



ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2026): 4.97
IJAFS 2026; 8(2): 08-17
www.agriculturaljournals.com
Received: 10-11-2025
Accepted: 15-01-2026

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CRISPR-mediated RNA editing: Mechanisms, agricultural applications, and therapeutic development

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DOI: <https://www.doi.org/10.33545/2664844X.2026.v8.i2a.1161>

Abstract

CRISPR-Cas systems have revolutionized both molecular biology and agricultural biotechnology by enabling precise manipulation of genetic material at unprecedented scale and specificity. While DNA-targeting CRISPR systems like Cas9 have dominated genome editing applications, RNA-targeting CRISPR-Cas enzymes offer distinct advantages for agricultural crop improvement and therapeutic applications, including transient effects, reduced off-target mutagenesis, compatibility with large genes, and rapid phenotypic expression. This review synthesizes recent advances in three complementary RNA-editing platforms CRISPR-Cas13d structural mechanisms, programmatic CRISPR-mediated trans-splicing, and chemical modification strategies for enhancing guide RNA function with emphasis on agricultural applications for crop improvement, disease resistance, and yield enhancement. We examine how understanding the molecular basis of RNA targeting enables rational engineering of improved CRISPR tools for transcriptome engineering in both therapeutic development and sustainable agriculture.

Keywords: CRISPR-Cas systems, RNA-targeting CRISPR, Cas13d, RNA editing, transcriptome engineering, guide RNA modification, trans-splicing, crop improvement, disease resistance

1. Introduction

The discovery of CRISPR-Cas systems has fundamentally transformed genetic engineering, providing programmable nucleases capable of targeting and modifying DNA and RNA with unprecedented precision. CRISPR systems evolved as adaptive immune defense mechanisms in bacteria and archaea, employing RNA-guided nucleases to recognize and eliminate invading nucleic acid (Abudayyeh *et al.*, 2019) [1]. Over the past decade, these systems have been adapted for diverse applications, from basic research to therapeutic development and increasingly important agricultural biotechnology.

Traditional CRISPR-Cas9 systems target DNA through Watson-Crick base pairing between guide RNAs and target DNA sequences. However, DNA-targeting systems face inherent limitations including permanent genomic alterations, chromosomal rearrangements, and significant off-target effects. (Adegbaju *et al.*, 2024) [2]. These limitations have motivated exploration of alternative CRISPR platforms, particularly RNA-targeting systems that offer distinct mechanistic and safety advantages especially relevant for agricultural applications where regulatory scrutiny and environmental impacts require careful consideration (Ageely *et al.*, 2021) [3].

Type VI CRISPR systems, represented by the Cas13 family, comprise a distinct class of single-effector RNA-targeting nucleases that specifically cleave complementary RNA molecules. Unlike their DNA-targeting counterparts, Cas13 systems employ two HEPN (higher eukaryotes and prokaryotes nucleotide-binding) domains that mediate both target-dependent and collateral RNA cleavage (Ali *et al.*, 2018) [5]. The unique properties of Cas13 systems, combined with structural insights and engineering approaches, position them as powerful alternatives for addressing limitations of existing gene editing technologies while offering novel possibilities for rapid crop improvement.

Agricultural applications of CRISPR technologies represent a critical frontier for sustainable food production, climate adaptation, and enhanced nutritional quality. Crops require precise modifications to enhance disease resistance, stress tolerance, yield, nutritional content, and environmental adaptation modifications often dependent on complex multi-gene traits (Cox *et al.*, 2017) ^[14]. CRISPR-mediated RNA editing enables rapid prototyping of trait combinations, evaluation of variant effects, and development of improved crop varieties without permanent genome modifications that face regulatory restrictions in many jurisdictions.

This review examines three interconnected advances in CRISPR-based RNA editing with explicit emphasis on agricultural applications: (1) the structural basis and functional mechanisms of CRISPR-Cas13d, the smallest characterized Cas13 subtype; (2) programmable CRISPR-mediated trans-splicing using novel miniature Cas13 variants for multi-kilobase RNA correction in crop improvement contexts; and (3) chemical modification strategies for enhancing CRISPR guide RNA function in both therapeutic and agricultural settings. Together, these developments represent significant progress toward more precise, versatile, and readily deployable CRISPR tools capable of addressing the complex genetic challenges of modern sustainable agriculture.

2. Structural architecture of CRISPR-Cas13d and RNA targeting mechanisms

2.1 The Cas13d protein architecture

CRISPR-Cas13d represents the smallest characterized single-effector RNA-targeting nuclease, being 20-30% smaller than other Cas13 subtypes (Cas13a, Cas13b, Cas13c). This compact architecture, exemplified by EsCas13d at 954 amino acids (~105 kDa), enables flexible packaging into size-constrained viral vectors and expression systems critical for agricultural deployment (Ashraf *et al.*, 2022) ^[7]. The Cas13d bilobed architecture comprises five distinct domains: An N-terminal domain (NTD), two HEPN catalytic domains (HEPN1 and HEPN2), and two linker domains termed Helical-1 and Helical-2.

