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CRISPR-Cas Variants in Crop Improvement – Beyond CAS9

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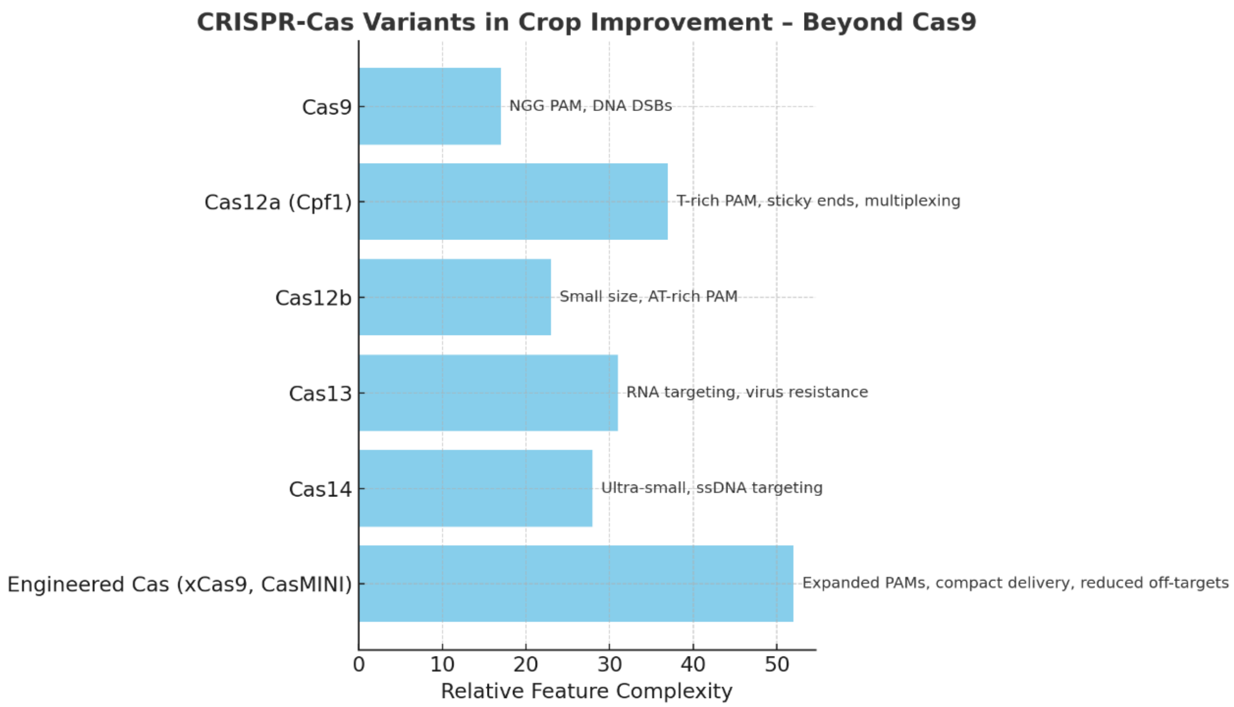
SUMMARY

CRISPR-Cas9 revolutionized plant science but possess some limitations such as large size of cascade, strict NGG PAM site requirements, and permanent DNA edits. New CRISPR variants likeCas12a, Cas12b, Cas13, Cas14, and engineered forms such as xCas9, HypaCas9, and CasMINI overcome these barriers with broader targeting, higher precision, base editing, RNA editing and compact size. Advanced tools like base editing, prime editing, and epigenome editing enable precise and heritable, or reversible trait modifications. These technologies promise disease resistance, stress tolerance, improved yield, and enhancement of nutritional quality in crops. However, challenges in delivery, regulation, and off-target monitoring system are always remain researchable issues in genome editing approach. Integrating CRISPR with multi-omics and AI will drive next-generation genome editing for sustainable agriculture development in climate changing scenario.

