Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

ADVANCES IN PLANT BIOTECHNOLOGY FOR CROP IMPROVEMENT: TRANSLATING LABORATORY INNOVATIONS INTO FIELD APPLICATIONS

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DOI: https://doi.org/10.5281/zenodo.21451550

Keywords

CRISPR-Cas9; Genome Editing; Plant Microbiome; Synthetic Microbial Communities (SynComs); Precision Breeding; Climate-Smart Agriculture.

Article History

Received: 01 February 2026
Accepted: 17 March 2026
Published: 30 March 2026

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Abstract

Plant biotechnology is undergoing a paradigm shift from empirical traditional breeding to precision genome editing and holistic microbiome engineering, driven by the urgent need for climate-resilient, nutrient-dense crops. This review synthesizes the transition from first-generation transgenic approaches with random foreign DNA insertion to refined cisgenic and intragenic methods that maintain genetic integrity, and spotlights the transformative role of CRISPR-Cas9 in enabling targeted nucleotide-level modifications knockouts, allele replacements, base edits often indistinguishable from natural mutations. Beyond the host genome, the plant-associated microbiome is recognized as a heritable "extended genome" crucial for nutrient acquisition and stress tolerance. Host-mediated microbiome engineering, wherein plant genes controlling microbial assembly are edited, allows the recruitment of rationally designed synthetic microbial communities (SynComs) that provide emergent properties like enhanced disease suppression and drought resilience, overcoming the limitations of single-strain inoculants. Field-validated genome-edited crops showcase real-world impact: the high-GABA tomato in Japan for blood pressure regulation, drought-tolerant rice in India via promoter editing, powdery mildew-resistant wheat in China through engineered susceptibility genes, and multi-pathogen-resistant VPM-0008 maize in the USA. The lab-to-field pipeline is accelerated by multi-omics integration (genomics, transcriptomics, metabolomics, phenomics) for target discovery, advanced delivery systems including DNA-free RNP complexes and morphogenic regulators (Baby boom, Wuschel2) to overcome genotype dependency, and evolving regulations such as the UK Precision Breeding Act 2025, the EU's differentiated NGT framework, and product-based models in the Americas. Looking ahead to 2050, co-optimizing crop genomes and microbiomes through CRISPR precision breeding, synthetic biology for photosynthetic efficiency, and SynComs for nutrient cycling charts a path to climate-smart agriculture. Realizing this vision depends on equitable technology access, internationally harmonized science-based regulations, and responsible environmental stewardship.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

1.INTRODUCTION

The convergence of climate change, population growth, and diminishing arable land has created an unprecedented imperative for agricultural innovation. Global population is projected to reach nearly 10 billion by 2050, requiring a 50% increase in food production from fewer resources and under increasingly hostile growing conditions [29]. This challenge is compounded by the fact that agricultural productivity gains have been plateauing in many major crop species, with yield ceilings approaching biological limits in some high-input production systems [25]. Abiotic stresses—drought, salinity, extreme temperatures, and flooding—already reduce average crop yields by 50% or more, and the frequency and intensity of these stresses are escalating with climate change [26]. Climate models predict that for every 1°C increase in global mean temperature, wheat yields will decline by approximately 6%, rice by 3.2%, maize by 7.4%, and soybean by 3.1%, creating an urgent need for thermotolerant germplasm [30]. Simultaneously, biotic pressures from emerging pathogens and insect pests are expanding into new geographies as warming temperatures enable their migration poleward and to higher elevations, exposing naive crop populations to novel disease complexes [32]. The paradigm of agricultural intensification has undergone a dramatic conceptual shift: from the "Green Revolution" (input-intensive, focused on irrigation and chemical inputs) of the 1960s-1980s, to the "Gene Revolution" (genetic potential-driven, focused on developing resilient germplasm through molecular breeding and transgenics) of the 1990s-2010s, and now to the "Microbiome Revolution"—a holistic approach recognizing the plant-soil-microbiome continuum as an integrated system for sustainable yield enhancement. This latest paradigm acknowledges that crops do not exist in isolation but rather as holobionts, complex symbiotic entities whose phenotypes are determined by the combined genetic potential of the plant host and its associated microbial communities [8].

Conventional breeding, while highly successful during the Green Revolution, is constrained by several fundamental limitations that have become increasingly apparent as the pace of climate change outstrips the rate of genetic gain achievable through traditional methods. Linkage drag—the coinheritance of undesirable traits with desired genes due to physical proximity on chromosomes—requires extensive backcrossing over multiple generations, prolonging breeding cycles to 10–15 years for new varieties and making the introgression of traits from wild relatives particularly cumbersome and time-intensive [33]. The available gene pool is limited to cross-compatible species, excluding many potentially valuable resistance or tolerance genes found in wild relatives that cannot be readily crossed with cultivated varieties due to sexual incompatibility barriers. This limitation is especially acute for traits such as salt tolerance or disease resistance, where the most potent genetic resources often reside in wild species with completely different ploidy levels or incompatible reproductive biology [31]. Mutation breeding, which emerged as the first-generation biotech tool using chemical or radiation mutagenesis, introduced random, uncharacterized mutations throughout the genome, necessitating large-scale screening populations to identify rare beneficial variants. While useful for generating genetic diversity—over 3,200 mutant varieties have been officially released worldwide since the 1950s [34]—this approach lacks precision and often introduces multiple unintended mutations that must be painstakingly eliminated through subsequent backcrossing, adding years to the development timeline and potentially compromising agronomic performance through cryptic genetic damage.