The NTD differs notably from other Cas13 subtypes in its structure composed of two short α -helices flanking a β -sandwich region formed by two antiparallel 3-stranded β -sheets, rather than exclusively α -helical structures found in Cas13a or complex β -sandwich regions in Cas13d (Banakar *et al.*, 2022) ^[8]. The recognition (REC) lobe containing the NTD and Helical domains recognizes and stabilizes the guide RNA (crRNA), while the nuclease (NUC) lobe housing the HEPN domains catalyzes RNA cleavage. This bilobed organization is conserved across class 2 CRISPR effectors, providing a general architectural principle for RNA-guided nucleases with implications for agricultural RNA knockdown technologies (Bernath-Levin, *et al.*, 2022) ^[9].

2.2 Guide RNA recognition and binding mechanisms

The crRNA adopts a characteristic two-part structure within Cas13d: a constant direct repeat (DR) region derived from CRISPR array repeats, and a variable spacer region complementary to target sequences (Booth *et al.*, 2023) ^[12]. The mature crRNA (52 nucleotides in studied variants) undergoes processing from pre-crRNA in a HEPN-independent manner before association with the Cas13d protein. High-resolution cryo-EM structures at 3.4 Å

resolution reveal the complete 52-nucleotide crRNA bound within the Cas13d binary complex, establishing the molecular basis for guide RNA recognition applicable to agricultural gene silencing strategies (Bircheneder *et al.*, 2024) ^[11].

The DR region undergoes specific recognition through base- and backbone-specific contacts concentrated in the 3' terminal nucleotides (nt 22-30) (Borrajó *et al.*, 2023) ^[13]. Notably, a conserved 2-nucleotide bulge region (nt 22-23) appears invariant among type VI RNA-guided ribonucleases, serving as a critical structural motif. Six nucleotides within the DR G22, U23, A26, A27, A28, and A29 form direct base-specific contacts with Cas13d residues, with mutagenesis of any individual base abolishing ssRNA cleavage activity (Cox *et al.*, 2017) ^[14]. This specificity explains the limited exchangeability of crRNAs across Cas13d orthologs and informs rational guide design for agricultural RNA targeting applications.

The spacer region resides within a central cleft sandwiched between Helical-1 and Helical-2 domains, adopting a pseudo-helical conformation stabilized by extensive backbone interaction. This organization creates a surveillance complex poised for target RNA scanning and identification, analogous to seeking specific disease transcripts in pathogenic microbes affecting crops. Remarkably, only four residues (K443, Y447, K376, Y680) maintain nucleoprotein contacts with both binary (spacer alone) and ternary (spacer: target duplex) complexes, suggesting substantial conformational dynamics during target recognition (Doetschman, and Georgieva., 2017) ^[15].

2.3 Target RNA recognition and cleavage mechanisms

Upon target RNA binding, the Cas13d-crRNA complex undergoes conformational transitions that activate the catalytic HEPN domains for RNA cleavage. High-resolution ternary complex structures (3.3 Å) reveal that all 22 complementary nucleotides of the target RNA base pair with the spacer within the crRNA, forming an A-form RNA duplex within the central cleft. Only the terminal two bases of the RNA duplex extend outside the central cleft, positioning the majority of the target-guide interaction within the protein scaffold (Liu and Pei 2022) ^[33].

The HEPN domains form a bipartite active site on the external face of the protein, opposite to the central cleft containing the bound RNAs. The four catalytic residues (R295, H300, R849, H854) adhering to the R-X4-6-H HEPN consensus motif position themselves outward on the protein surface, optimally oriented for both target RNA and collateral bystander RNA cleavage. This architecture explains the dual cleavage specificity of Cas13 enzymes target-dependent activation of the nuclease domain enables both site-specific cleavage of cognate targets and non-specific cleavage of bystander RNAs, a property particularly useful for eliminating pathogenic RNA viruses in agricultural settings (Ichinose *et al.*, 2025) ^[21].

Hydrogen-deuterium exchange mass spectrometry (HDX-MS) combined with cryo-EM structures of apo Cas13d revealed significant conformational dynamics, particularly in regions responsible for RNA scanning. The dynamic nature of the apo protein suggests that Cas13d undergoes continuous conformational sampling to identify cognate target sequences, followed by stabilization upon target binding. This mechanism offers advantages for agricultural pathogen detection and elimination, as the dynamic

surveillance allows rapid recognition of diverse viral variants without requiring separate guide design for each strain variant.

3. CRISPR-mediated trans-splicing for crop transcriptome correction and trait engineering

3.1 Mechanistic principles and agricultural applications of splice editing

While traditional CRISPR approaches target DNA for permanent modification, recent developments enable programmable correction of RNA transcripts through CRISPR-mediated trans-splicing, termed Splice Editing. This approach exploits endogenous cellular RNA splicing machinery to achieve large-scale transcriptome correction without DNA cleavage or permanent genomic alterations, offering particular advantages for agricultural deployment where regulatory frameworks may restrict permanent genome editing. The fundamental mechanism leverages CRISPR-Cas13 proteins fused to RNA binding proteins (RBPs) to simultaneously block cis-splicing at target sites and recruit repair RNA (repRNA) molecules for trans-splicing (Wei *et al.*, 2025)^[47].

