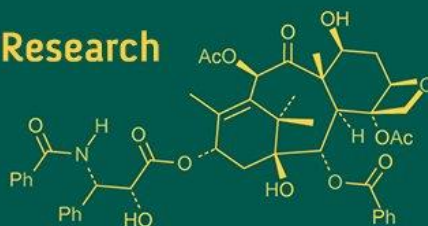


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Precision breeding in rice: Integrating CRISPR-Cas genome editing, pangenomics and artificial intelligence for climate-resilient cultivar development

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Abstract

Rice remains the staple calorie source for more than half the global population, yet the genetic gains delivered by conventional and marker-assisted breeding have not kept pace with the compounding pressures of heat, drought, salinity and flooding associated with a changing climate. This review examines whether the convergence of three distinct technological streams CRISPR-Cas genome editing, pangenome-scale comparative genomics, and artificial intelligence constitutes a genuinely integrated precision-breeding paradigm, or whether the field is still assembling components that have yet to function as a single pipeline. Genome editing has matured from simple knockout applications toward base and prime editing and multiplexed trait stacking, but genotype dependency, delivery bottlenecks and regulatory uncertainty continue to constrain deployment beyond model backgrounds. Pangenomic resources built from wild and cultivated rice accessions have exposed how much functional variation a single reference genome conceals, reframing candidate-gene discovery around presence-absence variation and structural rearrangement rather than single-nucleotide polymorphism alone. Machine learning and deep learning architectures now support genomic prediction, image-based phenotyping and multi-omics integration, but their reliability degrades sharply outside the environments and germplasm on which they were trained. The central argument developed here is that the three domains are conceptually complementary AI proposes hypotheses, pangenomics supplies allelic raw material, and genome editing tests and delivers it but that this loop is presently closed only in isolated proof-of-concept studies rather than as routine breeding practice. Persistent obstacles include trait complexity arising from epistasis and pleiotropy, poor cross-environment transferability of predictive models, and regulatory frameworks that vary widely by jurisdiction. The review closes by identifying realistic near-term priorities, including explainable prediction models, expanded pangenome representation of underused wild relatives, and editing systems less dependent on genotype background, as the conditions under which integration could move from conceptual promise toward operational breeding infrastructure.

Keywords: Rice breeding, CRISPR-Cas genome editing, pangenomics, artificial intelligence, genomic prediction, climate resilience, precision agriculture

1. Introduction

Rice occupies a peculiar position in the global food system: it is simultaneously one of the best-characterized crop genomes and one of the most vulnerable to the specific stresses that climate change is intensifying. Because paddy rice is grown under standing water in low-lying, often coastal or delta environments, it is disproportionately exposed to submergence and salinity intrusion, while upland and rainfed systems face increasingly erratic drought [3]. Conventional breeding, built on phenotypic selection across successive generations, is slow relative to the rate at which stress regimes are shifting, and marker-assisted selection, while faster, still depends on a reasonably tractable genetic architecture something that traits like yield stability under combined stress rarely offer [24, 35]. The gap between the urgency of the climate problem and the tempo of trait deployment is the basic justification for precision breeding as a category distinct from marker-assisted or genomic selection approaches that preceded it.

Three technological developments have each, independently, been proposed as accelerants for rice improvement. CRISPR-Cas genome editing allows targeted modification of specific alleles rather than reliance on existing standing variation [1, 2].

Pangenomic sequencing of diverse cultivated and wild accessions has revealed that a disproportionate share of functionally relevant variation particularly structural variants and gene presence-absence differences is invisible to analyses anchored on a single reference genome^[15, 17, 22]. Artificial intelligence, spanning classical machine learning to deep neural architectures, offers a route to integrate genomic, phenotypic and environmental data at a scale unmanageable by earlier statistical genetics^[9, 16]. Each of these has generated a substantial and growing literature considered on its own terms. What is less settled, and is the actual subject of this review, is whether treating them as separate toolkits undersells their value, and whether the field has produced credible evidence that they function as a coupled system rather than three parallel research programmes that happen to share a target species. This review does not attempt an exhaustive cataloguing of every reported application. It instead asks, for each domain and then for their intersection, what has been convincingly demonstrated, what remains provisional, and what structural barriers biological, computational or regulatory stand between current practice and a genuinely integrated precision-breeding pipeline for climate-resilient rice.