The era of transgenics began with the introduction of Bt crops expressing insecticidal proteins from *Bacillus thuringiensis* and herbicide-tolerant crops such as Roundup Ready soybean, representing the first commercial applications of recombinant DNA technology in agriculture [37]. The adoption of insect-resistant corn and cotton has significantly reduced insecticide use on these crops by an estimated 40–60% [35], while glyphosate-tolerant soybeans simplified weed management through broad-spectrum post-emergence herbicide application. However, widespread adoption of herbicide-tolerant crops has also driven a dramatic increase in glyphosate use, raising serious concerns

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ISSN: (e) 3007-1607 (p) 3007-1593

about herbicide resistance evolution in weed populations—with glyphosate-resistant weeds now documented in over 50 species across more than 30 countries [36]—as well as potential environmental impacts on non-target organisms and soil health. More fundamentally, transgenesis introduced the concept of "foreign DNA"—genes from distantly related or unrelated species—which triggered significant public concern, particularly in European and Asian markets, and led to stringent regulatory frameworks that have severely limited the release of many promising transgenic varieties. The cost of regulatory approval for a transgenic crop event, estimated at $35–136 million depending on the jurisdiction and trait, has effectively restricted transgenic crop development to a handful of multinational corporations working on a limited set of high-value commodity crops, leaving many nutritionally important orphan crops and minor staples without access to biotechnological improvement [20].

CRISPR-Cas genome editing has fundamentally transformed the plant breeding landscape by enabling precise, targeted modifications of endogenous genes without the introduction of foreign DNA, thereby blurring the line between natural variation and deliberate genetic improvement. This technology is uniquely suited for developing climate-smart crops [2] because of its inherent programmability, high specificity, multiplexing capability (allowing simultaneous editing of multiple genes controlling complex traits), and crucially, its ability to recapitulate naturally occurring beneficial alleles that already exist in germplasm collections but would require decades to introgress through conventional breeding [1]. The regulatory status of genome-edited crops has been a major driver of adoption and investment: in the United States, the USDA SECURE rule determined that most CRISPR-edited crops (specifically those created through SDN-1 and SDN-2 mechanisms, which generate small deletions or templated edits without foreign DNA integration) are not subject to biotechnology regulations if they contain no recombinant DNA and produce phenotypes that could otherwise be achieved through conventional breeding. This product-based regulatory approach has been adopted by Canada, Brazil, Argentina, Chile, Colombia, and several other nations [11,15], dramatically shortening commercialization timelines to an estimated 4–6 years compared to 10–16 years for transgenic events [27]. The UK's Precision Breeding Act came into full effect in November 2025, making the United Kingdom the first major European economy to establish a dedicated science-based regulatory framework for gene-edited crops, creating a distinct pathway that separates precision-bred organisms from genetically modified organisms (GMOs) while maintaining appropriate safety oversight through field trial notifications and trait-specific risk assessments. Meanwhile, in December 2025, EU lawmakers reached a landmark provisional agreement on the regulation of plants developed using new genomic techniques (NGTs), establishing a two-tier system that eases regulatory burdens for certain gene-edited plants (Category 1, with modifications equivalent to those achievable through conventional breeding) while maintaining stricter controls for more complex modifications (Category 2, including transgenics and cisgenics with more extensive alterations). This policy evolution in Europe, historically the most restrictive market for biotech crops, signals a potential convergence toward harmonized global regulatory frameworks that prioritize product characteristics over process methodology [40].

2. Core Molecular Toolbox for Crop Engineering

2.1 The Workhorse: RNA Interference (RNAi) and Virus-Induced Gene Silencing (VIGS)

2.1.1 RNA Interference in Plants: From Silencing Mechanism to Crop Protection

RNA interference is a sequence-specific gene silencing mechanism mediated by double-stranded RNA (dsRNA) and conserved across eukaryotes, from fungi and plants to animals [41]. The discovery of RNAi, recognized by the 2006 Nobel Prize in Physiology or Medicine awarded to Fire and Mello, transformed plant functional genomics by providing a simple, rapid method for reducing gene expression without the need for stable transformation or the isolation of mutant alleles. The molecular mechanism involves Dicer, an RNase III family endonuclease, which cleaves

