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CRISPR in Plant Breeding: Precision and Possibilities

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Abstract

Plant breeders have always nudged genomes in the direction of better harvests first with selection, then with crosses and mutagenesis and more recently with marker-assisted and genomic selection. CRISPR changes the pace and the precision of that journey. Instead of waiting for the right allele to show up in a segregating population, editors can reproduce a naturally occurring variant in an elite background, stack subtle promoter tweaks to “tune” gene expression, or remove a single susceptibility gene with surgical accuracy. In the last decade, plant editing has moved from lab benches to greenhouses and, in a few cases, to grocery shelves. Beyond obvious win–wins like disease resistance and quality, the technology is being harnessed to accelerate de novo domestication of hardy wild relatives and to build climate resilience into mainstream crops. This article translates the core ideas, toolset, delivery routes, real-world examples, regulatory contours and practical caveats into breeder-friendly language. The aim is not to sell a miracle but to show where CRISPR genuinely fits in a modern breeding pipeline, where it still struggles and how to tell apart hype from progress. The upshot: as editing efficiencies rise and regulation clarifies, CRISPR is becoming one more reliable instrument in the breeder’s kit powerful, yes, but most effective when paired with careful phenotyping, agronomy and genetics.

Keywords: CRISPR, Cas9, Cas12a, base editing, prime editing, promoter editing, transgene-free editing, disease resistance, quality traits, climate resilience, de novo domestication, regulatory policy, plant breeding, doubled haploids

Introduction

For most of agricultural history, improving a crop meant shuffling entire genomes and hoping valuable traits came along for the ride. With CRISPR, the conversation shifts from

“Which lines carry a trait?” to “Which nucleotide change gives it and can we make that change cleanly in our best line?” That is the practical promise breeders are now testing in field plots and product pipelines. What makes CRISPR compelling is not magic, but control: the ability to make targeted edits knockouts, single-base conversions, or promoter tweaks while largely preserving the rest of the elite genome. The tool doesn’t replace breeding; it shortens detours.

How CRISPR works boiled down for breeders

At its heart, CRISPR pairs a programmable RNA “address label” (the guide) with a DNA-cutting or DNA-editing enzyme (the Cas or editor). In plants, three edit styles are most useful:

- **Cut and repair (NHEJ):** create small insertions/deletions that knock out a gene. Think removing a susceptibility gene (S-gene) to gain disease resistance.
- **Base editing (C→T or A→G):** install predictable single-base conversions without making a double-strand break; ideal for herbicide-target alleles or quality tweaks.
- **Prime editing:** a guided “search-and-replace” that can write small insertions, deletions, or precise substitutions promising for fine-tuning, though still maturing in many crops.

Cas9 remains the workhorse, while Cas12a (Cpf1) brings a T-rich PAM and naturally supports multiplexing, which is handy for stacking edits or scanning promoter elements. CRISPRa/CRISPRi (dead Cas fused to activators or repressors) adds a reversible, expression-level dial. _

Delivery: getting edits in and transgenes out

Three routes dominate:

- **Agrobacterium** or **particle bombardment** to deliver DNA constructs; later segregate away the transgene in progeny to yield “transgene-free” edited plants.
- **Protoplast transfection** with plasmids or ribonucleoproteins (RNPs) to create DNA-free edits from the start appealing where regulations distinguish edits from GMOs that contain foreign DNA.
- **Viral vectors** and new transformation tricks (like morphogenic regulators) that raise editing efficiency and help crack difficult genotypes.

Off-target risk is managed by careful guide design, whole-genome-scale off-target prediction and the use of high-fidelity Cas variants; in plants, off-targets are generally low and can be removed during line development. _

What precision looks like in practice

Knocking out a gene can be transformative (for example, MLO genes for powdery mildew resistance in cereals). But the most elegant gains often come from promoter editing altering transcription factor binding or pathogen effector binding elements to “tune” expression

without touching the coding sequence. In rice, editing effector binding elements in SWEET gene promoters has produced broad-spectrum resistance to bacterial blight and multiplexing multiple promoters helps outsmart rapidly evolving pathogens. Base editors are handy for making single-nucleotide changes such as ALS herbicide-tolerance alleles or recreating flavourful or quality variants like BADH2 for aromatic rice precision that used to require rare chance events. Prime editing aims to do the same with fewer by-products and while plant prime-editing efficiencies have lagged behind mammalian systems, engineering advances over the last few years are steadily improving performance in major crops.