2. From Phenotype to Genotype to Design: The Evolution of Precision Breeding

Classical phenotypic selection in rice relies on recurrent evaluation of segregating populations under target-representative conditions, an approach that works reasonably well for traits with simple architecture and visible expression but performs poorly for traits expressed only under intermittent or compound stress. Marker-assisted selection and gene pyramiding addressed part of this problem by tagging known quantitative trait loci and tracking them through backcrossing programmes, and this approach has produced durable, field-validated gains for traits such as submergence tolerance and specific disease resistances^[24, 53, 55]. Its limitation is architectural: pyramiding works cleanly when the target traits are governed by a modest number of large-effect loci with limited epistatic interaction, and becomes progressively less tractable as breeders move toward traits drought tolerance under variable timing and intensity being the clearest example that are polygenic, environment-dependent, and subject to trade-offs against yield and phenology^[30, 40].

Genomic selection reframed the problem statistically, using genome-wide marker data to predict breeding values without requiring that causal loci be individually identified. This is a meaningful conceptual shift because it sidesteps the need for prior biological understanding of the trait, but it substitutes one dependency for another: predictive accuracy depends heavily on the size, diversity and phenotypic depth of the training population, and models trained in one environment or genetic background transfer poorly to another^[34, 43]. Genome editing constitutes a further shift again, moving from selection among existing variants toward creation of new, targeted variants, which in principle removes the constraint that useful alleles must already exist somewhere in the breeding pool^[1, 31]. AI-assisted breeding is not a discrete stage in this sequence so much as an accelerant layered across the others it can inform which loci are worth pyramiding, improve the accuracy of genomic selection models, and prioritise candidate targets for editing^[9, 14]. Understood this way, precision breeding is less a

single new method than a recombination of the strengths of what preceded it, with the specific claim of the current period being that computational prediction, comprehensive genomic reference material, and precise allele modification can now be brought into the same workflow rather than operating as sequential, loosely connected stages.

3. CRISPR-Cas Genome Editing: Precision Without Universality

The appeal of CRISPR-Cas systems for rice breeding rests on their capacity to introduce a defined change at a defined locus without the multi-generation linkage drag that accompanies introgression from a donor line^[2, 10]. Cas9-based knockout editing, exploiting the non-homologous end-joining repair pathway to generate small indels, remains the most widely deployed format because it is comparatively simple and tolerant of imperfect guide design, but it is fundamentally a loss-of-function tool: it is well suited to disabling susceptibility genes or negative regulators of a desired trait, and poorly suited to installing a specific beneficial substitution^[6, 13]. Base editing addresses part of this gap by enabling precise nucleotide conversions without double-strand breaks, reducing the reliance on error-prone repair and lowering the risk of large unintended deletions, while prime editing extends this further toward arbitrary small insertions, deletions and substitutions, at the cost of generally lower efficiency and a more demanding design process^[29, 31]. Cas12-based systems, including recently reported Cas12i variants, offer an alternative PAM compatibility and, in some contexts, reduced off-target activity relative to Cas9, expanding the accessible target space within the rice genome rather than replacing Cas9 outright^[38].

Multiplex editing targeting several loci simultaneously within a single transformation event is arguably the format most directly relevant to complex climate-resilience traits, since it allows stacking of alleles governing, for example, both stress tolerance and yield-component traits without the sequential crossing that pyramiding would otherwise require^[33]. The evidence that multiplexing accelerates trait combination is reasonably strong; what is less resolved is whether efficiency scales linearly as the number of simultaneous targets increases, since guide RNA competition and finite transformation capacity impose practical ceilings that are rarely made explicit in reports of successful multiplex outcomes.