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ISSN: (e) 3007-1607 (p) 3007-1593

dsRNA into small interfering RNAs (siRNAs) of 21–24 nucleotides in length, characterized by their distinctive two-nucleotide 3' overhangs. These siRNAs are subsequently loaded into the RNA-induced silencing complex (RISC), where the Argonaute (AGO) protein acts as the catalytic engine, retaining one strand of the siRNA duplex and discarding the passenger strand. The guide strand then directs sequence-specific recognition and cleavage of complementary mRNA targets through AGO's endonucleolytic Slicer activity, resulting in post-transcriptional gene silencing (PTGS) [42]. In plants, RNAi has three core applications that span the full spectrum from functional genomics research to practical crop protection. Virus-induced gene silencing (VIGS) uses engineered viral vectors—commonly derived from Tobacco rattle virus (TRV), Barley stripe mosaic virus (BSMV), or Apple latent spherical virus (ALSV)—to deliver dsRNA into plant cells during viral replication, achieving transient gene silencing in as little as 2–3 weeks, dramatically faster than traditional genetic modification methods that require months to years [43]. This technology has become indispensable for high-throughput reverse genetics screens, enabling researchers to rapidly assess gene function in diverse plant species without the need for stable transformation protocols. Host-induced gene silencing (HIGS) generates transgenic crops that stably express dsRNA or hairpin RNA constructs targeting essential genes in fungal pathogens, viruses, or insect pests, providing durable, sequence-specific resistance. This approach has been successfully deployed against Fusarium head blight in wheat, cotton bollworm, and soybean cyst nematode, with the plant's own RNAi machinery processing the transgene-derived dsRNA into siRNAs that are then taken up by the invading pest or pathogen during the infection or feeding process, silencing critical genes required for pathogenesis or survival [44].

2.1.2 SIGS: Extracellular Application of dsRNA for Transgene-Free Control

Spray-induced gene silencing (SIGS) represents the most recent and potentially transformative application, applying dsRNA directly to plant surfaces as a foliar spray, trunk injection, or soil drench, offering a non-transgenic, environmentally friendly approach to crop protection that circumvents the lengthy regulatory processes associated with genetically modified organisms. This method leverages the natural capacity of many fungal pathogens to take up environmental RNA from their surroundings, and has shown particular promise against Botrytis cinerea, Sclerotinia sclerotiorum, and Fusarium graminearum [43]. However, RNAi-based strategies face significant practical challenges that currently limit widespread field deployment: dsRNA stability in field conditions is severely compromised by UV radiation, rainfall, and microbial degradation, with half-lives often measured in hours rather than days, while effective delivery through the cuticle and leaf barriers into target cells and organisms remains a substantial technical hurdle requiring innovative formulation technologies such as layered double hydroxide nanoparticles, cell-penetrating peptides, and cationic liposomes to enhance both stability and cellular uptake [45].

2.2 Precision Genome Editing: The CRISPR-Cas Arsenal in Plants

2.2.1 CRISPR-Cas9-Mediated Genome Editing in Plants: Mechanisms, Repair Pathways, and Precision Challenges

The CRISPR-Cas9 system, derived from the type II adaptive immune system of Streptococcus pyogenes, has been the most widely deployed platform for plant genome editing due to its simplicity, efficiency, and extensive characterization across diverse species [38]. The SpCas9 protein (1,368 amino acids) forms a bilobed complex with a chimeric single guide RNA (sgRNA) that directs the nuclease to a specific genomic target through Watson-Crick base pairing between the 20-nucleotide spacer sequence and the complementary DNA strand. Target recognition also requires the presence of a short protospacer adjacent motif (PAM)—specifically 5′-NGG-3′ for SpCas9—which restricts targeting to sequences containing this motif and serves as a critical self versus non-self discrimination mechanism preventing cleavage of the bacterial

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ISSN: (e) 3007-1607 (p) 3007-1593

CRISPR array itself [38]. Upon binding and formation of the R-loop structure, Cas9 generates a blunt-ended double-strand break (DSB) precisely three nucleotides upstream of the PAM via the concerted action of its two nuclease domains: RuvC, which cleaves the non-target strand, and HNH, which cleaves the target strand [39]. This DSB then triggers cellular DNA repair pathways that form the mechanistic basis for all genome editing outcomes. In somatic plant cells, the error-prone non-homologous end joining (NHEJ) pathway is the dominant repair mechanism, operating throughout the cell cycle to directly religate broken DNA ends, frequently generating small insertions or deletions (indels) of 1–10 base pairs that disrupt the open reading frame and create loss-of-function mutations through frameshifts [46]. For applications requiring precise allele replacement, gene correction, or controlled small insertions, homology-directed repair (HDR) can be harnessed by providing an exogenously supplied donor repair template containing the desired modification flanked by homology arms. However, HDR efficiency in somatic plant cells remains frustratingly low (typically less than 1%, often 0.1–0.5%), as it is restricted to the S and G2 phases of the cell cycle and must compete with the overwhelmingly dominant NHEJ machinery. This efficiency bottleneck represents one of the most active areas of current research, with strategies including chemical inhibition of NHEJ components, cell cycle synchronization, viral replicon-based donor delivery, and the use of geminivirus-based vectors to amplify repair templates, all showing promising improvements in specific experimental contexts. Beyond these approaches, the recent emergence of base editing and prime editing has begun to bypass the HDR roadblock entirely by enabling precise nucleotide changes without requiring double-strand breaks or donor repair templates. Base editors (cytosine and adenine deaminases fused to a Cas9 nickase) directly convert C•G to T•A or A•T to G•C, while prime editing uses a Cas9 nickase–reverse transcriptase fusion guided by a prime editing guide RNA (pegRNA) to write new sequence information directly into the target site. Although delivery challenges and variable efficiencies remain, these DSB-free tools are rapidly expanding the scope of precision plant genome editing and offer a practical alternative for generating targeted point mutations, insertions, and deletions in somatic tissues.

