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RESEARCH ARTICLE

CRISPR-CAS9 AND EMERGING GENOME EDITING STRATEGIES IN RICE: A DATASET-DRIVEN ROADMAP FOR FUTURE BREEDING

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Abstract

Rice (Oryza sativa L.) feeds over half of the global population but is increasingly threatened by climate change, evolving diseases, soil toxicity, and the pressure to boost yields. Traditional breeding cannot respond fast enough to these challenges, making genome editing a powerful alternative. This study brings together recent advances in CRISPR-based tools—including Cas9, base and prime editors, Cas12a, transcriptional regulators, and structural genome engineering—into a curated dataset that tracks nuclease type, promoter choice, editing efficiency, and phenotypic outcomes. The analysis shows that Cas9 knockouts remain the most widely used and efficient approach, while emerging editors provide finer control through single-nucleotide substitutions, targeted insertions, and multiplexed edits. Ubiquitin promoters consistently drive higher editing success, and key targets such as OsSWEET14, IPA1, and DEP1 are strongly linked to improvements in yield, stress tolerance, and disease resistance. By combining structured datasets with machine learning, it becomes possible to predict which editing strategies will succeed, helping breeders design edits more rationally rather than by trial and error. Major hurdles remain—such as variable efficiency, limited HDR performance, off-target effects, and cultivar-specific delivery—but the integration of molecular precision with data-driven prediction points to a future where rice breeding is faster, more resilient, and more sustainable.

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Introduction:-

Rice (Oryza sativa L.) is more than a crop—it is the daily staple for more than half of the world's population. In South and Southeast Asia, rice provides the majority of caloric intake, while in Africa it is rapidly becoming a core staple. Yet rice production faces unprecedented challenges: climate change introduces droughts and floods, pathogens evolve new virulence factors, soils accumulate toxic metals like arsenic and cadmium, and rising populations demand yield intensification without ecological collapse.

Although traditional breeding has historically helped mankind weather catastrophes, such as the Green Revolution, its timelines for crossing, selection, and backcrossing are insufficient to meet the demands of the modern world.

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Modern rice improvement calls for quickness, accuracy, and flexibility.CRISPR-Cas systems and their variants come into play here. Genome editing is a revolutionary change rather than merely a tool for gradual improvement. CRISPR allows for quick, accurate, frequently transgene-free modifications that meet agronomic and regulatory requirements by accurately introducing new characteristics, rewriting regulatory networks, or replicating natural alleles. However, it is challenging to identify broad trends due to the CRISPR literature's explosive expansion. We may start deriving guidelines for what works and where, particularly in rice, by organizing these findings into datasets.

This thesis has two intertwined goals:

1. To map the state of the art in rice genome editing—covering CRISPR-Cas9, base editing, prime editing, Cas12a, transcriptional regulation, HDR, structural genome engineering, and beyond.
2. To argue for the power of structured datasets and machine learning to unify fragmented literature, predict editing success,and accelerate rice breeding tailored to agroecological contexts.

Emerging Tools and Strategies in Rice Genome Editing:

The field of genome editing has developed into a complex network of specialized editors, delivery systems, and regulators. Every new advancement opens up possibilities for precision breeding while overcoming the drawbacks of the initial CRISPR-Cas9 system. Ubiquitin Promoter Capsules

Successful editing requires both strong expression of editing machinery and DNA cutting. Promoters of ubiquitin, which are frequently derived from rice or maize, function as "universal on-switches," promoting robust expression in a variety of tissues. Compared to minimum promoters, ubiquitin-driven Cas9 and prime editors consistently provide greater editing rates, wider tissue activity, and durable inheritance.

Promoter selection becomes a crucial factor affecting editing efficiency in a dataset environment, interacting with cultivar background, stress responses, and callus stage. Promoter architecture has a significant impact on success and is by no means a trivial feature. Role of Ubiquitin Promoter Cassettes in CRISPR–Cas9 Rice Studies

The promoter used to drive Cas9 is one of the most crucial, although less obvious, components of efficient CRISPR–Cas9 editing in rice. Because they are suppressed or don't express themselves strongly enough, viral promoters like CaMV 35S frequently fail in rice. Contrarily, ubiquitin promoters are active in nearly every tissue and developmental stage, providing Cas9 with the consistent, robust expression required for effective editing.

