Genetic Engineering of Transgenic Crops

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Abstract. Crop genetic engineering has moved well beyond conventional breeding. Researchers now routinely rewrite plant genomes with nucleotide precision, though the underlying biology still resists easy transfer. This review traces how the tools work, where they have succeeded, and where they fall short. A particular focus is the tension between Agrobacterium-mediated delivery and host defense—plants recognize the pathogen through pattern-triggered immunity (PTI), and resistant accessions may mount effector-triggered immunity (ETI), either response capable of aborting transformation. We also contrast the error-prone non-homologous end joining (NHEJ) pathway with template-dependent homology-directed repair (HDR), which remains inefficient in most species.The field has produced scattered but tangible outcomes: CLE promoter editing raises maize yields; base editing yields herbicide-resistant oilseed rape; HDR-derived rice tolerates glyphosate; and metabolic engineering pushes vitamin C higher in tomatoes. Looking ahead, speed breeding merged with precise editing, multiplex engineering, and AI-guided design could push agriculture toward greater sustainability. These advances suggest genetic engineering is becoming indispensable—for food security and for reducing environmental harm.

Keywords: Crop genetic engineering, CRISPR/Cas9, Genome editing, *Agrobacterium*-mediated transformation, Base editing, Plant immunity, DNA repair, Sustainable agriculture

1. Introduction

Genetic engineering in crops has moved far beyond its early proof-of-concept stage. Breeders once worked with whatever natural variation they could find, crossing lines over years—work slowed by reproductive barriers between species. Molecular biology has since let scientists move genes across species and tweak specific loci directly. CRISPR/Cas, in particular, has redrawn the boundaries of what breeders can attempt: precise edits without introducing foreign DNA, though off-target effects remain debated. These tools can raise yields and resilience, but success is patchy—some species transform easily, others barely at all [1].

The two main DNA delivery routes, such ad Agrobacterium and biolistics—have both been refined, though gains are uneven. Agrobacterium now infects cereals it once failed to penetrate, while gene guns produce less fragmented DNA than earlier models. Beyond these workhorses, base editing and prime editing add precision that CRISPR cleavage alone could not achieve, and marker-free systems using recombinases or CRISPR excision are close to routine in some labs. The bottleneck is less the tools themselves than getting them to work reliably across the hundreds of species that matter for agriculture.Engineered maize, rice, oilseed rape, and tomato now yield more, survive harsher conditions, and carry better nutrition than conventional lines. The same tools are being turned to food security, lower-input farming, and more nutritious harvests. Whether these gains scale beyond proof-of-concept remains the open question [2].

2. Molecular Mechanisms

2.1. Layered Immunity Mechanisms in Plants

Plant immune defenses routinely block Agrobacterium infection, making transformation a battle against host resistance. Pattern-triggered immunity (PTI) recognizes conserved signals like flagellin and shuts down T-DNA transfer before it begins. effector-triggered immunity (ETI), triggered by intracellular receptors that spot bacterial effectors, often ends in hypersensitive cell death — transformation fails alongside the dying tissue. RNA interference adds another hurdle, shredding foreign DNA before it integrates. Researchers now dampen these defenses with immunosuppressants or silence key resistance genes, pushing transformation rates up in species once considered intractable [3].

2.2. Homology-Directed Repair and Non-Homologous End Joining

When plant DNA breaks, two repair pathways compete: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ operates throughout the cell cycle, ligating broken ends directly without a template. It is efficient but error prone. It is well suited for gene knockout but inadequate for precise editing. HDR, by contrast, copies from a homologous template to achieve exact repair, though it is restricted to S and G2 phases and remains inefficient in most plant species.

CRISPR/Cas9 can improve HDR frequency by generating targeted breaks while supplying a donor template; synchronizing cuts with S or G2 phase further helps. This approach has yielded one of the clearer crop successes to date: precise EPSPS edits in rice that confer glyphosate resistance. Extending such HDR-mediated improvements beyond rice, however, remains a major challenge [4].