2.2.2 Beyond Cas9: Expanding the Plant Genome Editing Toolkit with Base Editors, Prime Editors, and Type V CRISPR Systems

Beyond these approaches, the recent emergence of base editing and prime editing has begun to bypass the HDR roadblock entirely by enabling precise nucleotide changes without requiring double-strand breaks or donor repair templates. Base editors (cytosine and adenine deaminases fused to a Cas9 nickase) directly convert C•G to T•A or A•T to G•C, while prime editing uses a Cas9 nickase–reverse transcriptase fusion guided by a prime editing guide RNA (pegRNA) to write new sequence information directly into the target site. Although delivery challenges and variable efficiencies remain, these DSB-free tools are rapidly expanding the scope of precision plant genome editing and offer a practical alternative for generating targeted point mutations, insertions, and deletions in somatic tissues.Cas12a (formerly Cpf1), derived from Acidaminococcus species (AsCas12a) and Lachnospiraceae bacterium (LbCas12a), represents a distinct type V-A CRISPR system with several features that complement Cas9 for plant genome editing applications. Unlike Cas9, Cas12a generates double-strand breaks with staggered 5' overhangs of 4–5 nucleotides, recognizes T-rich PAM sequences (5′-TTTV-3′), and importantly, processes its own CRISPR RNA (crRNA) array [50] through an intrinsic RNase domain without requiring trans-activating crRNA (tracrRNA) or host RNase III. This autonomous crRNA processing capability enables simplified multiplex editing from a single promoter driving a tandem crRNA array, making Cas12a particularly advantageous for targeting multiple genes or multiple sites simultaneously and for editing AT-rich genomic regions such as intergenic regulatory sequences and repetitive elements. Cas12f (also known as CasΦ or Cas14), a miniaturized single-effector nuclease originally identified in

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

hyperthermophilic archaea and the genome of a huge phage metagenomic dataset, is approximately half to one-third the size of SpCas9 (~400–700 amino acids versus 1,368), representing the smallest functional CRISPR-Cas enzyme discovered to date. This dramatically reduced size could enable delivery via viral vectors such as adeno-associated viruses (AAVs) that have limited packaging capacity, as well as more efficient delivery as ribonucleoprotein complexes, but extensive characterization, efficiency optimization, and PAM requirement determination in diverse plant systems remain at very early stages with only a handful of proof-of-concept publications to date [51].

Looking forward, the continued mining of microbial (meta)genomes and metagenomic datasets is yielding a rapidly expanding catalogue of compact Cas effectors, including Cas12j (CasΦ-2) and Cas12l, as well as even smaller systems like IscB and TnpB that are believed to be evolutionary ancestors of Cas9 and Cas12.These ultra-compact nucleases, coupled with innovations in delivery from engineered viral vectors to cell-penetrating peptide fusions and nanocarrier-based RNP delivery are progressively lowering the barriers to tissue-culture-independent editing and direct germline modification in plants. Furthermore, the integration of these tools with RNA interference strategies, such as host-induced gene silencing (HIGS) andspray-induced gene silencing (SIGS), opens the possibility of multi-layered protection stacks that combine stable gene knockouts with inducible, sequence-specific pest and pathogen suppression. While significant work remains to achieve predictable editing efficiencies, high specificity, and amenable regulatory frameworks, the convergence of base editing, prime editing, and diverse Caseffectors is setting the stage for a new era of precision molecular breeding in crops.