From plots to plates: early products and field-forward traits

In Japan, a high-GABA tomato developed with CRISPR is available to home gardeners and consumers, marketed for its elevated gamma-aminobutyric acid content.

- In the United States, a CRISPR-edited mustard green with reduced pungency launched through limited channels an example of editing for flavour rather than agronomic performance.
- Waxy corn edited at the Wx locus (high amylopectin starch) proceeded through U.S. regulatory confirmation as not regulated under plant pest rules when first evaluated an early industrial trait use case.

Regulation in 2025: same destination, different roads

United States: For several years, certain edits that could be achieved through conventional breeding were exempted from permits under USDA's SECURE framework. In December 2024, a federal court vacated the 2020 SECURE updates, prompting APHIS to adjust how it implements those provisions; developers should check current APHIS guidance during product planning.

- **European Union:** In February 2024, the European Parliament voted to establish a category for "new genomic techniques" (NGTs) to ease the path for some edited plants, but final rules depend on ongoing negotiations and implementation steps.
- **United Kingdom:** The Genetic Technology (Precision Breeding) Act 2023 created a distinct route for precision-bred organisms (including many gene-edited plants) in England, with implementing rules rolling out for trials and commercialization.
- **India:** In 2022, India issued guidelines distinguishing SDN-1/SDN-2 edits from transgenic GMOs, easing research and, potentially, commercialization for certain edit types.
- **Japan:** Since 2019, gene-edited foods without inserted foreign DNA have followed a notification pathway; this opened the door for the high-GABA tomato.

Bottom line: the same edit could be regulated very differently depending on where you plan to grow or sell it. Keep regulatory due diligence on the project checklist from day one.

Breeding pipeline: where CRISPR sits

1. **Trait-to-target mapping:** Confirm that a clear causal gene/allele (or set of promoter elements) underlies your trait. If not, use QTL mapping or pangenome resources to narrow candidates.
2. **Edit design:** Decide whether to knock out, base-edit, or tune a promoter. For disease targets, ask if a susceptibility gene is dispensable; for quality traits, ensure edits won't trigger agronomic penalties.
3. **Transformation strategy:** Pick a genotype with good transformability or use morphogenic regulators to expand your options; plan for transgene-free recovery if that changes the regulatory path.
4. **Screening at scale:** Expect mosaicism in T0; plan high-throughput genotyping, off-target checks for elite lines and early phenotyping under relevant stresses.
5. **Speed and fixation:** Pair editing with speed breeding and, where appropriate, doubled haploids (DH). Edits to haploid induction genes (e.g., MTL/PLA1) have even been used to create or improve haploid inducers in cereals useful for fixing edited alleles fast.
6. **Field validation:** Greenhouse gains don't always travel; test under realistic management and disease pressure across environments.
7. **Stacking and stewardship:** Multiple small edits can deliver more durable gains than a single big hammer. Document each edit clearly for stewardship and market acceptance.

Limits, risks and the human factor

CRISPR is not a free lunch. Multi-gene traits (yield, flavour bouquets, complex stress resilience) often need multiplex edits or promoter tuning, which raises delivery and regeneration challenges. Some crops remain transformation-recalcitrant, which can be a bigger bottleneck than editing itself. Edits can have pleiotropic effects knocking out an S-gene may carry a growth cost, or a flavour edit might impact shelf life. And while off-target edits are typically rare in plants and can be bred out, they're not zero. None of these are deal breakers; they're reminders that editing is powerful genetics, not sorcery and still needs the same careful phenotyping and agronomy that any good variety requires.