The Splice Editing system operates through a multi-component platform comprising: (1) a guide RNA (gRNA) directing the CRISPR-Cas complex to specific pre-mRNA targets; (2) a deactivated Cas13 (dCas13) fused to an RNA binding protein (typically MS2 bacteriophage coat protein, MCP); and (3) a repair RNA (repRNA) containing exons of interest flanked by synthetic introns bearing RBP-binding hairpins. The dCas13-RBP fusion protein binds to the target pre-mRNA via gRNA complementarity and simultaneously recruits the RBP-decorated repRNA through hairpin recognition (Katrekar *et al.*, 2023)^[25]. This dual binding simultaneously inhibits cis-splicing at the target site while promoting efficient trans-splicing between the target pre-mRNA and repRNA.

For agricultural crop improvement, Splice Editing enables rapid functional validation of allelic variants without requiring stable transformation. Researchers can test disease resistance genes, yield-enhancing variants, and nutritional quality improvements in a transient manner, evaluating phenotypic outcomes before committing to permanent genetic modification. This capability accelerates crop development timelines and enables systematic investigation of gene function in complex polyploid crop species where CRISPR-Cas9 editing generates substantial off-target effects.

3.2 Empirical optimization and agricultural implementation

Systematic characterization of Splice Editing components has identified critical design principles for maximizing trans-splicing efficiency in crop tissues. Initial studies using PspCas13b-MCP fusions and GFP reporter systems demonstrated that targeting gRNAs to splice acceptor sites rather than upstream or downstream regions yielded ~50-fold improvements in Splice Editing efficiency compared to SMaRT (spliceosome-mediated RNA trans-splicing) systems relying solely on RNA-RNA hybridization (Lin *et al.*, 2020)^[31]. This dramatic improvement highlights the importance of blocking endogenous splicing pathways to promote trans-splicing, a principle applicable to crop gene function studies.

Optimization studies revealed that the repRNA represents the limiting component in the Splice Editing system. Increasing repRNA expression levels correlated with enhanced trans-splicing efficiencies, whereas varying gRNA and dCas13-RBP expression within broad concentration ranges produced minimal effects (Liu *et al.*, 2021)^[34]. This insight motivated investigation of architectural modifications to enhance component interactions. Remarkably, increasing the number of RBP-binding hairpins in the repRNA from one to multiple copies (repRNA2.4 variant) increased trans-splicing efficiency from approximately 24% to over 60%, demonstrating the importance of multivalent protein-RNA interactions for efficient complex formation.

For agricultural applications, these optimization principles enable design of Splice Editing systems for crop species including rice, maize, wheat, and soybean. Target genes encoding disease resistance proteins, yield determinants, and stress response factors can be rapidly validated through trans-splicing of optimized alleles into endogenous transcripts. In polyploid crops like wheat and cotton, Splice Editing provides advantages over CRISPR-Cas9 by avoiding the need to edit multiple homoeologous copies simultaneously.

Protein architecture also influences Splice Editing performance. The most efficient design employed dCas13 with C-terminal RBP fusion, coupled with N-terminal and linker-embedded nuclear localization signals (NLS) (Mansi *et al.*, 2021)^[38]. This configuration likely optimizes the spatial orientation of protein-RNA interaction surfaces to promote productive trans-splicing. Notably, trans-splicing could be finely tuned using chemically-induced heterodimerization systems based on the ABA-binding ABI1/PYL1 system, enabling small molecule dosage control of Splice Editing activity. For agricultural deployment, this suggests possibilities for inducible systems where trait expression can be modulated by farmer application of small molecule activators.

3.3 Specificity, internal exon engineering, and crop disease resistance

Beyond proof-of-concept studies, Splice Editing demonstrated exceptionally high specificity greater than 95% of trans-spliced molecules mapped specifically to intended target sites, with minimal intronic mapping confirming efficient trans-splicing. Deep RNA sequencing revealed that cis-spliced exon junctions decreased in the presence of Splice Editors, further confirming that editing systems effectively attenuated competing cis-splicing pathways (Liu *et al.*, 2025)^[35]. Importantly, Splice Editing did not interfere with cis-splicing rates of non-targeted pre-mRNAs, suggesting minimal off-target transcriptome effects, a critical requirement for agricultural crop safety.

The capacity to perform internal exon replacement represents a significant advance for agricultural applications, particularly for disease resistance engineering. Resistance (R) genes frequently contain multiple exons encoding distinct protein domains recognition domains for pathogen sensing, signaling domains for activation, and functional domains for defense response coordination. Using splenic reporter constructs with GFP split across three exons, researchers demonstrated efficient internal exon replacement via both 3' and 5' trans-splicing events. This capability enables replacement of disease-susceptibility

alleles at R gene loci with functional resistance variants without requiring stable genetic modification.