2.2.3 Base Editors Enable DSB-Free C•G-to-T•A and A•T-to-G•C Conversions via Chemical Deamination

Beyond standard Cas9 nucleases, the CRISPR toolkit has expanded dramatically through protein engineering and the discovery of novel CRISPR systems from diverse microbial sources. Base editors represent a transformative class of precision editing tools that chemically convert one nucleotide to another without creating a double-strand break. Adenine base editors (ABEs), evolved from the RNA-editing enzyme TadA of Escherichia coli, catalyze A·T to G·C conversions through inosine intermediate formation, while cytosine base editors (CBEs) fusing catalytically impaired Cas9 (nCas9 or dCas9) to cytidine deaminases such as APOBEC1 convert C·G to T·A within a precise activity window. This approach is ideal for introducing premature stop codons (CAG to TAG, CAA to TAA, or CGA to TGA) for creating precise gene knockouts without DSBs, or for correcting deleterious nonsense mutations in agronomically important genes. Both ABEs and CBEs have been successfully deployed across major crop species including rice, wheat, maize, tomato, and potato, enabling targeted amino acid substitutions that can modulate enzyme activity, alter protein stability, or fine-tune regulatory functions. However, base editors have a narrow editing window (typically positions 4–8 within the protospacer, counting the PAM as positions 21–23) and can introduce unwanted bystander edits when multiple target nucleotides are present within the activity window [47], and they fundamentally cannot create transversions (purine-to-pyrimidine or pyrimidine-to-purine changes) [48]. Prime editors represent an even more versatile and powerful approach, fusing a catalytically impaired Cas9 nickase (H840A mutant) with an engineered Moloney murine leukemia virus reverse transcriptase (M-MLV RT) domain. The prime editing guide RNA (pegRNA) serves a dual function: its spacer sequence specifies the genomic target site, while its 3' extension contains a primer binding site (PBS) that hybridizes to the nicked DNA strand and an RT template that encodes the desired edit, enabling precise insertion, deletion, and all 12 possible types of base-to-base conversions without requiring double-strand breaks, donor DNA templates, or HDR machinery. In principle, prime editors can correct up to 89% of known genetic variants catalogued in human disease databases [49], and in plants, prime editing has been successfully applied to install small regulatory motifs in gene

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ISSN: (e) 3007-1607 (p) 3007-1593

promoters, introduce precise single-nucleotide polymorphisms associated with agronomic traits, and generate small defined deletions for cis-regulatory element disruption, though editing efficiency in many crop species remains suboptimal and requires extensive optimization of pegRNA design parameters including PBS length, RT template structure, and nicking sgRNA positioning to achieve practical rates of precise modification.

Cas12a (formerly Cpf1), derived from Acidaminococcus species (AsCas12a) and Lachnospiraceae bacterium (LbCas12a), represents a distinct type V-A CRISPR system with several features that complement Cas9 for plant genome editing applications. Unlike Cas9, Cas12a generates double-strand breaks with staggered 5' overhangs of 4–5 nucleotides, recognizes T-rich PAM sequences (5′-TTTV-3′), and importantly, processes its own CRISPR RNA (crRNA) array through an intrinsic RNase domain without requiring trans-activating crRNA (tracrRNA) or host RNase III. This autonomous crRNA processing capability enables simplified multiplex editing from a single promoter driving a tandem crRNA array, making Cas12a particularly advantageous for targeting multiple genes or multiple sites simultaneously and for editing AT-rich genomic regions such as intergenic regulatory sequences and repetitive elements. Cas12f (also known as CasΦ or Cas14), a miniaturized single-effector nuclease originally identified in hyperthermophilic archaea and the genome of a huge phage metagenomic dataset, is approximately half to one-third the size of SpCas9 (~400–700 amino acids versus 1,368), representing the smallest functional CRISPR-Cas enzyme discovered to date. This dramatically reduced size could enable delivery via viral vectors such as adeno-associated viruses (AAVs) that have limited packaging capacity, as well as more efficient delivery as ribonucleoprotein complexes, but extensive characterization, efficiency optimization, and PAM requirement determination in diverse plant systems remain at very early stages with only a handful of proof-of-concept publications to date. Figure 1 is showing that The CRISPR-Cas9 system for targeted crop improvement. (A) The editing construct is delivered into plant cells via Agrobacterium-mediated transformation, carrying genes for Cas9, a single guide RNA (sgRNA) targeting a gene of interest (e.g., a negative yield regulator), and a selectable marker. (B) Inside the nucleus, the sgRNA-Cas9 complex creates a site-specific double-strand break (DSB). The DSB is repaired by the error-prone Non-Homologous End Joining (NHEJ) pathway, causing insertions/deletions that create a gene knockout (e.g., disabling a susceptibility factor). (C) If a donor repair template is provided, the DSB is repaired by Homology-Directed Repair (HDR), enabling precise allele replacement or trait stacking at the target locus.