Three limitations recur across the CRISPR literature on rice regardless of editor format. First, genotype dependency: transformation and regeneration efficiency vary substantially across rice subspecies and elite cultivars, meaning that a protocol validated in a model japonica background does not automatically transfer to the indica lines dominant in much of tropical Asian production^[37, 39]. Second, delivery remains a bottleneck. Agrobacterium-mediated and particle bombardment methods both depend on tissue-culture regeneration steps that are genotype-sensitive and labor-intensive, and DNA-free or transient delivery alternatives are not yet routine at scale^[10, 29]. Third, off-target activity and unintended structural consequences, while generally lower than early critics feared, are not zero, and comprehensive whole-genome characterization of edited lines is inconsistently reported across studies, which makes cross-study comparison of true off-target rates difficult^[29]. Regulatory treatment of edited rice varies

considerably between jurisdictions depending on whether the final product contains foreign DNA, and this divergence has practical consequences for which editing strategies breeders consider viable for eventual release rather than research use alone [37, 48].

4. Pangenomics: What a Single Reference Genome Cannot Show

The rice reference genome, however thoroughly annotated, represents one accession's genomic content, and structural comparison across diverse cultivated and wild accessions has repeatedly shown that a meaningful fraction of genes present in the wider species pool are simply absent from that reference [17, 27]. Pangenome construction assembling core genes shared across essentially all accessions alongside a dispensable genome variable between them reframes the practical question for breeders from "which SNP is associated with this trait" to "which structural or presence-absence variant, potentially invisible to reference-based mapping, might explain it" [15, 22]. Large-scale pangenome efforts spanning hundreds of cultivated and wild rice genomes have shown that structural variation, including large insertions, deletions and presence-absence differences, is pervasive enough that reference-genome-only genome-wide association studies plausibly miss a substantial share of functionally relevant loci [17, 23].

Wild rice relatives are of particular interest here because domestication is understood to have narrowed genetic diversity substantially, meaning that stress-tolerance alleles lost during domestication or improvement may still be recoverable from wild germplasm collections rather than requiring de novo creation [12, 49]. Pangenome resources that specifically incorporate wild accessions alongside cultivated diversity are consequently positioned as a route to candidate-gene discovery that neither a single reference genome nor a cultivated-only diversity panel could offer [22, 26]. Recent large pangenome references combining wild and cultivated rice illustrate the direction the field is moving, toward graph-based representations that can capture structural variation directly rather than inferring it indirectly from short-read alignment to a linear reference [22].

The practical limitation is that pangenome construction remains resource-intensive relative to the resequencing approaches it supplements: long-read, high-contiguity assembly across hundreds of accessions is not yet as routine as short-read resequencing, and functional annotation of dispensable-genome content lags well behind its structural characterization [12, 23]. A gene's presence in the pangenome is not the same as a validated functional or agronomic contribution, and the pipeline from pangenome-identified candidate to functionally confirmed, breeding-relevant allele still typically requires the kind of targeted validation that genome editing, rather than pangenomics itself, is positioned to provide [26]. This is the first concrete point of intersection between the three domains under review: pangenomics generates candidates that editing is needed to test.

5. Artificial Intelligence in Precision Breeding: Prediction, Not Proof

Machine learning methods applied to rice breeding data span a spectrum from relatively interpretable models (random forests, support vector machines, Bayesian regression approaches) to less interpretable but often higher-

capacity architectures such as gradient-boosted ensembles, convolutional neural networks and, more recently, graph-based and transformer architectures applied to genomic or multi-omics data [32, 34]. Genomic prediction is the application area with the longest track record: models trained on marker genotype and phenotype data attempt to estimate breeding values for lines not yet phenotyped, and ensemble methods such as gradient boosting have been reported to improve predictive accuracy over classical genomic best linear unbiased prediction in specific rice datasets [32]. Deep neural network approaches incorporating multi-omics inputs combining genomic, transcriptomic and sometimes metabolomic layers have likewise been proposed as a means of capturing non-additive genetic effects that marker-only models struggle with [34, 43].

