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CRISPR-Cas9 Genome Editing in Crop Improvement: Progress, Regulation and Limitations

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ABSTRACT

CRISPR-Cas9 has become the working tool of plant genome editing within little more than a decade of its first use in crops, and claims about what it will deliver for agriculture now run well ahead of what has been demonstrated in the field. This narrative review follows the technology from its bacterial origin to its present range of nucleases, base editors and prime editors, describes how editing machinery is delivered into plant cells, and summarises the traits that have been edited in cereals, tomato and other crops. It then sets out how regulators in different jurisdictions treat edited plants and why their answers diverge. The published record shows rapid technical progress and many convincing proofs of concept, but most reports describe single genotypes, greenhouse conditions and short follow-up. Transformation and regeneration, not editing itself, still limit many species, unintended changes are documented less thoroughly in plants than the enthusiasm suggests, and field evidence of agronomic benefit is thin. Regulation remains uneven, which shapes which products reach farmers at all. The review draws on published literature only, reports no new data, and ends with the evidence needed before the promise can be judged.

Keywords: CRISPR-Cas9; Genome editing; Crop improvement; Base editing; Off-target effects; Regulation; Narrative review

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Article Class: Review Article

1. Introduction

Plant breeding has always depended on variation. Breeders look for useful differences between plants, combine them, and select the offspring that perform best, and when natural variation runs short they have turned to radiation and chemicals to create more. Those methods work, but they

are blunt, scattering changes across the genome. A tool that could change one chosen gene in one chosen variety would shorten the process considerably, and CRISPR-Cas9 has been widely presented as exactly that tool (1, 2, 3).

The system began as a bacterial defence against viruses (4). Its adaptation into a



programmable DNA-cutting enzyme guided by a short RNA was reported in 2012 (5), and within a year it had been shown to work in human cells (6, 7) and in plants, including rice, wheat and tobacco relatives (8, 9, 10). A technique that is cheap to set up, easy to retarget and effective in many species spread through plant laboratories faster than almost any before it.

Enthusiasm and evidence are not the same thing, however, and the title of this review names three separate questions: what progress has been made, how regulators have responded, and where the limits lie. The article is a narrative review of published work. It reports no data collected by the authors and makes no claim about the performance of any variety in any particular country or region.

2. Literature Review

2.1 From Bacterial Defence to Plant Tool

The key step was to show that the Cas9 enzyme could be directed to a chosen DNA sequence by a single guide RNA, which cut the target at a predictable position (5). Reviews described it as a new frontier for genome engineering (11), and plant scientists were quick to test it: within months, groups reported targeted changes in model plants and in crops, and showed that several genes could be edited at once (8, 9, 10, 12). The speed of uptake had a simple explanation. Earlier tools for targeted changes, zinc-finger nucleases and TALENs, needed a new protein to be engineered for every target, whereas Cas9 needs only a new guide sequence, a far cheaper and simpler change. The same logic made it practical to target several genes in a single experiment, which had been laborious before. Figure 1 places the main developments discussed in this review on a single timeline. Much of what

followed was a variation on this simple two-part design.

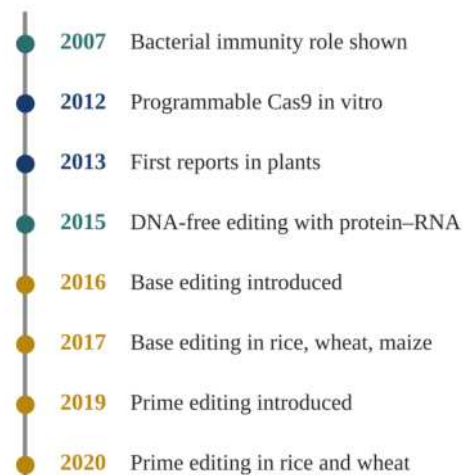


Figure 1. Milestones in the development of CRISPR-based editing in plants, with the year of each cited report (schematic timeline, not data).

Vector systems built for both monocots and dicots soon made multiplex editing, in which several targets are changed in one plant, a routine option (13). In hexaploid bread wheat, where each gene normally exists in three copies, all three copies of a susceptibility gene were edited together, giving heritable resistance to powdery mildew (14).

2.2 Beyond the Double-Strand Break

Standard Cas9 cuts both strands of DNA, and the cell repairs the break in a way that usually leaves small insertions or deletions. That is excellent for switching a gene off and much less useful for making a precise change. Base editors, which pair a damaged Cas9 with an enzyme that rewrites one DNA letter, were introduced to solve this (15) and were quickly moved into rice, wheat and maize (16), with adenine-based versions following (17). Prime editing goes further, using a guide that also carries a template for a short, designed edit (18). Its first plant results in rice and wheat were encouraging in principle but modest in efficiency (19). Table 1 summarises the approaches.