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

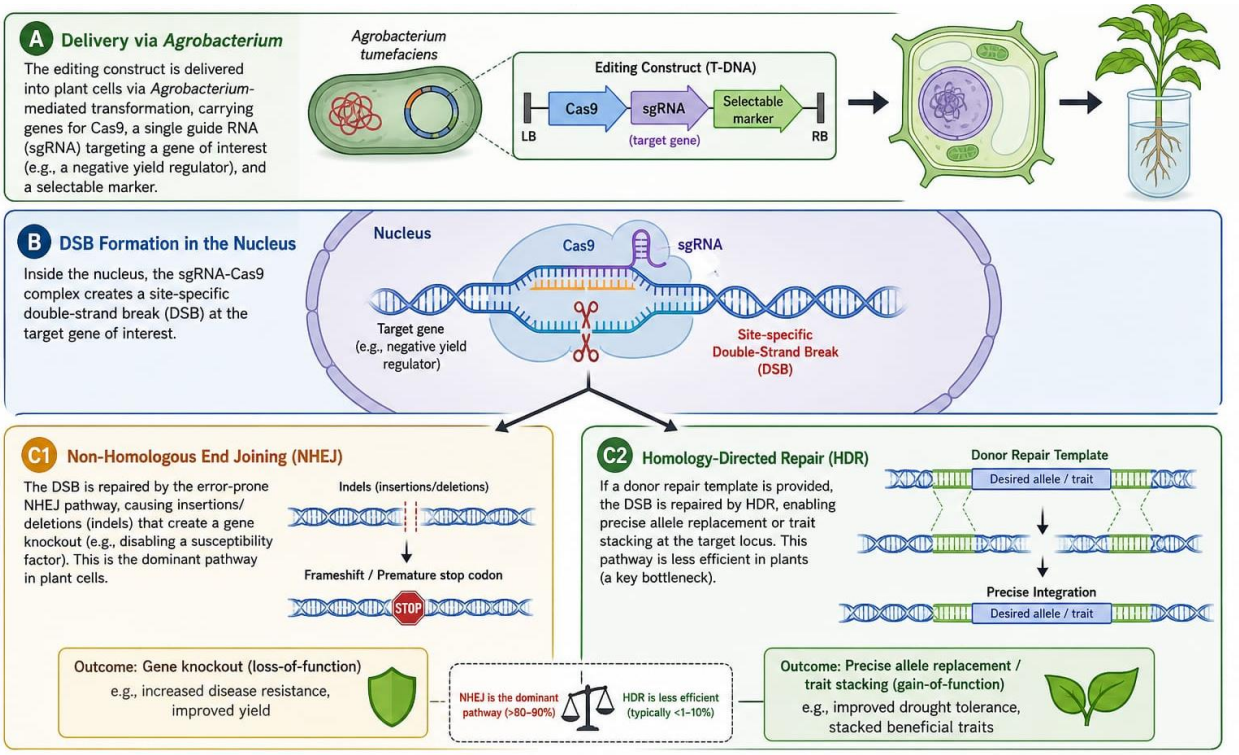


Figure 1: Molecular Mechanism of CRISPR-CAS Genome Editing in Plants

2.3 The Enabling Platform: In Vitro Regeneration and Transformation

2.3.1 Traditional Regeneration and Transformation Platforms: Bottlenecks and Genotype Dependency

The delivery of genome editing reagents into plant cells and the subsequent regeneration of intact, fertile plants from edited cells remain significant technical bottlenecks that constrain the full potential of CRISPR technologies across diverse crop species. Tissue culture—specifically somatic embryogenesis and organogenesis—is a prerequisite for most established transformation protocols, requiring the isolation of explant tissues (typically immature embryos, mature embryo axes, leaf discs, or cotyledonary nodes), their culture on defined media containing specific hormone combinations (auxins such as 2,4-D or picloram to induce embryogenic callus, and cytokinins such as BAP or kinetin to stimulate shoot regeneration), and the stepwise regeneration of rooted plantlets over periods ranging from 3 to 12 months depending on the species. The canonical method for stable integration remains *Agrobacterium tumefaciens*-mediated transformation, which exploits the soil bacterium's natural ability to transfer a defined segment of its tumor-inducing (Ti) plasmid, the T-DNA, into the plant nuclear genome through a type IV secretion system [53]. This method is generally preferred for its simplicity, low copy number integration reducing the likelihood of transgene silencing, and capacity to transfer relatively large DNA fragments of 20–150 kb when using binary or superbinary vector systems. Alternative methods include biolistics (particle bombardment), which accelerates DNA-coated gold or tungsten microparticles into plant cells using high-pressure helium gas, and remains important for organellar (chloroplast) transformation where T-DNA delivery is inapplicable, and for recalcitrant monocots such as certain maize and sorghum genotypes where Agrobacterium transformation is inefficient due to limitations in bacterial attachment, vir gene induction, or host defense responses. However, the most profound and persistent obstacle is

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ISSN: (e) 3007-1607 (p) 3007-1593

genotype dependency: many elite commercial varieties, particularly of major cereal crops that have been intensively selected for yield and quality rather than tissue culture responsiveness, are highly recalcitrant to in vitro regeneration, severely limiting the applicability of genome editing to the very germplasm that is most urgently needed for commercial impact [52]. The recalcitrance problem is further compounded by the physiological state of the explant; even within a single variety, regeneration competence varies dramatically with donor plant age, growth conditions, and seasonal fluctuations, making reproducible transformation a logistical challenge. Moreover, the prolonged tissue culture phase itself is inherently mutagenic,inducing somaclonal variation—unintended genetic and epigenetic changes—that can confound phenotypic analysis of edited lines and complicate regulatory approval.