Image-based, high-throughput phenotyping is the second major application area, using computer vision to quantify traits such as canopy architecture, panicle characteristics or stress symptom progression at a scale and consistency that manual scoring cannot match, which matters directly for climate-resilience breeding because stress phenotyping (wilting progression under drought, chlorosis under salinity) is precisely the kind of continuous, time-resolved trait that benefits from automated image capture [16, 47]. AI applications also extend upstream of prediction into candidate-target prioritization, where models trained on genomic, expression or evolutionary-conservation features attempt to rank candidate genes by their likely contribution to a trait of interest, effectively proposing where a genome-editing effort might be directed before wet-lab validation begins [9, 14].

The critical qualification that applies across essentially all of these applications is generalization. Models validated within a single population, environment or growing season routinely show degraded accuracy when applied to a different genetic background or a different environment, a pattern consistent enough across genomic prediction studies that it should be treated as a default expectation rather than an occasional caveat [34, 35]. Deep learning architectures, by virtue of their parameter count, are particularly prone to overfitting when the available training data often on the order of hundreds to a few thousand phenotyped lines in rice breeding programmes is small relative to genomic dimensionality, and the field's use of held-out cross-validation within a single dataset does not fully substitute for genuinely independent, cross-environment validation [32, 34]. Interpretability compounds this problem: black-box deep learning predictions are difficult for breeders to audit or trust when a prediction conflicts with agronomic expectation, and this is a genuine barrier to adoption independent of raw predictive accuracy [47, 54]. None of this negates the value of AI methods in rice breeding, but it does mean that current evidence supports AI as a prioritization and prediction aid operating alongside conventional evaluation, rather than as a validated replacement for field-based confirmation.

6. Where the Three Domains Actually Meet

The strongest form of the integration argument runs as follows: AI-based prediction and target prioritization narrow a genome-wide search space down to a tractable set of candidate loci; pangenomic resources reveal which structural or presence-absence variants at those loci exist across cultivated and wild diversity, including variants

invisible to a single reference; and CRISPR-Cas editing then either validates candidate gene function directly or introduces the favourable allele into an elite background without the linkage drag of conventional introgression [9, 26, 33]. Framed this way, the three domains are not competing strategies but sequential stages addressing different failure points of the others. AI reduces the search space that pure pangenomic screening would otherwise leave too large, pangenomics supplies the allelic diversity that AI models trained on a narrow cultivated panel would not otherwise see, and editing supplies the causal validation and precise deployment that neither computational prediction nor comparative genomics can provide on its own. Where this argument is weaker is in demonstrated practice rather than principle. The great majority of studies reviewed

here operate within one or, at most, two of the three domains; end-to-end pipelines in which an AI-prioritized, pangenome-supported candidate is subsequently edited and field-validated in rice remain the exception rather than the norm in the current literature, and where such pipelines are reported, they tend to involve single genes or small gene sets rather than the polygenic, environment-dependent architecture that characterizes most climate-resilience traits [7, 11]. This is not a criticism of the underlying logic so much as an observation about where the field currently sits: the conceptual case for integration is considerably more mature than the empirical case, and describing the three domains as already operating as a unified pipeline would overstate what has actually been demonstrated at scale.