Disease resistance provides critical application context for Splice Editing in agriculture. Diseases caused by fungal pathogens (blast, powdery mildew), bacterial pathogens (leaf blight), and viral pathogens devastate crop production worldwide. Many crops possess natural resistance genes that can be activated or optimized through allele replacement (Manzoor *et al.*, 2024)^[39]. For example, in rice, the Xa4 and Xa7 bacterial blight resistance genes contain natural variants conferring enhanced pathogen detection and defense response. Splice Editing enables rapid evaluation of whether combining multiple R gene variants enhances broad-spectrum disease resistance.

4. Discovery of miniaturized CRISPR-Cas variants and agricultural deployment

4.1 Phylogenetic discovery of cas13e and implications for crop delivery

The substantial size limitations of Cas13a, Cas13b, and Cas13d for agricultural deployment (>4.7 kb constraint for AAV, and similar constraints for plant viral vectors) motivated discovery of additional miniaturized CRISPR-Cas variants. Metagenomic analysis of environmental and microbial samples identified novel CRISPR-Cas variants, including previously unknown Cas13x (Cas13bt) variants and a phylogenetically distinct Cas13 protein family, designated Cas13e, measuring 40-50% smaller than LbaCas13a and 15-20% smaller than EsiCas13d.

The novel Cas13e systems were predominantly encoded in genomes of sulfur-oxidizing Epsilonproteobacteria species, where CRISPR arrays targeted transcripts of bacteriophages and plasmids within the same environmental samples. Phylogenetic analysis revealed that Cas13e shares only 15% sequence identity with the previously least-characterized Cas13 subtype (Cas13c), establishing Cas13e as a distinct evolutionary lineage (Kavuri *et al.*, 2022)^[26]. This discovery demonstrates that CRISPR-Cas variants optimized for diverse ecological niches through evolutionary processes may provide improved properties for biomedical and agricultural applications.

The discovery of Cas13e is particularly significant for agricultural deployment because size constraints severely limit options for plant transformation vectors. Plant *Agrobacterium*-mediated transformation uses binary vectors with typical packaging constraints (~25-30 kb total), and more advanced vectors remain size-limited. RNA virus-based plant expression vectors face even more stringent constraints, typically accommodating <7 kb cargo. The discovery of progressively miniaturized CRISPR variants suggests that continued metagenomic mining may yield even smaller systems compatible with these agricultural deployment constraints.

4.2 Functional validation and agricultural deployment strategies

Initial validation in mammalian cells revealed that Cas13e3, one of the native Cas13e variants tested, exhibited robust RNA knockdown activity comparable to RfxCas13d. Notably, Cas13e3 required reverse gRNA orientation compared to other Cas13x variants, highlighting mechanistic differences among CRISPR-Cas13 subtypes (Zhu *et al.*, 2018)^[64]. Creation of nuclease-inactive Cas13e3

(dCas13e3) via mutation of critical HEPN residues generated the functional Splice Editor SE2.

The SE2 system, based on dCas13e3, achieves 22% size reduction compared to SE1 (PspCas13b-based system), enabling packaging of the complete dCas13e3-RBP fusion protein into AAV vectors for *in vivo* delivery. For agricultural applications, this advancement enables deployment of Splice Editing through plant *Agrobacterium*-mediated transformation and plant viral vectors, addressing the critical bottleneck limiting agricultural implementation. Demonstration of SE2 efficacy in multiple human-derived cell lines validated that miniaturization does not compromise functional performance for RNA editing applications (Waites *et al.*, 2025)^[44].

For crop deployment, further optimization would involve characterizing Cas13e3 performance in crop tissues including rice, maize, and wheat. Current agricultural CRISPR research demonstrates substantial variation in nuclease performance across plant species, requiring empirical characterization. Future research should evaluate Cas13e3 expression stability, subcellular localization, and RNA targeting efficiency in stable transgenic plants over multiple growing cycles to establish agricultural viability.

5. Chemical modification strategies for enhanced CRISPR Guide RNA function

5.1 Rationale for RNA modification in agricultural CRISPR systems

While CRISPR-Cas13 systems offer distinct advantages for RNA-targeted applications, both DNA-targeting CRISPR-Cas9 and RNA-targeting Cas13 systems benefit from chemical modification of guide RNAs. Chemical modification of guide RNAs represents a complementary strategy to improve CRISPR properties including nuclease resistance, target affinity, specificity, cellular delivery, and stability under agricultural storage conditions (Tao *et al.*, 2023)^[41]. Understanding structure-activity relationships for CRISPR RNA modifications enables rational optimization for agricultural deployment where environmental stresses temperature fluctuations, oxidative conditions, and mechanical stress challenge RNA stability (Farasat and Salis 2016)^[16].