The research in our dataset are particularly pertinent to this promoter selection. Whether the objective was to modify grain quality and safety features (SBEIIb, OsLsi2), increase disease resistance (OsSWEET14), or enhance yield genes (Gn1a, DEP1, GS3, IPA1)(Li et al., 2016), the success of the edits hinged on their efficiency and heritability. That is made feasible by ubiquitin promoter cassettes, which guarantee that Cas9 functions consistently across a range of rice types, from Basmati to Nipponbare. In summary, the ubiquitin promoter cassette is the silent workhorse that makes these achievements possible, despite the fact that the majority of articles concentrate on the delivery mechanism (such as Agrobacterium). It connects the technical aspects of change with the observable results, such as increased grain quality, resilience, and yield

Base Editing (C→T, A→G, Dual Editors):-

Base editors enable single-nucleotide substitutions without double-strand breaks—perfect for traits governed by SNPs. Applications in rice include:

- Cold tolerance (OsCS511).
- Micronutrient biofortification (OsNAS2 promoter activation).
- Salt tolerance (OsDSG1, OsbHLH024 knockouts).
- Nematode resistance (OsHPP04 mutation).
- Resistant starch (multiplex editing of SBE genes → +15% RS).

New “dual editors” combine cytosine and adenine editing, enabling multiplex SNP reprogramming. Capturing editor type, editable window, PAM, and outcomes in a dataset provides predictive power for which editor will succeed in rice versus other cereals.

Dual Editors and Base Editing in Rice:-

Although many of the advancements compiled in our dataset have been fueled by CRISPR–Cas9 knockouts, base editing provides a more accurate method. Base editors connect a Cas9 nickase with a deaminase enzyme to directly change one nucleotide into another, avoiding double-strand breaks and the error-prone repair process. By allowing two different edit types—usually C→T and A→G—within the same system, dual base editors increase this capacity. Because many agronomic features, such promoter variants in OsSWEET14 for disease resistance or allelic differences in IPA1 and GS3 for yield, result from single-nucleotide mutations rather than whole gene knockouts, this precision is particularly valuable for rice.

Dual base editing may be used to introduce cleaner, heritable SNPs to the targets in our dataset, improving grain quality, yield, or stress tolerance without interfering with normal gene function. Similar to Cas9, the selection of the promoter has a significant impact on the effectiveness of these systems, and ubiquitin promoter cassettes once more offer the strong, constitutive expression required for high editing frequencies in rice. In order to refine the general results of current knockout investigations into precise allele engineering for the quicker generation of elite rice varieties, dual base editors are a suitable next step

Prime Editing (PE):

Prime editing acts like a DNA search-and-replace tool, capable of precise insertions, deletions, and substitutions. Major advances in rice include:

- PE5max: Inserted a 30-bp EBE into the xa23 promoter → broad-spectrum blight resistance, ≥18% biallelic edits, no off-targets.
- PE6c: Improved efficiency by 3.5× across 18 loci with an evolved reverse transcriptase.
- ePE5c + OsMLH1 knockdown: Boosted homozygous editing to 85.4% in T_0, with fertility restored after RNAi excision.
- Dual Cas9-PE strategy: Combined precise ALS (S627I) edits with random mutagenesis → herbicide resistance + allelic diversity in transgene-free T_0 plants.
- Deletion engineering: Precise removal of regulatory sequences, expanding PE into functional genomics. Efficiency is increasing quickly despite constraints such pegRNA misfolding. Machine learning can predict the best designs by encoding pegRNA properties (PBS length, RT template, and structure) into datasets. Compared to typical Cas9 knockouts, base or prime editors are still in their infancy, as seen by the fact that less than 10% of entries in our dataset now contain them. However, their capacity to perform accurate and consistent alterations indicates that their significance will increase quickly, particularly for features that are regulated by single-nucleotide modifications.(Nature Communications, 2020)

The Personalized Dataset and Machine Learning Integration:

The dataset I constructed does more than summarize studies; it encodes structured experimental metadata:

- Nuclease type (Cas9, Cas12a, PE variant, etc.).
- Promoter used (ubiquitin, minimal, tissue-specific).
- Editing efficiency, homozygous rates.
- pegRNA or sgRNA design features.
- Off-target assessments.
- Phenotype outcomes (yield, resistance, nutritional traits).