Representative CRISPR edits in crops (real-world and lab-stage)

Crop	Trait/Goal	Target gene(s) or element	Edit approach	Outcome/Status
Tomato	Higher GABA (nutritional quality)	SIGAD	Knockout of auto-inhibitory region / CRISPR	Commercial product in Japan

Mustard greens	Milder taste / reduced pungency	Multiple taste/bitterness loci (undisclosed)	Multiplex CRISPR	Limited U.S. market launch
Maize (corn)	High-amylopectin (waxy) starch	Wx	Knockout (NHEJ)	U.S. confirmation as not regulated under plant-pest rules when assessed
Wheat	Powdery mildew resistance	TaMLO homoeologs	Knockout (NHEJ)	Strong resistance in greenhouse/field testing
Rice	Aromatic grain	BADH2	Knockout/base edit	2-AP fragrance restored; breeding lines under development
Rice	Glutinous grain (low amylose)	Wx	Knockout (NHEJ)	Waxy rice lines generated; quality validated
Rice	Herbicide tolerance	ALS/ALS1	Base editing	Specific amino-acid substitutions confer tolerance
Rice	Blast resistance	OsERF922	Knockout (NHEJ)	Increased resistance demonstrated
Rice	Bacterial blight resistance	SWEET promoter EBEs	Promoter editing, multiplex	Broad-spectrum resistance in elite backgrounds
Tomato (wild → improved)	Compact habit, higher yield	SP, SP5G, others	Multiplex coding/promoter edits	De novo domestication of wild tomato
Citrus	Canker resistance	CsLOB1 promoter	Promoter editing	Resistant citrus genotypes validated
Potato	Lower acrylamide after frying	VInv	Knockout	Reduced cold-induced sweetening
Soybean	High oleic acid	FAD2 family	Knockout/base edit	Oil profile improved in edited lines
Cassava	Virus resistance (CBSV/UCBSV)	eIF4E family	Knockout	Reduced virus accumulation in greenhouse tests
Tomato	Salinity tolerance	HKT1;2	Precise allele edits	Increased Na ⁺ /K ⁺ selectivity; enhanced tolerance
Wheat	Reduced gluten immunogenicity	α-gliadin genes	Multiplex knockouts	Lower immune-reactivity in flour
Rice	Salinity tolerance	OsRR22	Knockout	Salinity-tolerant, transgene-free

				progeny recovered
Maize	Faster DH line creation	MTL/PLA1 (MATRILINEAL)	Knockout	Haploid induction enabled/improved
Tomato	Powdery mildew resistance	SIMLO1	Knockout	Resistant, transgene-free mutants recovered
Barley	Naked (hull-less) grains	NUD	Knockout	Hull-less trait recreated for food/feed use

A practical checklist for teams starting an edit

- **Start with the agronomy.** What pain point are you solving for growers or consumers? Can you quantify it in the field?
- **Pick targets you can measure quickly.** Disease scores, a lab assay for oil profile, or a simple grain quality metric help you iterate.
- **Design guides for multiplexing.** Many traits benefit from hitting homoeologs or promoter elements together; Cas12a can simplify this.
- **Aim for transgene-free recovery if it helps adoption.** That can streamline regulation and public acceptance in several markets.
- **Partner early on policy.** Regulatory status can shift; stay aligned with current guidance in your target countries.

Conclusion

CRISPR doesn't replace the craft of breeding it rewards it. The teams that win with editing are doing the same things great breeders have always done: grounding decisions in field data, thinking two or three product generations ahead and balancing bold ideas with careful line development. What CRISPR adds is the ability to move straight at the genetic levers that matter, at a speed that makes business sense. Use it to recreate a proven allele in your best background, to remove a single liability without dragging along a dozen linked ones, or to tune gene expression rather than smashing pathways on or off. Pair the edits with smart selection and keep your eyes open to pleiotropy and stewardship. Done well, CRISPR can help deliver varieties that are a little hardier, a little tastier, a little more profitable and, crucially, delivered to farmers and consumers with clarity about what was changed and why. That's not science fiction; it's simply good breeding with a sharper tool. _

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