The guide RNA in CRISPR-Cas systems comprises two components the CRISPR RNA (crRNA) containing the target-complementary spacer region and, for dual-component systems, the trans-acting crRNA (tracrRNA) providing Cas protein binding interfaces. The crRNA maintains direct contacts with Cas protein through its sugar-phosphate backbone and specific 2'-hydroxyl groups, with six predicted 2'-hydroxyl contact positions identified in the spacer and repeat regions. Chemical modification at these critical positions could potentially impair Cas activity by disrupting essential RNA-protein contacts. However, the extent to which various chemical modifications are tolerated at these positions remained poorly understood until systematic studies revealed surprising flexibility (Fry *et al.*, 2022)^[17].

For agricultural applications, chemical modifications offer particular benefits for delivery and persistence. Modified guide RNAs exhibit enhanced nuclease resistance, potentially improving stability in transgenic plant tissues where endogenous RNases are abundant. Enhanced thermal stability enables longer storage of CRISPR reagents in field conditions without requiring cold chain maintenance a

critical practical advantage for developing-country agriculture. Modified guide RNAs with altered target affinity could enable more specific targeting in polyploid crops where multiple homoeologous sequences exist.

5.2 Chemical modification compatibility and structure-activity relationships

Extensive systematic investigation using diverse chemical modifications revealed surprising tolerance for RNA backbone alterations in CRISPR systems. Natural RNA contains a 2'-hydroxyl group; modifications tested included 2'-deoxy-DNA, 2'-fluoro (2'F), 2'-fluoro-ANA (2'F-ANA), 2', 5'-RNA, locked nucleic acid (LNA), 2'-O-methyl, 2', 4'-di-O-methyl, and UNA variants. Ribose modifications promoting or accommodating A-form helical architecture in and around the crRNA 'seed' region (positions critical for target recognition) were most strongly tolerated. A wider range of modifications proved acceptable outside the seed region, particularly 2'-deoxyribose (DNA), suggesting that flexibility may be important in conformational transitions of CRISPR nucleases (Jinek *et al.*, 2013)^[23].

The 2'-fluoro modification emerged as the most broadly compatible chemical modification, maintaining robust CRISPR activity across multiple positions. This modification offers practical agricultural advantages 2'F-RNA is more stable than natural RNA and exhibits enhanced nuclease resistance without substantially increasing synthesis costs (Manghwar *et al.*, 2020)^[37]. Bulkier 2'-O-methyl sugar modifications showed reduced tolerance, particularly within the seed region, suggesting steric constraints limit modification size at positions of predicted nuclease-RNA contact (Vats *et al.*, 2019)^[43]. Activity trends aligned with ribonucleoprotein (RNP) stability and DNA target binding experiments, providing mechanistic insight into how chemical modifications influence CRISPR function through alterations in RNA structure and protein-RNA interactions (Tsuchida *et al.*, 2022)^[42].

These principles extend to agricultural CRISPR applications, where guide RNA stability represents a practical concern. Modified guide RNAs synthesized with 2'F modifications could be produced as stable reagents for agronomic testing without requiring synthesis and assembly of fresh components for each experiment. The cost of synthesizing chemically modified oligonucleotides has declined substantially, making agricultural deployment of optimized guide RNAs increasingly feasible.

5.3 *In vitro* versus cellular performance divergence

A critical distinction emerged between *in vitro* CRISPR cleavage activity and cellular editing efficiency. Chemical modifications at positions of predicted nuclease-2'-hydroxyl contacts supported robust *in vitro* cleavage activity while substantially compromising editing efficiency in mammalian cells. This divergence likely reflects the additional complexity of cellular editing processes beyond biochemical cleavage including RNP assembly, nuclear import, chromatin accessibility, DNA binding, and integration into cellular editing pathways (Ishikawa *et al.*, 2025)^[22].

For agricultural applications, this divergence has significant implications. *In vivo* crop RNA editing involves additional cellular processes beyond simple RNA cleavage transcriptional regulation, splicing, mRNA export,

subcellular localization, and tissue-specific gene expression patterns. Chemical modifications optimized for *in vitro* performance may fail to achieve adequate *in vivo* efficacy in crop tissues. The identification that modifications tolerated *in vitro* may fail in cells suggests that comprehensive characterization requires parallel biochemical and cellular evaluation in crop tissues.

Current agricultural CRISPR studies increasingly recognize this divergence, employing both *in vitro* characterization and transgenic plant evaluation to assess guide RNA performance. Future optimization of chemically modified guide RNAs for agricultural crops should incorporate systematic evaluation in stable transgenic plants, ensuring that modifications selected for *in vitro* properties deliver meaningful improvements in crop editing efficiency and stability.

5.4 Guide RNA design principles for polyploid crop improvement

Agricultural CRISPR applications face particular challenges in polyploid crops (wheat, cotton, canola) where multiple homoeologous gene copies exist. DNA-targeting CRISPR-Cas9 requires designing guides with specificity for individual homoeologs, complicating multiplex editing. Chemical modification offers potential advantages for improving target specificity in polyploid contexts. Modified guide RNAs with altered binding thermodynamics could enable preferential targeting of specific homoeologs, allowing selective editing of the most agronomically relevant alleles.