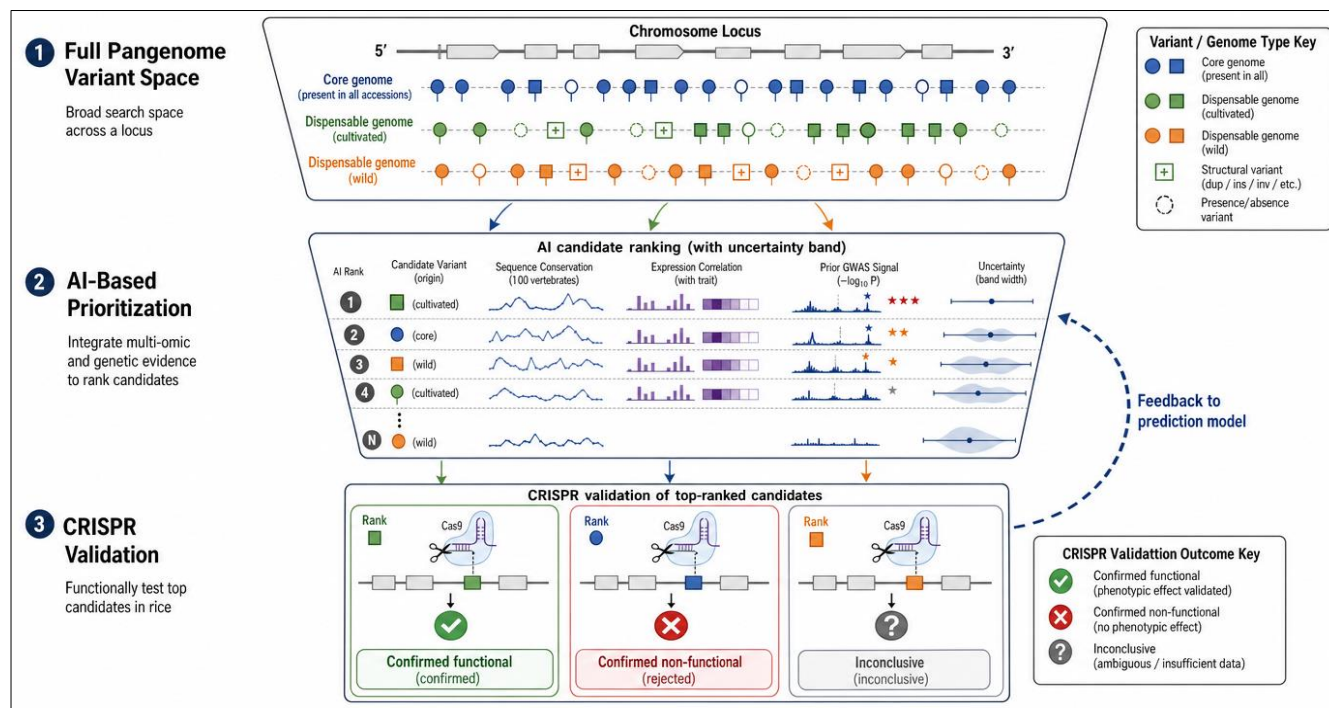


Fig 1: Allelic search space collapse through AI-guided pangenome screening prior to CRISPR validation.

7. Climate-Resilient Traits Through a Precision-Breeding Lens

Drought tolerance in rice is polygenic and strongly influenced by the developmental timing and intensity of water deficit, which is precisely why marker-assisted introgression of individual quantitative trait loci has yielded real but incremental gains rather than transformative resilience, and why genomic-selection and multiplex-editing approaches capable of addressing multiple contributing loci simultaneously are considered more promising for this trait than single-gene strategies [30, 35, 40]. Heat tolerance intersects with reproductive-stage sensitivity in particular, where even short periods of elevated temperature during flowering can disproportionately affect yield, making high-resolution, time-resolved phenotyping of reproductive processes a natural application area for image-based AI phenotyping [16]. Salinity tolerance has a comparatively better-characterized molecular basis than drought, with ion transport and exclusion mechanisms offering more tractable single- or few-gene targets, which is part of why it has been an earlier and more frequent target for both marker-assisted and editing-based approaches relative to more diffusely polygenic traits [3, 53]. Submergence and flooding tolerance