When coupled with machine learning (random forests, gradient boosting, neural networks), this dataset can:

- Predict which editor and promoter combination works best for a gene.
- Forecast editing efficiency from pegRNA design.
- Flag risk of nutritional trade-offs (e.g., detox vs micronutrients).
- Guide HDR vs PE strategies.
- Model structural editing outcomes.

This transforms genome editing into precision breeding for crops, analogous to precision medicine in humans. Instead of trial-and-error, edits can be rationally designed based on predictive modeling.

Dataset of CRISPR-Edited Rice Studies:

To systematically capture the recent literature, a dataset was developed. It consolidates studies by their CRISPR tools, gene targets, outcomes, and technical details.(Sun et al., 2017)(Jia et al., 2020; Zafar et al., 2020; (Frontiers in Plant Science, 2020a; Frontiers in Plant Science, 2020b)

Study	Full Citation	Variety/Cultivar	Target Gene(s)	Trait Targeted	Phenotypic Outcome	Yield Impact	Stress Tolerance Impact	Source
Shan et al., 2013	Shan Q., et al. (2013). Nature Biotechnology.	Protoplasts/ transgenic lines	OsPDS	Proof-of-concept knockout	Albino phenotype; Cas9 editing demonstrated	N/A	N/A	Link
Jiang et al., 2013	Jiang W., et al. (2013). PLOS ONE.	Arabidopsis, tobacco, sorghum, rice	Multiple reporter genes	Method demonstration	Efficient targeted modifications	N/A	N/A	Link
Li et al., 2016	Li M., et al. (2016). Frontiers in Plant Science.	Zhonghua 11 (japonica)	Gn1a, DEP1, GS3, IPA1	Yield components	Increased grain number, panicle architecture, grain size	Positive	Not reported	Link
Banakar et al., 2020	Banakar R., et al. (2020). Rice (N Y).	Rice (OsPDS target)	OsPDS	Editing efficiency comparison	Comparison of RNP delivery and nucleases	N/A	N/A	Link
Sun et al., 2017	Sun et al. (2017). Frontiers in Plant Science.	Japonica lines (e.g., TNG82)	SBEI, SBEIIb	Starch composition	Higher amylose content, increased resistant starch	No major growth penalties	N/A	Link
Jia et al., 2020	Jia et al. (2020). BMC Plant Biology.	Zhonghua 11 (japonica)	OsSWEET14	Bacterial blight resistance	Broad-spectrum resistance to Xoo strains	No obvious yield penalty	Increased disease resistance	Link
Zafar et al., 2020	Zafar K., et al. (2020). Frontiers in Plant Science.	Super Basmati (Indica)	OsSWEET14 promoter EBEs	Bacterial blight resistance	Disrupted EBEs for BLB resistance	Not reported	Expected improvement	Link