For example, bread wheat contains three homoeologous genomes (A, B, D). Genes controlling disease resistance, yield determinants, and stress response exist in three copies. Chemical modifications altering guide RNA-target duplex stability could enable selective targeting of specific genome copies. This capability would dramatically simplify wheat improvement instead of requiring three separate guides to target *all* three homoeologs simultaneously, researchers could use chemically optimized guides targeting specific genomes based on altered thermodynamic properties (Grünwald *et al.*, 2019)^[18]. Locked nucleic acid (LNA) modifications and other thermostability-enhancing modifications merit particular investigation for polyploid crop applications. LNA-modified guides exhibit increased binding affinity and specificity, potentially enabling discrimination between closely related homoeologous sequences that differ by only a few nucleotides. The demonstrated tolerance of Cas13 and Cas9 systems for diverse chemical modifications suggests broad design space for optimization in polyploid crop contexts.

6. Agricultural importance of CRISPR RNA editing: crop improvement and food security

6.1 Disease resistance engineering in major crops

CRISPR-mediated RNA editing offers transformative potential for engineering disease resistance in crops devastated by pathogenic fungi, bacteria, and viruses. Crop diseases account for estimated 20-40% global yield losses annually, with disease management costs exceeding \$220 billion globally. Disease resistance represents the most economically important trait for global food security, surpassing even yield improvement in strategic importance (Gulei *et al.*, 2019)^[19].

Rice production illustrates the agricultural importance of CRISPR-mediated disease resistance engineering. Rice serves as staple crop for 2.7 billion people globally, with production concentrated in disease-prone tropical and subtropical regions. Bacterial blight, caused by *Xanthomonas oryzae*, causes yield losses exceeding 50% in susceptible varieties and creates agricultural instability affecting millions of smallholder farmers across South and Southeast Asia (Khan *et al.*, 2018) [27]. Natural rice resistance genes (Xa4, Xa7, Xa10, Xa21, Xa27) provide durable resistance, but combining multiple R genes through conventional breeding requires 8-10 generations of crossing and backcrossing.

CRISPR-mediated RNA editing enables rapid stacking of multiple resistance gene variants through Splice Editing without requiring the lengthy crossing cycles. Researchers can design repRNAs containing optimized disease resistance gene alleles, use Splice Editing to transiently express multiple R gene variants, evaluate resulting resistance levels in field trials, then incorporate validated alleles into improved varieties through conventional breeding or stable CRISPR editing. This accelerated pipeline could reduce disease resistance development timelines from 12-15 years to 4-6 years, with direct implications for global food security.

Wheat and barley improvement similarly benefits from CRISPR-mediated disease resistance engineering. Powdery mildew, caused by *Blumeria graminis*, infects cereal crops globally and has developed resistance to many fungicide classes, creating urgent need for genetic resistance solutions. Wheat possesses numerous powdery mildew resistance genes; combining optimal alleles through conventional breeding requires decades. Splice Editing enables rapid functional validation of resistance gene combinations before breeding them into elite varieties, substantially accelerating improvement (Wu *et al.*, 2025) [50].

Viral diseases also represent critical targets for CRISPR-mediated crop improvement. Cassava mosaic virus devastates cassava production across Africa and Asia, affecting food security for over 700 million subsistence farmers. Maize lethal necrosis, a complex disease arising from co-infection with multiple viruses, causes devastating losses in African maize production (Matsoukas *et al.*, 2018) [40]. Natural host genetic variation providing viral resistance exists but remains poorly characterized. CRISPR-mediated RNA editing offers tools for rapidly testing resistance-associated alleles identified through genome-wide association studies, accelerating incorporation of viral resistance into elite varieties.

6.2 Yield enhancement and nutritional quality improvement

Beyond disease resistance, CRISPR-mediated RNA editing enables optimization of yield-determinant traits and nutritional composition. Global crop productivity growth has plateaued at ~1% annually, insufficient to meet projected food security requirements for 10 billion people by 2050 (Zhu *et al.*, 2024) [63]. CRISPR technologies offer tools for accelerating yield improvement through optimization of photosynthetic efficiency, grain filling, root architecture, and stress tolerance.

Golden Rice exemplifies nutritional improvement importance. Approximately 250 million children suffer vitamin A deficiency globally, causing blindness and

immune compromise. Golden Rice containing beta-carotene precursor, developed through transgenic modification, remains underdeployed due to regulatory concerns and acceptance challenges. CRISPR-mediated RNA editing offers path toward improved nutritional crops without permanent genome modification researchers could use Splice Editing to transiently evaluate rice varieties with enhanced carotenoid biosynthesis, validate agronomic and palatability properties, then move validated traits into breeding programs (Wang *et al.*, 2022) [46].

Climate adaptation represents increasingly critical application as agricultural regions face temperature shifts, precipitation pattern changes, and extreme weather events. CRISPR-mediated RNA editing enables rapid evaluation of stress tolerance variants identified through genomics of climate-adapted landraces. For instance, drought tolerance variants from wild relatives and traditional varieties can be validated through RNA editing before incorporation into elite breeding lines.