INTRODUCTION

A New Era in Plant Science-When CRISPR-Cas9 arrived in 2012, it was not just a discovery but it was the revolution in molecular biology to achieve the traits of interest with very high precision. This molecular approach earning its title as the molecular scissors with high precision of modern biological sciences. In plants, the Cas9 quickly became the tool of choice for improving yields, developing pest-resistant varieties, and accelerating breeding programs for various agro-economical traits. Besides modern breakthrough, it possesses some limitations such as large size of cascade, strict NGG PAM site requirements, and permanent DNA edits. The Cas9 is relatively large in size, works only at certain DNA sites, and permanently alters the plant genome, which sometimes arise potential regulatory and ethical issues. These challenges have sparked the search for new as next-generation CRISPR systems that should be small in size, more flexible for recognition sites, and offer new editing capabilities.



Why Go Beyond Cas9?

Cas9 is efficient tool in genome editing approach widely used in various application in biological sciences including plant science, however, it possesses several limitations mention as below:

1. Strict targeting rules: It requires an **“NGG”** PAM site in DNA sequence nearby, which restricts where edits can occur.

In CRISPR-Cas9, 'NGG' refers to a short DNA tag called the PAM (protospacer adjacent motif). For Cas9 to cut DNA, this tag must appear right next to the target sequence. In the case of the commonly used *Streptococcus pyogenes* Cas9 (SpCas9), the PAM is always 'NGG,' where 'N' can be any DNA nucleotide (A, T, C, or G) followed by two guanosines (G). Without this NGG tag, Cas9 simply not able to recognize or cut the DNA.

2. Bulky size: The large size of the cascade (~4.2 kilobases) make harder to deliver into plant cells using standard transformation methods.

3. DNA-only editing: The Cas9 system cannot perform the editing in RNA molecules, which carry genetic messages.

4. Off-target edits: Mismatched guide RNAs can sometimes cut unintended sites, which may produce unwanted traits.

To circumvent these limitations, the other variants of Cas proteins such as Cas12, Cas13, Cas14, and engineered derivatives have been developed as novel tools. These variants significantly broadening the scope of genome and transcriptome editing in plants.

CRISPR-Cas Variants in Crop Improvement

1. Cas12a (Cpf1)

Cas12a, derived from *Acidaminococcus* and *Lachnospiraceae* species, is one of the most prominent alternatives to Cas9.

Distinct Features:

- Recognizes T-rich PAMs (TTTV), expanding targetable regions in plant genomes.
- Produces staggered DNA cuts with sticky ends, potentially improving precision in homology-directed repair (HDR) of DNA recombination.
- Requires a shorter CRISPR RNA (crRNA), simplifying multiplex genome editing.

Applications:

Rice: Cas12a-mediated editing of *OsPDS* and *OsSWEET* genes for disease resistance (ref.).

Maize: Targeted knockouts in multiple loci to improve kernel yield.

Soybean and Wheat: Development of herbicide tolerance and stress-resilient genotypes.

Cas12a is thus proving to be an efficient tool for broadening the target range in monocot and dicot crops.

2. Cas12b

Cas12b, a smaller nuclease than Cas12a and Cas9, offers an excellent balance between editing efficiency and compactness.

Distinct Features:

- Recognizes AT-rich PAM sequences.
- Smaller size makes it easier to deliver using viral vectors.
- Exhibits high specificity, reducing off-target mutations.

Applications:

In rice and wheat, Cas12b has been used for precise mutagenesis of yield-related genes (ref.). Its compact size makes it a promising tool for crops that are difficult to transform.

3. Cas13 – RNA Targeting

Unlike Cas9 and Cas12, Cas13 enzymes (Cas13a, Cas13b, Cas13d) target RNA rather than DNA, enabling transient and reversible modifications.

Distinct Features:

- Bypasses DNA editing, reducing risks of permanent mutations.
- Recognizes and cleaves single-stranded RNA with high specificity.
- Useful for transcriptome engineering, viral resistance, and RNA diagnostics.

Applications in Crops:

Virus Resistance: Cas13a has been used in rice and potato to confer resistance against RNA viruses like *Potato virus Y* and *Rice stripe virus*.

Gene Regulation: Cas13 systems enable silencing of undesirable transcripts without altering the genome, opening avenues for dynamic trait regulation.

Cas13 offers a new frontier in managing plant viral diseases, a major bottleneck in global agriculture.

