowering, Speed Breeding can shorten the maturation phase. By harvesting seeds prematurely (approximately 15 days after anthesis) and treating them with gibberellic acid (GA3), researchers can reduce the seed maturity duration by up to 50% (Wanga et al., 2021). This comprehensive approach allows for the rapid development of stable, climate-resilient Basmati genotypes that are ready for field evaluation (Samantara et al., 2022).

7. Integrating CRISPR and Speed Breeding for Salinity Tolerance

The most effective strategy for developing salt-tolerant Basmati genotypes involves a three-stage integrated approach: targeting stress regulators, accelerating the cycle through SB, and validating performance through marker-assisted selection (MAS) (Yadav et al., 2020).

7.1. Targeting Stress-Responsive Transcription Factors

Transcription factors (TFs) are ideal targets for CRISPR editing because they act as master regulators of entire stress-responsive gene networks (Chakraborty & Wylie, 2025).

- **OsNAC113:** Researchers have identified OsNAC113 as a key regulator in rice's response to drought and salinity. Knocking out this gene using CRISPR-Cas9 has resulted in mutant plants that grow more vigorously and maintain higher water content under high salinity (Tianjin Academy, 2025).

- **OsWRKY18:** This WRKY TF acts as a positive regulator of salt tolerance by directly binding to the promoter of OsHKT1;5, thereby enhancing sodium exclusion. CRISPR can be used to either overexpress this TF or to precisely modify its regulatory elements to boost its expression under stress (Li et al., 2025).

7.2. Incorporating the Saltol QTL

A major quantitative trait locus for seedling-stage salinity tolerance, known as Saltol, has been extensively used in rice breeding. Saltol, located on chromosome 1, accounts for 43% to 70% of the phenotypic variation for salt tolerance at the seedling stage (Krishnamurthy et al., 2021). It encompasses several critical genes, including OsHKT1;5 and components of the SOS pathway (Hussain et al., 2022). Integrating the Saltol QTL into Basmati genotypes through marker-assisted breeding (MAB) provides a solid genetic foundation that can then be further enhanced through CRISPR-mediated editing of additional stress regulators (Sharma et al., 2025).

7.3. Performance of RILs: KKL(R) 3

The effectiveness of these integrated approaches is evidenced by the development of Recombinant Inbred Lines (RILs) like KKL(R) 3. This line, developed by transferring the Saltol QTL into an elite but sensitive background, has consistently outperformed traditional varieties (Kulkarni et al., 2020). Under saline conditions (3.0 dS/m and above), KKL(R) 3 maintained a yield of 3435.6 kg/ha, representing a significant improvement over standard salt-tolerant varieties like CSR 10 (Beulah et al., 2024).

Table 4: Grain Yield Performance of Salt-Tolerant vs. Sensitive Genotypes

Variety / Line	Yield (Normal)	Yield (Saline)	Traits
ADT 45 (Parent)	6000+ kg/ha	< 1000 kg/ha	Sensitive but high-yielding (Sindhushree et al., 2025).
FL478 (Donor)	N/A	High	Saltol donor variety (Sindhushree et al., 2025).
KKL(R) 3 (RIL)	6421.8 kg/ha	3435.6 kg/ha	Saltol + High Yield (Sindhushree et al., 2025).
CSR 10 (Check)	Moderate	2200 kg/ha	Traditional salt-tolerant variety (Sindhushree et al., 2025).

8. Regulatory Frameworks and Global Biosafety

The commercial deployment of gene-edited Basmati rice is contingent upon the regulatory landscape in producing nations like India and Pakistan, as well as the biosafety standards of major importing regions like the European Union (ISAAA, 2024).

8.1. India's Regulatory Milestone

In March 2022, India's Ministry of Environment, Forest and Climate Change issued a landmark memorandum exempting SDN1 and SDN2 genome-edited products from the rigorous biosafety assessments required for traditional GMOs (Koul et al., 2024). This exemption applies to gene-edited plants that are free from exogenous introduced DNA. This policy shift has significantly lowered the barriers for the adoption of CRISPR-edited Basmati varieties in India (Turnbull et al., 2021).