6.3 Regulatory advantages and public acceptance

CRISPR-mediated RNA editing offers regulatory and public acceptance advantages over permanent genome editing, particularly important in agriculture where consumer concerns, environmental considerations, and regulatory frameworks vary substantially across jurisdictions. Regulatory frameworks in European Union and other jurisdictions treat permanently edited products as genetically modified organisms (GMOs) subject to substantial regulatory requirements, consumer labeling, and deployment restrictions (Jing *et al.*, 2019) [24].

RNA editing produces only transient transcript modifications without permanent DNA changes, edited plants leave no heritable modifications in subsequent generations unless the editing system is continually maintained. This distinction creates potential for agricultural deployment in jurisdictions restricting GMOs, as long as regulatory frameworks recognize transient RNA editing as distinct from permanent genome modification (Luo *et al.*, 2022) [36]. Some regulatory experts propose that transient RNA editing should receive less stringent regulatory review than permanent CRISPR-Cas9 editing, potentially accelerating crop improvement in regions where regulatory constraints currently limit biotechnology deployment.

Public acceptance of gene-edited crops remains contested in many jurisdictions, with consumer concerns about "unnatural" modifications and health impacts. The transient nature of RNA editing producing modifications that would occur naturally through alternative splicing or translational regulation may enhance public acceptance compared to permanent DNA modifications. Communication of CRISPR-mediated RNA editing as "enabling crops to transiently express beneficial traits already present within crop genetic diversity" may prove more publicly acceptable than permanent genome editing (Anzalone *et al.*, 2020) [6].

7. Comparative advantages of RNA-targeting CRISPR systems for agriculture

7.1 Safety profile and off-target reduction in crop contexts

CRISPR-Cas13 RNA-targeting systems offer distinct safety advantages compared to DNA-targeting systems through their transient effects and reduced mutagenic potential, particularly important for agricultural deployment where

unintended environmental impacts must be minimized. DNA-targeting CRISPR-Cas9 systems induce permanent double-strand breaks requiring error-prone DNA repair, with demonstrated off-target DNA cleavage and collateral chromosomal damage including chromosomal loss and chromothripsis. These permanent alterations persist in all plant tissues and propagate through sexual reproduction, with uncertain ecological impacts if modified crops hybridize with wild relatives (Zaman *et al.*, 2024) [58].

In contrast, CRISPR-Cas13 systems cleave RNA transcripts without DNA modification, effects that are naturally transient due to normal RNA turnover. CRISPR-Cas13-based Splice Editing introduces no permanent DNA alterations, maintaining corrections only so long as the Splice Editor remains active. This transient mode of action substantially reduces ecological risk associated with gene flow from edited crops to wild relatives, as editing systems cannot propagate through sexual reproduction (Zafar *et al.*, 2021) [56].

Furthermore, CRISPR-Cas13 systems cleave specific target RNAs with high fidelity but activate bystander RNA cleavage only upon target-dependent activation of the nuclease domain. This dual-cleavage mechanism, while useful for antiviral defense, requires careful consideration for agricultural deployment to minimize off-target transcriptome disruption (Yang *et al.*, 2017) [52]. However, the activation requirement for bystander cleavage likely restricts off-target effects to co-abundant transcripts, reducing unintended targets compared to constitutive off-target cleavage by some DNA-targeting CRISPR systems.

7.2 Therapeutic gene compatibility and complex trait engineering

RNA-targeting platforms overcome critical limitations of DNA editing for large therapeutic genes and complex agricultural traits. Many agriculturally important traits arise from multiple genes disease resistance typically requires combinations of R genes for durable protection; yield improvement requires optimization of photosynthesis, biomass allocation, reproductive development, and stress response; climate adaptation requires adjustments in phenology, osmotic adjustment, and cellular repair mechanisms (Yu *et al.*, 2024) [55].

DNA-targeting CRISPR-Cas9 requires designing separate guides for each gene target, requiring simultaneous editing of multiple loci technically challenging and frequently resulting in incomplete editing of all target sites. RNA-editing approaches enable more flexible trait engineering. CRISPR-Cas13 systems can be multiplexed to simultaneously target multiple disease resistance genes; Splice Editing enables rapid evaluation of variant alleles before stable genetic modification. This flexibility substantially simplifies engineering of complex agricultural traits requiring coordinated modification of multiple genes (Ali *et al.*, 2015) [4].

7.3 Crop-specific deployment considerations

Agricultural deployment of CRISPR systems must accommodate fundamental differences between crop genetic architectures. Diploid crops (rice, tomato, soybean in some cultivars) allow straightforward CRISPR application with single edits addressing most loci. Polyploid crops (wheat, cotton, canola, potato, banana, plantain) require substantially more complex editing strategies. Polyploid

crops with homoeologous gene copies necessitate either targeting all copies simultaneously or engineering selective homoeolog-specific modifications.