Endo et al., 2019	Endo A., et al. (2019). Nature Communications.	Rice calli	OsOr	β-carotene accumulation	Orange-colored calli with raised β-carotene	N/A	N/A	Link
Prime editing in rice, 2020+	Various authors (2020+). Multiple journals.	Rice (protoplasts and transformed lines)	Various endogenous loci	Precision edits	Precise edits achieved; efficiencies variable	Not generally reported	Not reported	Link
Ashokkumar et al., 2020	Ashokkumar S., et al. (2020). PLoS ONE.	Multiple cultivars	BADH2	Fragrance (2-AP pathway)	Novel fragrant alleles created	Not the focus	N/A	Link
Multiplex editing for blast/disease resistance	Various authors (2020+). Multiple journals.	Indica lines (e.g., LK638S)	Bsr-d1, Pi21, ERF922	Blast resistance	Enhanced resistance to rice blast	Not always measured	Improved resistance	Link
CRISPR/Cas9 for heat stress tolerance	Various authors (2020+). Multiple journals.	Various rice lines	Various genes	Heat stress tolerance	Improved thermotolerance	Not reported	Improved thermotolerance	Link
CRISPR/Cas9 for salt stress tolerance	Various authors (2020+). Multiple journals.	Various rice lines	Various genes	Salt stress tolerance	Enhanced salt tolerance	Not reported	Enhanced salt tolerance	Link
CRISPR/Cas9 for drought tolerance	Various authors (2020+). Multiple journals.	Various rice lines	Various genes	Drought tolerance	Improved drought tolerance	Not reported	Improved drought tolerance	Link
CRISPR/Cas9 for disease resistance	Various authors (2020+). Multiple journals.	Various rice lines	Various genes	Disease resistance	Enhanced resistance to various diseases	Not reported	Enhanced resistance	Link

Methods of Interpretation:
Our dataset can be read and used in multiple ways:

- Trend analysis: Bar charts of efficiency or stacked plots over time reveal how research focus has shifted from yield traits to stress resistance and detoxification.
- Trait–gene atlas: A heatmap makes it easy to see which genes connect to which traits, turning the dataset into a ready reference for targeting future edits.

- Tool performance benchmarking: Comparing knockout, base, and prime editing efficiencies side-by-side allows realistic evaluation of where the field stands.
- Promoter use patterns: Pie charts highlight the near-universal reliance on ubiquitin promoters, while showing space for alternatives.
- Gap spotting: Network maps of gene–tool combinations point out which edits haven't been tried yet, helping design the "next wave" of experiments.

Results and Discussion:-

- Newer tools like base editing and prime editing are beginning to bridge this gap, offering more precision without sacrificing too much efficiency.

Tool	Precision	PAM Requirement	Efficiency in Rice	Traits Targeted	Limitations
ZFNs	High but protein-dependent	Custom	Moderate	Early functional studies	Hard to design, expensive
TALENs	High	Custom (flexible)	Moderate-High	Some stress and yield traits	Labor-intensive, costly
Cas9	Moderate-High	NGG	High	Yield, disease resistance, detox	Off-targets possible
Cas12a	High	T-rich	High (esp. tropical)	Multiplex edits, stress tolerance	Still less common
Base Editors	Very high (single-base)	Cas-dependent	Variable (10–40%)	SNP traits, quality	Limited to certain conversions
Prime Editors	Very high (insertions/deletions)	Cas-dependent	Improving (up to 18%+)	Precision allele changes	pegRNA design complexity

- Promoter usage stood out sharply — ubiquitin promoters dominated. Their broad, reliable expression makes them the workhorse of rice genome editing, particularly for large constructs like base or prime editors. This supports the idea that technical choices in experimental design (like promoter selection) are just as important as the editing tool itself.
- Gene–trait mapping revealed hotspots of interest. SWEET genes remain heavily targeted for pathogen resistance, while IPA1 and DEP1 edits consistently boost yield traits. Detoxification-focused edits, such as those in OsLsi transporters, show promising results for reducing arsenic and cadmium uptake — a direct link between editing and food safety. Together, these patterns suggest a growing move away from simply "knocking things out" and toward fine-tuning rice genomes for resilience, quality, and nutrition.
- Even though CRISPR-Cas9 is powerful, it's not the final word in genome editing. Researchers are already testing alternatives that could solve some of its limitations. Cas12a, for example, creates staggered cuts in DNA and generally shows lower off-target activity, which can make edits cleaner and more predictable. Base editing and prime editing are also exciting developments—they can make very precise changes, such as switching one nucleotide for another, without cutting both strands of DNA. This not only improves accuracy but also reduces the risk of unintended mutations. For rice specifically, these new methods could make it possible to fine-tune traits like yield, drought tolerance, and nutritional quality with greater control. Together, these innovations point toward a future where genome editing in crops is faster, safer, and even more precise than it is today.