represents one of the field's clearer success stories for marker-assisted introgression specifically, with QTL-based approaches having already delivered field-validated, farmer-adopted varieties, which is worth noting precisely because it illustrates that not every climate trait requires the full weight of genome editing or AI-driven prediction to achieve meaningful deployment architecture, not technological sophistication alone, determines which toolkit is proportionate [24, 53]. Nutrient-use efficiency and durable disease resistance round out the trait set most frequently targeted, with the former increasingly framed as a climate-adaptation trait in its own right given the emissions and cost burden of nitrogen fertiliser, and the latter benefiting particularly from CRISPR-based approaches that disable susceptibility genes rather than relying on race-specific resistance genes that pathogens can eventually overcome [6, 13].

8. Challenges and Limitations That Cut Across All Three Domains

Several limitations recur regardless of which single domain or combination is under discussion. Biologically, epistasis and pleiotropy mean that editing or selecting for one

favourable allele can have unanticipated effects elsewhere in the phenotype, and gene-by-environment interaction means that an allele beneficial under one stress regime may be neutral or even deleterious under another, which is a substantive complication for any breeding strategy that treats climate resilience as a fixed target rather than a moving one [28, 36]. Computationally, the generalization and interpretability problems already discussed for AI models are compounded by the comparatively modest size of most rice breeding datasets relative to the genomic and phenotypic dimensionality involved, and by demonstrable bias where models trained predominantly on well-represented germplasm groups perform less reliably on underrepresented wild or landrace material precisely the material pangenomics is trying to bring into the fold [34, 54]. Technically, genotype-dependent transformation efficiency and delivery constraints for genome editing mean that a validated editing protocol in one background cannot be assumed to transfer directly to the diverse elite lines actually

grown across different rice-producing regions, and the computational burden of pangenome-scale, long-read assembly and graph-based variant calling remains non-trivial relative to standard resequencing pipelines [12, 37]. Regulatory and deployment challenges are perhaps the least technical but most consequential: biosafety assessment frameworks for edited crops differ meaningfully across jurisdictions, public acceptance of genome-edited food crops is not uniform, and intellectual property considerations around both editing tools and proprietary prediction models can slow translation from research demonstration to farmer-available variety regardless of the underlying science being sound [37, 48]. Taken together, these four categories of limitation explain why so much of the literature surveyed here remains confined to controlled greenhouse or single-season field settings rather than multi-environment, multi-year validation at the scale required before a variety reaches release.