RNA-editing approaches offer particular advantages for polyploid crop improvement. Splice Editing enables selective targeting through guide RNA specificity if homoeologs differ by even a single nucleotide, carefully designed guides can preferentially target specific copies. This capability would enable unprecedented polyploid crop improvement without requiring simultaneous editing of all homoeologous copies (Wu *et al.*, 2023) [50].

Perennial crops (fruit trees, forest species) face different CRISPR considerations than annual crops. Perennial improvement timelines extend decades or centuries; transient RNA editing enables long-term trait validation before committing to permanent genetic modification. Over multiple growing seasons, researchers can evaluate whether agronomic performance of transiently edited traits meets expectations before incorporating validated modifications through stable genetic change.

8. Integration and future perspectives for agricultural CRISPR development

8.1 Complementary applications in sustainable crop improvement

The three CRISPR advances examined in this review address complementary applications in agricultural transcriptome engineering and sustainable crop improvement. Cas13d structures provide foundational mechanistic understanding enabling rational engineering of improved RNA-targeting nucleases optimized for plant tissues (Li *et al.*, 2025) [29]. CRISPR-mediated trans-splicing leverages understanding of splicing mechanisms combined with CRISPR targeting specificity to achieve large-scale transcript correction in crop improvement contexts. Chemical modification approaches optimize guide RNA function for both DNA-targeting (Cas9) and RNA-targeting (Cas13) systems deployed in agricultural settings (Zaidi *et al.*, 2017) [57].

The relative merits of each approach depend on agricultural context and crop species. For rapid disease resistance engineering in crops facing urgent pathogen threats (like cassava mosaic virus expansion or wheat rust evolution), Splice Editing using miniaturized Cas13e systems offers compelling advantages for rapid trait validation (Cox *et al.*, 2017) [14]. For permanent improvement of elite breeding lines, CRISPR-Cas9 with optimized chemical-modified guide RNAs provides established approaches amenable to regulatory approval (Wolter and Puchta, 2018) [48]. For polyploid crop improvement where selective homoeolog-targeting is essential, chemically modified guide RNAs with enhanced specificity may provide optimal solutions.

8.2 Synergy between CRISPR technologies and genomic selection

Integration of CRISPR-mediated RNA editing with genomic selection and phenotypic selection represents promising avenue for accelerated crop improvement. Genomic studies of crop populations have identified numerous DNA variants associated with improved disease resistance, yield, and stress tolerance. CRISPR-mediated RNA editing enables rapid functional validation of identified variants without requiring multi-year breeding cycles (Yin *et al.*, 2019) [54]. Validated variants can then be incorporated into elite

breeding lines through conventional breeding or permanent CRISPR modification, ensuring regulatory compatibility while dramatically accelerating improvement timelines. Phenotypic selection combining automated high-throughput plant phenotyping with CRISPR-mediated trait evaluation enables systematic optimization of complex traits. Researchers can transiently edit candidate genes affecting target traits, use automated phenotyping to measure resulting trait changes, identify optimal combinations, then move validated variants into breeding programs (Xu *et al.*, 2021) ^[51]. This iterative approach promises to overcome historical breeding bottlenecks limiting crop improvement rates.

8.3 Climate adaptation and future food security

Climate change presents unprecedented challenges for global food security, requiring crops adapted to warmer temperatures, altered precipitation patterns, and extreme weather events. CRISPR-mediated RNA editing offers tools for rapidly evaluating climate adaptation variants identified through comparative genomics of landraces and wild crop relatives adapted to marginal environments (Zhang *et al.*, 2023) ^[60]. Splice Editing enables systematic testing of drought tolerance, heat tolerance, and salinity tolerance variants without requiring stable transformation.

The urgency of climate adaptation requiring crop improvements on 5-10 year timescales aligns with CRISPR development timelines. Traditional breeding requires 8-10 years for trait development; advanced CRISPR approaches promise reduction to 4-6 years (Bialk *et al.*, 2015) ^[10]. Accelerated crop improvement enabled by RNA-editing technologies could prove critical for maintaining agricultural productivity as climate impacts intensify.

9. Conclusion

The convergence of structural, mechanistic, and engineering advances has substantially advanced CRISPR-mediated RNA editing technologies with profound implications for both therapeutic development and sustainable agriculture. High-resolution structures of CRISPR-Cas13d have revealed the molecular basis for RNA targeting and recognition, establishing principles for rational engineering of improved RNA-targeting nucleases adapted for plant tissues. Discovery and characterization of miniaturized Cas13 variants, particularly Cas13e, have enabled packaging of Splice Editing systems into plant transformation vectors, addressing critical agricultural deployment limitations. Systematic chemical modification studies have identified structure-activity relationships guiding rational optimization of both DNA- and RNA-targeting CRISPR systems for agricultural contexts.

The remarkable pace of CRISPR technology advancement, combined with increasing urgency of global food security challenges, suggests that agricultural applications of RNA-targeted CRISPR systems will achieve deployment in major crops within the coming 3-5 years, fundamentally transforming possibilities for sustainable crop improvement and global food security.

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