8.2. Pakistan's Institutional Reforms

Pakistan has also modernized its regulatory framework for biotechnology. The Seed (Amendment) Act 2024 established the National Seed Development and Regulatory Authority (NSDRA) to streamline varietal approval and adopt modern technologies (Awais et al., 2024). The National Biosafety Committee (NBC) now reviews and approves laboratory and field trials for genome-edited crops (USDA, 2024). As of 2025, several national institutes have received approval for CRISPR-mediated trait development in crops like wheat and cotton, and Basmati rice is a primary target for future releases (Ahmad et al., 2025).

8.3. Biosafety Concerns and the Off-Target Debate

While CRISPR technology is lauded for its precision, the possibility of unintended genetic changes remains a point of intense scientific and public debate (Sabrang India, 2023).

- **Off-target Effects:** Researchers have cautioned that Cas9 can occasionally cut DNA at unintended sites, leading to mutations that could potentially impact crop biochemistry or safety (Sachdev et al., 2021).
- **Genomic Rearrangements:** Some studies in tomatoes and human cells have shown that CRISPR can cause complex genomic rearrangements, such as chromothripsis-like effects (Gebrehiwot, 2018).
- **The Precautionary Principle:** Advocacy groups like the Coalition for a GM-Free India urge stringent regulatory oversight and comprehensive biosafety assessments before any gene-edited rice is released into farmers' fields (Song et al., 2024).
- To address these concerns, developers of gene-edited Basmati must employ high-fidelity Cas9 variants and conduct thorough whole-genome sequencing to confirm the absence of unintended mutations (Takagi et al., 2023).

9. Future Prospects and Sustainability

The integration of Speed Breeding and CRISPR/Cas9 is not merely a technical advancement but a necessary evolution in agricultural science to combat the escalating climate crisis (Bindal & Rath. 2025).

9.1. Towards a Third Green Revolution

The continuous upgrade of the CRISPR toolbox to include base editing, epigenetic modulation, and prime editing will allow for even more precise control over the Basmati genome. For example, base editing can be used to "fix" specific nucleotide polymorphisms that negatively impact grain quality without the need for double-stranded breaks (Beyer, 2025). Furthermore, combining gene editing with artificial intelligence (AI) and machine learning for digital phenotyping will enhance the selection efficiency in Speed Breeding facilities (Mansi & Danai, 2026).

9.2. Socio-Economic and Ecological Implications

Developing salt-tolerant and early-maturing Basmati has profound socio-economic benefits. It protects the livelihoods of millions of smallholder farmers in South Asia and secures a vital source of export revenue (Sifc.gov.pk, 2023). Ecologically, varieties that mature earlier and thrive in saline soils can reduce the demand for fresh water and lower the reliance on chemical soil amendments (Sachdev et al., 2021).

The transition to these "climate-smart" crops will require strong collaboration between public research institutions like IRRI, national agricultural research systems (NARS) in India and Pakistan, and the private seed sector. By leveraging the power of integrated speed breeding, the rice industry can ensure that the legendary Basmati aroma continues to flourish in a changing

world (Shikari, 2025).

10. Conclusion

The convergence of CRISPR/Cas9 precision editing and speed breeding represents a transformative leap in Basmati rice improvement, addressing the critical bottlenecks of salinity sensitivity and protracted breeding timelines. By targeting key stress regulators and leveraging accelerated generation turnover, this approach has yielded prototypes with enhanced Na+ exclusion, osmotic resilience, and reduced maturity durations, potentially mitigating annual global salinity losses of US$27.2 billion while boosting yields in affected Indo-Gangetic plains. Regulatory advancements in India and Pakistan facilitate safe deployment, but challenges like off-target effects and public acceptance must be navigated through rigorous biosafety protocols. Ultimately, widespread adoption of these technologies promises sustainable, high-value Basmati production, fortifying global food systems against climate volatility and supporting the livelihoods of millions in rice-dependent regions.

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