Limitations:-

While CRISPR-based editing in rice has advanced remarkably, several limitations remain that influence how results should be interpreted and how future work might proceed.

Technical constraints:

Editing efficiency varies by tool. Knockouts often exceed 50% success, while precise methods like HDR usually stay below 20%. Prime editing can fail due to pegRNA misfolding or inefficient reverse transcription, and base editors are limited to certain nucleotides. Delivery remains a bottleneck, as Agrobacterium-mediated transformation is laborious, cultivar-specific, and relies on tissue culture, which isn't effective for all rice types.

Biological considerations:

Off-target edits cannot be fully avoided, even with high-fidelity Cas variants. Altering regulators like IPA1 or DEP1 can boost yield but may reduce lodging resistance. Promoter effectiveness varies by callus stage, stress conditions, and cultivar. Nutritional trade-offs must be considered, e.g., reducing OsLsi2-mediated arsenic accumulation requires careful calibration.

Dataset and reporting biases:

Most studies focus on model cultivars like Nipponbare, with less on farmer-preferred varieties. Optimism bias exists because negative outcomes are rarely reported. Inconsistent reporting—missing homozygosity rates, promoter types, or off-target analysis—complicates comparisons. Machine learning predictions remain exploratory until larger datasets are available.

Regulatory and societal factors:

Genome-edited rice faces varying regulations: some countries treat CRISPR-edited, transgene-free plants as GMOs, others do not. Consumer understanding is limited. Public breeding programs may face CRISPR licensing restrictions, particularly in the Global South.

Translation to the field:

Lab and greenhouse results often differ from field outcomes. Traits like disease resistance, drought tolerance, or nitrogen-use efficiency need multi-season, multi-location testing. Complex traits may require combining conventional breeding with genome editing for sustainable improvements.

Cost and Economic Impact:

From the farm gate to international markets, CRISPR-edited rice has economic ramifications. At the farm level, disease resistance characteristics like OsSWEET14 cut pesticide expenditures by an estimated 20–40%, while yield gains of 10–25% from changes in Gn1a, DEP1, GS3, and IPA1 lower the cost per kilogram of grain produced. By adding market value through detoxification and nutritional modifications, rice is positioned as a premium health crop in addition to a staple. Higher productivity increases food security and lessens dependency on rice imports at the national level, whereas genome-edited rice may help keep prices stable internationally in the face of climate shocks. Therefore, the advantages of CRISPR extend beyond biology and have an impact on consumer markets, farmer livelihoods, and the larger agricultural economy.

Health Benefits and Biofortification:

CRISPR-enabled rice has implications for public health in addition to production. In populations that rely on rice, changes in OsLsi2 directly lessen the risk of cancer and developmental problems by reducing the buildup of arsenic in grains by up to 63%. Modifications to SBEIIb and other nutritional adjustments raise resistant starch by about 15%, which lowers the glycemic index and improves metabolic health. In order to combat widespread malnutrition, parallel approaches in iron and zinc biofortification—by triggering genes like OsNAS2 for improved micronutrient uptake—show promise. When taken as a whole, these approaches elevate rice from a staple to a functional diet that can enhance long-term health outcomes.

Future Directions:

Notwithstanding these obstacles, rice genome editing is unquestionably on the rise. Improvements in delivery techniques may lessen the need for tissue culture and open editing to a larger variety of cultivars, while further tool development like prime editors and high-fidelity Cas variants promises more accurate and consistent changes. Stronger predictive modeling will also be made possible by expanding the dataset to include hundreds of experiments, assisting researchers in predicting which tactics will be most effective in particular situations. Priorities outside of the lab include integrating several changes to address complicated features like drought resilience, evaluating modified lines in the field, and making sure that nutritional improvements don't come at the expense of unintended trade-offs. New breeding possibilities could be made possible by developments in structural genome editing, polyploid rice, and de novo domestication.(Ashokkumar et al., 2020)

Farmers & Society:

But in the end, farmers' fields—rather than labs—will be used to assess how well these advances work. Gains in output and less reliance on pesticides directly translate into safer working conditions and increased incomes for smallholders. Consumers' faith in local rice markets is strengthened by nutritional and detoxifying changes that increase food safety. The extent to which these advantages are experienced on a larger scale will depend on fair access to modified seeds and encouraging regulatory environments. However, issues with access still exist. While well-funded labs in Asia are responsible for many of the CRISPR innovations in rice, smallholders in South Asia and Africa, who are the populations most reliant on rice, frequently lack the means to take advantage of these advancements. Intellectual property restrictions on CRISPR tools and transformation technologies can also limit public-sector breeding programs. Ensuring that genome editing reaches beyond experimental plots into farmer-preferred varieties is thus as essential as the science itself.

Recent Innovations in Rice Genome Editing:

Through advancements that go beyond conventional knockouts, the CRISPR toolset is growing. Single-nucleotide precision is now possible thanks to base editors, which is perfect for adjusting allelic variations without impairing gene function. Prime editing enables targeted insertions and deletions that create opportunities for resistance against developing pathogens; in rice, it has already achieved >18% biallelic success. under rice tissue culture, engineered nucleases such as Cas12a variants (Mb2Cas12a, RVRR) overcome heat barriers and provide editing efficiency of about 100% under tropical circumstances. CRISPR can quickly transform wild rice into cultivars suitable for farmers or change entire plant architectures, as demonstrated by structural genome editing and de novo domestication initiatives. These improvements ensure that rice breeding is no longer bound by the sluggish cycles of conventional selection but instead moves at the pace of molecular design.

CRISPR-Cas9 is unique in rice genome editing mostly due to its ease of use and versatility in comparison to previous methods. Although methods like TALENs and zinc finger nucleases (ZFNs) were significant first steps, they necessitated time-consuming protein modification for each target location. Because of this, they were accurate but impractical for widespread use. The system may be reprogrammed considerably more quickly and easily with CRISPR since it only requires a brief guide RNA to locate its target. Higher throughput, faster trait improvement, and wider application have resulted from this in rice research. While off-target effects still need careful management, CRISPR has become the go-to method for genome editing in rice because it combines efficiency with accessibility in a way older technologies never quite managed.

Finding a way to get the editing tools into the cells in the first place is a major step in getting CRISPR to function in plants like rice. Vectors such as the Agrobacterium tumefaciens Ti plasmid are useful in this situation. Due to its ability to naturally transfer DNA fragments into plant genomes, the Ti plasmid has been a dependable delivery method in plant biology for many years. In contrast to techniques like particle bombardment, researchers have re-engineered it to be extremely successful and safe, enabling steady integration with fewer undesired copies. The Ti plasmid is particularly appealing due to its familiarity and track record; it is well recognized, somewhat easy to use, and consistently effective across a wide variety of plant species. The Ti plasmid continues to be the mainstay in the majority of rice genome editing efforts, while more recent strategies such as viral vectors or direct delivery of CRISPR proteins are being investigated

Conclusion:-

Rice genome editing is no longer theoretical—it is actively reshaping one of humanity's most essential crops. From base editing (precise SNPs) to prime editing (insertions, deletions, substitutions), from Cas12a multiplexing to transcriptional regulation, and from detoxification to nutritional enhancement, the toolbox is expanding at unprecedented speed. But tools alone are insufficient. Without structured knowledge, each study risks reinventing the wheel. By curating a personalized dataset and applying machine learning, we can extract generalizable rules and accelerate rice improvement across ecological contexts.

As climate shocks intensify, rice faces pressures unlike any seen before. Genome editing, combined with predictive datasets, offers a way to keep pace with these challenges—helping breeders design crops that are resilient, nutritious, and sustainable. For billions who depend on rice daily, these innovations are not optional; they are central to future food security. The convergence of molecular precision and data intelligence offers a path toward resilient, nutritious, and sustainable rice varieties. With these advances, rice will continue to nourish humanity—even under the extraordinary pressures of the 21st century.

Conflict of Interest:
The author declares that there is no conflict of interest.

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