4. **Cas14 (Type V-F)**

Cas14, derived from uncultivated archaeal species, is an ultra-small nuclease (<700 amino acids) with unique editing potential.

Distinct Features:

- Capable of targeting single-stranded DNA without stringent PAM requirements.
- High potential for portable detection tools and precision editing in plant organelles (chloroplasts, mitochondria).

Applications:

Although its use in plants is still at an early stage, Cas14 may provide unprecedented flexibility in targeting complex genomes and organelle engineering.

Engineered Cas Variants

Beyond naturally occurring Cas systems, protein engineering has yielded powerful Cas derivatives with improved properties:

xCas9 & SpCas9-NG: Broadened PAM compatibility (NG, GAA, GAT), expanding editing scope.

eSpCas9 & HypaCas9: Reduced off-target activity for safer genome editing.

CasMINI: Ultra-compact Cas engineered for efficient delivery and editing in plants.

These engineered variants are particularly attractive for crop breeding programs requiring high precision and flexibility.

Advanced CRISPR Applications Beyond Nuclease Activity

Base Editing

- Combines Cas nickases with cytidine or adenine deaminases.
- Enables single nucleotide substitutions without double-strand breaks.
- Used in rice and wheat to create herbicide-resistant alleles and disease-resistance mutations.

Prime Editing

- Utilizes a fusion of Cas nickase with reverse transcriptase.
- Allows precise insertions, deletions, and base conversions without donor DNA templates.
- Demonstrated in rice for precise introduction of desired alleles.

Epigenome Editing

- Cas proteins fused with epigenetic modifiers (e.g., methyltransferases, acetyltransferases).
- Enable heritable or reversible regulation of gene expression without altering DNA sequence.
- Potential to fine-tune stress-responsive genes for climate-resilient crops.

Implications for Crop Improvement

CRISPR-Cas variants provide multiple opportunities to address global agricultural challenges:

Disease Resistance: Cas12a and Cas13 for durable resistance against bacterial, fungal, and viral pathogens.

Abiotic Stress Tolerance: Engineering drought-, heat-, and salinity-tolerant crops through precise gene modifications.

Nutritional Enhancement: Editing metabolic pathways for enhanced micronutrient content (e.g., zinc, iron, vitamin A).

Yield Improvement: Multiplex editing of yield-related genes in rice, wheat, and maize.

Sustainable Agriculture: Reducing pesticide dependence by developing pest-resistant crop varieties.

Challenges and Future Perspectives

Despite significant progress, several challenges remain in applying CRISPR-Cas variants to crop improvement:

Delivery Systems: Efficient transformation methods are still lacking for many recalcitrant crops. It also restrict to the crop specific transformation protocols.

Regulatory Frameworks: The SDN1 and SDN2 events of genome editing are acceptable as regulatory and ethical frameworks, however, the SDN3 of CRISPR-edited crops is still not acceptable globally; harmonization of policies is needed.

Off-target Risks: Though, the CRISPR meditated genome editing is very much precise and efficient, but off-target mutations must be carefully monitored.

Ethical and Societal Concerns: Public perception and bioethical debates may influence adoption of genome editing especially the case of SDN3 must be regularize and monitor.

Looking ahead, integration of multi-omics data, machine learning, and synthetic biology with CRISPR-Cas systems is expected to accelerate next-generation precision breeding in plant. With continued innovation, CRISPR variants beyond Cas9 will pave the way for climate-smart, nutritionally enhanced, and sustainable agricultural systems.

CONCLUSION

The advent of CRISPR-Cas systems has transformed the landscape of crop biotechnology, moving from random mutagenesis to precised editing. While Cas9 remains a cornerstone, emerging CRISPR-Cas variants such as Cas12, Cas13, Cas14, and engineered derivatives are expanding the scope of plant genome and transcriptome editing. These tools not only overcome the limitations of Cas9 but also open new opportunities for RNA editing, base editing, prime editing, and epigenome regulation. Harnessing these diverse CRISPR systems will be crucial in meeting the urgent demands of global food security, climate resilience, and nutritional enhancement. Beyond Cas9, the future of crop improvement lies in a versatile toolbox of CRISPR technologies, offering unprecedented precision and flexibility for development of sustainable agriculture.

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