2.3.2 Next-Generation Solutions: Morphogenic Regulators and Transgene-Free Editing

Emerging solutions leverage morphogenic regulators—developmental transcription factors that control meristem formation and cellular reprogramming—to overcome this genotype barrier in what represents one of the most significant advances in plant biotechnology in recent years. Ectopic expression of developmental regulators such as WUSCHEL2 (WUS2), a homeodomain transcription factor that maintains stem cell identity in shoot apical meristems, and BABY BOOM (BBM), an AP2/ERF family transcription factor that promotes somatic embryogenesis, induces de novo meristem formation from somatic cells, effectively enabling "genotype-independent" transformation across previously recalcitrant germplasm [16]. GROWTH-REGULATING FACTORS (GRFs) and their essential cofactors, GRF-interacting factors (GIFs), have shown particular promise for synergistically improving regeneration efficiency and transformation frequency in major cereal crops including maize, wheat, rice, and sorghum, with the GRF4-GIF1 chimeric fusion protein proving especially potent [6] when expressed transiently during the transformation and regeneration process. Optimizing BBM/WUS2 expression cassettes through cell type-specific promoters, tissue-specific excision systems such as CRE/loxP or heat-inducible removal, and transient expression strategies that avoid stable integration of the morphogenic regulators has enabled successful transformation and gene editing using seedling leaves as starting material rather than the traditional immature embryo explants, dramatically expanding the range of transformable genotypes to include elite commercial hybrids and locally adapted landraces. Viral-mediated delivery of morphogenic regulators using deconstructed RNA viruses as transient expression vectors has further been shown to achieve 75–79% embryonic calli formation in sorghum [16], a crop that has historically been among the most recalcitrant to transformation and where this advance opens the door to routine genome editing. Most excitingly from both a regulatory and public acceptance perspective, CRISPR-RNP (ribonucleoprotein) complexes—pre-assembled Cas9 protein and sgRNA molecules delivered directly into plant cells via biolistics, polyethylene glycol (PEG)-mediated protoplast transfection, electroporation, or lipid nanoparticles—enable completely transgene-free genome editing, producing edited plants that contain no foreign DNA and carry only the intended genetic modification. This approach avoids the regulatory and public acceptance hurdles associated with stable transgene integration, as the RNP complexes are rapidly degraded by endogenous proteases and nucleases after accomplishing the editing event, leaving no residual recombinant DNA in the final plant and resulting in genome-edited products that are molecularly indistinguishable from naturally occurring or conventionally bred variants [55], a critical consideration for regulatory classification in jurisdictions adopting product-based or tiered regulatory frameworks.

3. Strategic Approaches for Developing Stronger Crops

3.1 Cisgenesis Intragenesis: Rewriting the GM Narrative

3.1.1 Cisgenesis and Intragenesis: Conceptual Foundations and Regulatory Rationale

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Cisgenesis and intragenesis represent intermediate, yet highly strategic, approaches positioned between conventional transgenesis and modern precision genome editing, specifically engineered to address deeply rooted public concerns about foreign DNA while preserving the tangible benefits of genetic modification for crop improvement. The conceptual foundation of cisgenesis rests on the transfer of complete genes—encompassing their native coding sequences, introns, and fully intact regulatory elements (promoters, terminators)—exclusively derived from a cross-compatible donor species, meaning a species with which the recipient crop can naturally hybridize through conventional breeding. This methodology therefore respects the plant's natural generpool boundaries, accelerating traditional breeding cycles by decades through the direct introgression of desirable alleles without the penalty of linkage drag, the phenomenon where tightly linked undesirable alleles from wild relatives are co-inherited and require extensive backcrossing to eliminate. Intragenesis, by contrast, involves the innovative recombination of coding sequences and regulatory elements—promoters, enhancers, signal peptides—all sourced from the same or cross-compatible gene pool, to create novel, yet biologically permissible, expression patterns [57]. For example, a native resistance gene with a constitutive promoter could be re-engineered to be driven by a pathogen-inducible promoter, confining its expression to infection sites and minimizing any metabolic burden on the plant. The core scientific and regulatory advantage of both methods is their adherence to the plant's genetic boundaries; they introduce genetic material that could theoretically be exchanged through natural gene flow, thereby mitigating the foundational risk hypothesis associated with transgenic crops—the movement of genetic material across kingdom barriers. This genetic contiguity has been critical in persuading several regulatory bodies to create distinct, less burdensome pathways for cisgenic and intragenic crops, treating them more analogously to conventional breeding products [56].