**Table 1.** Main editing approaches used in plants and their principal limits.

Approach	Typical outcome	Main limit
Nuclease knockout	Small insertions or deletions that disable a gene (6, 8)	Outcome at the cut site is not controlled
Base editing	Single-letter change without a double-strand break (15, 16)	Narrow editing window; limited change types
Prime editing	Small, designed edits from a template on the guide (18, 19)	Efficiency in plants still modest
Cas12a nucleases	Staggered cut with a different recognition sequence (20, 21)	Fewer validated tools in crops
Template-guided repair	Precise gene replacement (22)	Rare in most plant cells

Note: Qualitative summary of the cited literature; efficiencies vary with species, target and delivery method and are deliberately not quoted.

Other enzymes widen the choice. Cas12a recognises a different sequence next to its target and makes a staggered cut (20), and it has been adapted for plants (21). Precise gene replacement through template-guided repair has been demonstrated, for example in an herbicide-tolerance gene of rice (22), but it remains rare because most plant cells favour the error-prone route.

2.3 Getting the Editor into the Cell

Editing is only half the task; the editor has to reach the right cells and those cells have to become whole plants. The traditional route introduces DNA encoding Cas9 and the guide, which usually leaves foreign DNA that must later be bred out. Alternatives deliver the editor as transient DNA or RNA (23) or as a ready-made protein–RNA complex, which acts briefly and then degrades (24, 25). Such

transgene-free routes matter both biologically and legally.

Regeneration is the stubborn part. Many elite varieties, and many species, respond poorly to tissue culture. Developmental regulators that boost regeneration have improved transformation in difficult monocots (26), and a different approach has induced new shoot meristems directly in whole plants to bypass much of the culture step (27). These advances are real, but they are still crop- and genotype-specific.

2.4 Specificity and Unintended Effects

A cut in the wrong place is the best-known worry. Early studies in human cells showed that Cas9 could tolerate mismatches between guide and target and cut elsewhere (28, 29), and engineered high-fidelity variants were developed in response (30). Plants appear somewhat reassuring: whole-genome sequencing of edited rice found very few off-target changes with carefully designed guides (31), and a review concluded that off-targeting is manageable but not zero (32).

That reassurance has limits. Most sequencing studies in plants involve a few lines of a few species. In mammalian cells, repair of a Cas9 cut has been seen to cause large deletions and rearrangements at the intended site itself (33), and comparable checks are seldom reported for crops. Prolonged expression of the editor can also add unplanned changes in later generations. It is a reminder that a clean sequence does not by itself guarantee a plant that behaves as intended.

3. Materials and Methods

3.1 Scope and Question

The question was broad on purpose: what has CRISPR-Cas9 and its close relatives actually delivered in crop plants, how is



that regulated, and what weakens the evidence? The review covers cereals, tomato and other crops.

3.2 Literature Assembly

This is a narrative synthesis. We did not run a formal systematic search, did not register a protocol and did not screen records against fixed inclusion rules. Sources were gathered by following foundational papers, expert reviews and their reference lists, with deliberate effort to include reports of difficulties as well as successes. Because selection was not systematic, relevant work will have been missed.

3.3 Appraisal Framework

Each source was read against a short list of questions, set out in [Table 2](#). They concern the stage of the work, the plant material, the controls, the search for unintended edits and the way the trait was measured. The questions guided judgement and were not turned into a score.

Table 2. Questions used to read each source in this review.

Domain	Question asked	Reason
Stage	Cell culture, greenhouse or field?	Field results travel furthest
Material	One genotype or several?	Genotype affects editing
Controls	Were unedited lines grown alongside?	Needed to attribute a trait
Unintended edits	Were they sought, and how?	Absence is easy to assume
Trait	Measured, or inferred from the target gene?	Gene function is not yield

Note: The framework guided reading; it was not a scoring system and no studies were excluded by a formal rule.

4. Results

4.1 Traits and Crops Reported

Edited traits fall broadly into disease resistance, stress tolerance, quality and

agronomic or domestication traits. [Table 3](#) gives representative examples. In rice, editing the promoters of genes that bacteria exploit gave broad resistance to bacterial blight (34), and targeting a transcription factor improved resistance to blast (35). In wheat, edits to grain protein genes reduced gluten reactivity (36). Maize lines with altered expression of an ethylene-response regulator were evaluated in the field under drought (37).

Table 3. Examples of edited traits and the stage of evidence reported.

Crop	Trait targeted	Evidence reported
Wheat	Powdery mildew resistance (14)	Heritable edits in all three gene copies
Rice	Bacterial blight resistance (34)	Edited promoters; broad resistance reported
Rice	Blast resistance (35)	Improved resistance in tested lines
Maize	Drought tolerance (37)	Field trials under drought stress
Wheat	Lower gluten reactivity (36)	Altered grain proteins; breeding use untested
Tomato	Domestication traits (38)	Several traits changed in a wild relative

Note: Examples were chosen to illustrate the range of published work, not to rank crops or targets.

Some of the most interesting work changes quantity rather than presence. Editing the regulatory regions of tomato genes produced a range of fruit and plant architecture phenotypes from a single locus (39), and mutating several genes in a wild tomato relative produced plants with domestication traits while keeping features of the wild parent (38). A similar route has been explored in wild allotetraploid rice (40). These are proofs of concept, not varieties in farmers' hands. Reports of this kind are encouraging, but each one covers a narrow slice of the genetic variation a crop actually contains.