2.3. CRISPR RNA-Guided Integrase Mechanism in Agrobacterium tumefaciens

Fusing CRISPR-Cas targeting to site-specific recombinases has given researchers a way to direct where Agrobacterium lands its DNA. A guide RNA brings nuclease-dead Cas9 to the locus; the attached recombinase then inserts donor DNA in one direction. The result is precise placement of large payloads, unlike the random scatter typical of natural T-DNA integration. Because the system bypasses host repair, it functions in non-dividing cells where HDR normally fails. Multiple loci can be targeted simultaneously, useful for stacking entire metabolic pathways. Whether it transfers stably to routine plant transformation—beyond proof-of-concept assays in model systems—remains the critical next step [5].

3. Genetic Engineering Tools

3.1. Agrobacterium-Mediated Gene Transfer Technology

Despite decades of alternatives, Agrobacterium-mediated transfer still dominates plant transformation. The bacterium naturally injects T-DNA from its Ti plasmid into plant nuclei through a type IV secretion system; researchers simply redirect this machinery to carry genes of interest. Recent work has pushed the system into monocots — cereals once considered intractable — by optimizing virulence induction and deploying super-binary and super-virulent vectors.

Yet efficiency still collides with plant immunity. Designers now suppress or evade these defenses rather than fight through them: acetovanillin boosts vir gene induction, silencing defense regulators weakens host resistance, and species-specific introns in selectable markers confirm genuine transformants. These tweaks have raised rates in previously stubborn cereals, bringing the system closer to routine use for both transgenesis and editing. [3].

3.2. Enhancement of Biolistic Transformation

Biolistic delivery bypasses the host-range constraints that limit Agrobacterium-mediated transformation, making it essential for species recalcitrant to bacterial infection. The method accelerates DNA-coated microparticles through compressed gas, propelling them through cell walls and membranes into the cytoplasm or nucleus. Recent barrel redesigns—aiming to stabilize particle acceleration and improve spatial distribution—have reduced the tissue damage that long plagued the technique while raising transformation consistency. Finer control over particle velocity and penetration depth now permits more uniform target coverage with less collateral injury to surrounding cells. These refinements are particularly valuable for delivering CRISPR/Cas ribonucleoproteins and large DNA constructs into organelles or into cereals and other crops that remain stubbornly resistant to Agrobacterium, advancing both transgenesis and genome editing applications [6].

3.3. CRISPR/Cas9 Genome Editing System

The CRISPR/Cas9 system comes from Streptococcus pyogenes, where it works as a bacterial immune defense. Plant genome editing has since made it a standard tool. A guide RNA leads the Cas9 nuclease to a specific DNA sequence, where it cuts. That double-strand break then triggers the cell's own repair machinery, and researchers use those pathways to knock out genes, insert new DNA, or swap single bases. Because the system is fairly straightforward, labs have adapted it quickly across many plant species—from model plants to major cereals like rice and maize.

Newer versions offer better precision. SSome Cas9 variants are more accurate, so they cut where intended and miss fewer off-target sites, addressing earlier worries about unintended changes. Also, tissue-specific promoters now restrict editing to certain organs (roots, leaves, or seeds). And delivering the Cas9 protein together with its guide RNA as a ribonucleoprotein complex avoids inserting any DNA into the plant genome. With these improvements, researchers have produced knockout lines, edited regulatory sequences, and made targeted gene replacements in rice, maize, wheat, and tomato. The real question now isn't whether the technology works—it clearly does. Whether CRISPR/Cas9 moves from lab demonstrations to routine commercial release depends more on regulations and public acceptance than on the tool itself [1].

3.4. Base Editing Technologies

Cytosine base editors (CBEs) convert C • G to T • A without cleaving both DNA strands, sidestepping the repair errors that plague conventional CRISPR/Cas9 knockouts. The tool fuses cytidine deaminase to a nickase-Cas9 variant; the deaminase strips an amino group from cytosine within a narrow editing window, producing uracil, which replication then reads as thymine. Researchers have introduced point mutations in acetolactate synthase genes of oilseed rape using this approach, yielding sulfonylurea-resistant lines. The edits are precise enough to avoid the scattershot damage of random mutagenesis, and because no foreign DNA remains in the final plant, the resulting varieties may encounter different regulatory treatment than transgenic crops. [7].