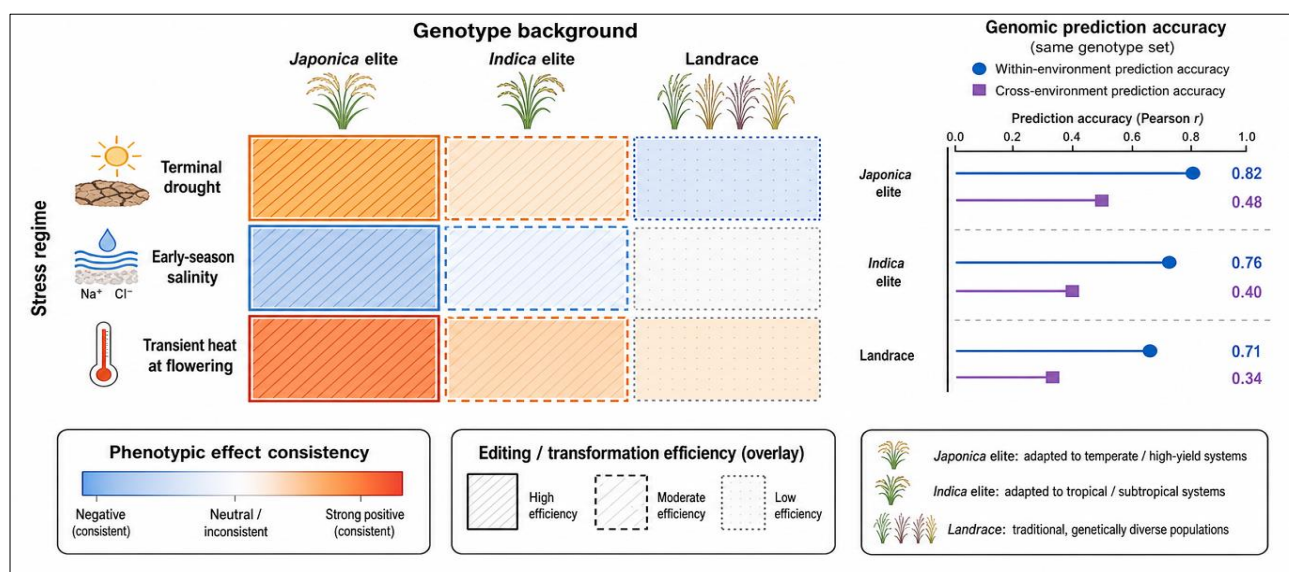


Fig 2: Interactions Among Genotype, Environment and Genome Editing Efficiency in Precision Rice Breeding

9. Future Perspectives

Several directions follow reasonably directly from the limitations identified above rather than requiring speculative extrapolation. Expanding pangenome representation specifically toward underused wild relatives and landraces, rather than further deepening resequencing of already well-represented elite cultivars, would address the allelic-diversity bottleneck more directly than incremental improvements to reference quality for material already characterized [12, 22, 49]. On the computational side, explainable AI approaches that expose which genomic or phenotypic features drive a given prediction, rather than treating the model purely as a black box, would help breeders audit predictions against agronomic knowledge and would make it easier to identify when a model's apparent accuracy is an artefact of the training population rather than a generalizable signal [47, 54]. Multi-omics integration combining genomic, transcriptomic, and phenotypic data streams within prediction frameworks rather than relying on genomic markers alone has already shown incremental gains in specific studies and represents a comparatively low-risk extension of current genomic-selection practice rather than a speculative leap [18, 20, 43].

Digital or "breeding-by-design" pipelines that explicitly formalize the sequence from AI-prioritized target through pangenome-informed allele identification to edited or introgressed deployment are a reasonable medium-term goal, but the evidence reviewed here suggests this should be framed as an aspiration the field is building toward rather than an existing operational reality [14, 42]. Editing systems less dependent on genotype background whether through improved transformation methods or expanded, more broadly compatible Cas variants would do more to widen practical deployment than further refinement of editing precision in genotypes where the technology already works well [37, 38]. Framed against the scale of the climate challenge, these are incremental rather than transformative steps, and that is arguably the more honest characterization of where the field genuinely stands.

10. Conclusion

CRISPR-Cas genome editing, pangenomics and artificial intelligence each address a genuine limitation of the technologies that preceded them in rice breeding, and there is a coherent conceptual argument for why they should function as complementary stages of a single precision-

breeding pipeline rather than as independent toolkits. The evidence assembled here supports that argument in principle more strongly than it demonstrates it in practice: genotype-dependent editing efficiency, poor cross-environment transferability of predictive models, and pangenome resources that remain more complete for structural characterization than for functional annotation are all obstacles that current studies acknowledge rather than resolve. Climate-resilience traits being polygenic, environmentally contingent, and often in tension with yield and phenology are precisely the traits least forgiving of an incomplete pipeline. The realistic path forward lies not in awaiting a single breakthrough but in closing specific, identifiable gaps: broader and more functionally annotated pangenome coverage of wild diversity, prediction models validated for genuine cross-environment transfer rather than within-dataset accuracy alone, and editing systems whose efficiency does not collapse outside a narrow set of model genotypes. Precision breeding in rice is a genuinely promising convergence of technologies, but it remains, at present, an assembly of powerful components rather than a finished machine.

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