4.2 From Laboratory to Field

Figure 2 shows the steps between choosing a target and a usable line. Editing is the quick part; regeneration, screening, segregation and field evaluation occupy most of the calendar. Most of the reports reviewed describe one or two genotypes, greenhouse conditions and a few generations of follow-up. Field data are the exception, and multi-site, multi-season evidence is rarer still. That mismatch matters, because a technique that works in one cultivar cannot be assumed to work in the next.

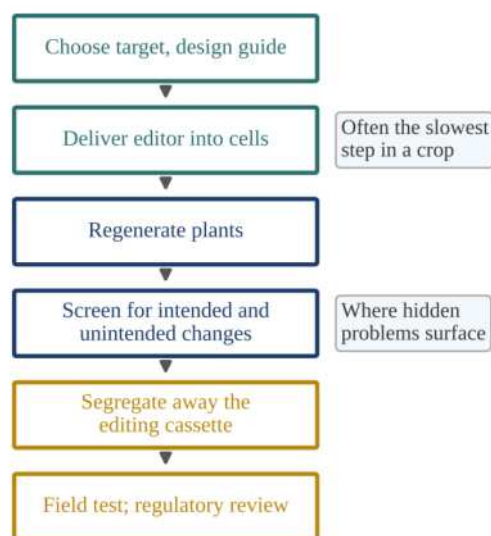


Figure 2. From design to field: the steps between choosing a target and a usable edited line (schematic, not data).

4.3 The Regulatory Picture

Regulatory systems divide mainly on whether they judge the process used to make a plant or the product that results. Some countries examine the product and exempt plants that carry no foreign DNA and a change that could have arisen naturally; others treat any plant made with the technique as genetically modified (41, 42). The European Union's highest court ruled in 2018 that organisms obtained by newer mutagenesis techniques fall under its genetically modified organism rules

(43), while other jurisdictions have moved towards case-by-case or exemption-based approaches (44, 45). Figure 3 shows the kind of decision path involved, simplified. A regulator has to decide on exactly this kind of ambiguity, often with little detection data to lean on.

Divergence has practical weight, since a product that reaches farmers in one country may be unsellable in another. It also raises a technical problem: a single-letter change is hard to tell apart from natural variation, so enforcing a rule that depends on the method of origin is difficult (42, 44).

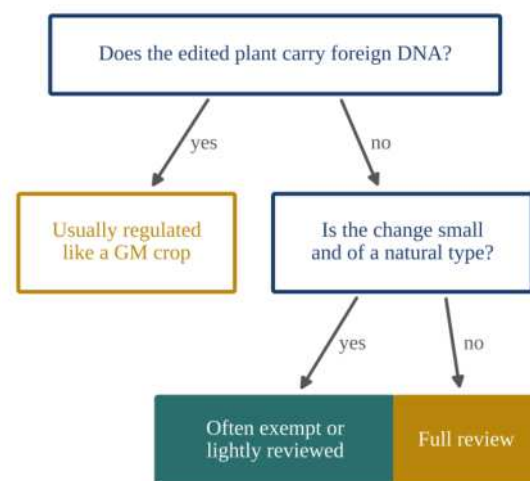


Figure 3. A simplified decision path of the kind regulators use; real systems differ by country (schematic, not data).

5. Discussion

5.1 Interpretation

The record shows that the technology works. Targeted changes can be made in many crops, the toolbox has grown well beyond the original nuclease, and delivery without lasting foreign DNA is possible in several species. The more careful reading is that these demonstrations are not the same as agronomic benefit. A resistance gene knocked out in a greenhouse tells us little about yield under real pathogen pressure (37).



The bottlenecks deserve more attention than they receive. Tissue culture and genotype dependence decide which crops benefit first, and they favour species with established protocols over the neglected crops smallholders often rely on (26, 27). Regulation compounds this, since the cost of approval weighs more heavily on small breeding programmes (42, 45).

5.2 Limitations

The limitations of this review mirror those of the evidence. It is a narrative synthesis without a systematic search, so the selection of studies reflects what the authors know and could access. It leans on high-profile reports, and publication bias would hide many unsuccessful attempts. Regulatory descriptions are simplified and change quickly, so current official sources should be consulted.

5.3 Directions for Future Work

Four needs stand out: multi-site, multi-season field trials with unedited controls; routine, fully reported whole-genome checking of edited lines, including large rearrangements at the target (31, 33); more general regeneration methods (26, 27); and transparent comparison of regulatory outcomes (44).

6. Conclusion

CRISPR-Cas9 and its descendants have given plant scientists precise and affordable control over genes, and the pace of technical improvement has been remarkable. What has not kept pace is the evidence that these edits translate into dependable gains on farms. Most published work describes greenhouse results in a few genotypes, unintended effects are checked unevenly, regeneration still restricts many crops, and the same plant can be regulated in opposite ways in different countries. The promise is real and largely unproven at scale, and closing that gap needs field evidence, fuller reporting

and clearer rules more than another editor.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

A.W. conceived the review and wrote the sections on editing technology and delivery. D.H. wrote the sections on crop traits and field evidence. I.S. wrote the sections on regulation and limitations. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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