4. Crop Improvement Achievements

4.1. Enhancing Grain Yield by CRISPR-Cas9 Promoter Editing of Maize CLE Genes

Editing promoters of CLE genes with CRISPR-Cas9 has raised grain yield in maize without the trade-offs typical of coding sequence knockouts. CLE peptides signal meristem maintenance and organ development; their expression levels set kernel number and size. Liu et al. tuned conserved regulatory elements in the ZmCLE7 and ZmCLE14 promoters, generating small expression shifts that increased yield while leaving plant architecture and stress response intact. The edits avoid pleiotropy because they alter dosage rather than protein structure. Field data showed more kernel rows and better ear development in edited lines. This points to promoter editing as a viable route for quantitative trait improvement [6].

4.2. Herbicide-Resistant Oilseed Rape

Cytosine base editing has produced sulfonylurea-resistant oilseed rape by altering conserved residues in the acetolactate synthase gene. The edits replace key amino acids in the herbicide binding pocket, cutting affinity for the chemical while leaving enough catalytic activity for normal growth. Because the final plant carries no foreign DNA, only the intended point change, it falls outside transgenic regulation in some jurisdictions. Greenhouse trials showed edited lines survive herbicide doses that kill wild-type plants, with growth metrics matching unedited controls [7].

4.3. Glyphosate Resistance in Rice

HDR with CRISPR-Cas9 has produced glyphosate-resistant rice by editing the EPSPS gene. The edits swap threonine and proline residues in the glyphosate binding pocket, cutting herbicide affinity while leaving enough enzymatic activity for aromatic amino acid synthesis. The repair used a donor template carrying the intended mutations, yielding plants with no foreign DNA and no selectable markers. Edited lines survived glyphosate doses lethal to wild-type plants in greenhouse tests. The same strategy should transfer to other cereals, though efficiency in monocots remains lower than in rice [3].

4.4. Ascorbate Content Enhancement in Tomato

Overexpressing GDP-L-galactose phosphorylase and L-galactose-1-phosphate phosphatase in tomato has raised fruit vitamin C levels two- to threefold. These enzymes control flux through the Smirnoff-Wheeler pathway; pushing both steps simultaneously diverts more carbon into ascorbate without stunting growth or distorting fruit. Transgenic lines accumulated up to 500 mg ascorbate per 100 g fresh weight in some reports, roughly matching kiwifruit. Thus, metabolic engineering offers a possible route for vegetable biofortification. Nevertheless, field trials and consumer responses remain to be evaluated [8].

5. Future Perspectives and Conclusions

Speed breeding and genome editing are coming together to shorten how long it takes to develop new crop varieties. With rapid generation advance in greenhouses or growth chambers, you can grow six generations of rice per year instead of just one. Add CRISPR to the mix, and breeders can lock in desired traits in far fewer cycles. Prime editing makes things even more precise. It changes single bases without cutting both DNA strands, which avoids the repair mistakes that often come with standard CRISPR. That said, this combination is still mostly at the proof-of-concept stage. Only a few varieties have been released so far. But early work in wheat and rice suggests the whole breeding timeline could drop from ten years down to three or four [10].

Multiplex editing that hits several yield genes at once is moving from theory to practice in rice and tomato. Regulatory network engineering, though harder to control, could tune drought tolerance or nitrogen use by rewiring transcription factors rather than single genes. Organelle editing remains stuck at proof of concept; inserting DNA into chloroplasts or mitochondria works in tobacco but not yet in cereals.

AI is already guiding CRISPR guide RNA design and predicting off-target sites from sequence data. Whether it can model full genotype--o-phenotype maps remains doubtful, given the noise in field trials. Some regulators now treat base-edited plants as non-transgenic, while others still require case-by-case review.

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