3.1.2 The Late Blight-Resistant Potato: A Global Exemplar and Regulatory Milestone

The global exemplar of cisgenic crop development is the late blight-resistant potato, engineered to combat Phytophthora infestans, the oomycete pathogen responsible for the Irish Potato Famine and still the most destructive disease of potato worldwide, causing annual losses exceeding $6 billion [58] and necessitating intensive fungicide regimes of 10–15 applications per growing season. These cisgenic potatoes carry resistance genes (Rpi genes, such as Rpi-vnt1.1, Rpi-blb2, Rpi-mcq1) cloned directly from diverse wild potato relatives like Solanum venturii, S. bulbocastanum, and S. mochiquense. By stacking multiple Rpi genes, each recognizing distinct effector repertoires, breeders have achieved broad-spectrum and genetically durable resistance against all known races of late blight currently circulating [58] in the UK and continental Europe. In November 2025, UK regulations for precision-bred plants came fully into effect under the Precision Breeding Act, and the first formal applications opened specifically for these cisgenic potato lines, marking a historic milestone as the first major European economy to establish a dedicated pathway for such products. Parallel field trials conducted in Switzerland throughout 2025 rigorously evaluated these same and similar cisgenic potato lines for their agronomic performance, tuber quality, and resistance durability against locally adapted strains of the pathogen under natural infection pressure, generating the comprehensive environmental risk assessment data required for future EU-level authorization. The large-scale commercial growing of such durably resistant potatoes could dramatically slash fungicide usage by an estimated 80–90%, delivering immense environmental and economic benefits, though a persistent point of friction remains the EU's regulatory framework for organic farming, which currently does not permit genetically modified organisms of any category, and the resulting debate over coexistence, traceability, and consumer labeling continues actively among member states.

Complementing the European developments, field evaluations in East Africa—where late blight can destroy up to 100% of a smallholder's

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ISSN: (e) 3007-1607 (p) 3007-1593

harvest—have adapted the same Rpi gene stacks into locally preferred potato varieties using a cisgenic approach, with participatory variety selection trials showing high farmer and consumer acceptance when the cisgenic nature of the improvement is clearly communicated [59]. In parallel, a cisgenic version of the popular Russet Burbank variety carrying Rpi-vnt1.1 has demonstrated stable resistance over four consecutive field seasons in the United States with no detectable changes in tuber processing quality, suggesting that the integration of cisgenic resistance into industrial potato production pipelines is agronomically feasible.

Table 1: Comparison of Genetic Modification Strategies in Crop Biotechnology

Features	Transgenesis	Cisgenesis	CRISPR-Cas9 Gene Editing
Gene Source	Unrestricted (cross-kingdom)	Cross-compatible species	Endogenous, cross-compatible species, or can generate novel alleles
Modification Type	Random genomic insertion of foreign DNA	Random insertion of native DNA	Precise knockout, SNP substitution, or small insertion/deletion at a targeted locus
Allelic Option	Gain-of-function (dominant traits)	Gain-of-function (dominant traits)	Loss-of-function (S-gene knockout), gain-of-function (promoter swap), allele replacement
Regulatory Status (e.g., USA)	Regulated as a biotech event	Largely deregulated in specific cases	SDN-1/SDN-2 (null segregant) considered equivalent to conventional breeding
Scientific Challenge	Position effect variation, public concern	Limited to traits present in the gene pool	Delivery and regeneration bottleneck; low HDR efficiency
Example	Bt Cotton (cry1Ac)	Late blight resistant potato (Rpi-vnt1)	High-oleic soybean (FAD2 knockout)

3.2 Mastering Abiotic Stress Tolerance

3.2.1 Engineering Drought Resilience: Stomatal Regulation and ABA Signalling.

Drought resilience has been a primary target of CRISPR-based crop improvement due to the devastating impact of water deficit on global yields. In rice, researchers have focused on editing negative regulators of stomatal development and patterning, including the OsEPF1, OsEPF2, and OsEPFL9 genes. These genes encode epidermal patterning factor-like peptides that signal through the ERECTA family receptor kinase pathway to suppress stomatal lineage cell division; their functional knockout or downregulation reduces stomatal density on the leaf epidermis. A lower stomatal count directly decreases water loss through transpiration, improving water-use efficiency under deficit conditions without proportionally compromising carbon dioxide uptake, as photosynthetic rates are often limited more by mesophyll conductance and biochemical capacity than by stomatal aperture under moderate stress. The central challenge lies in striking an optimal balance: overly severe reductions in stomatal density can critically limit intercellular CO_2 concentration and photosynthesis under well-watered, high-light conditions, leading to a significant yield penalty that negates the drought survival advantage. Promoter editing of stress-responsive genes offers

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a more nuanced, tunable approach. By modifying cis-regulatory elements in the promoters of key drought-responsive transcription factors (such as DREBs, NACs, or MYBs) or aquaporins, it is possible to elevate gene expression specifically under stress conditions while maintaining near-normal basal levels. This conditional expression avoids the constitutive activation that often drains metabolic resources and causes growth retardation, achieving drought tolerance without a concomitant yield penalty under normal or moderate conditions. Complementary approaches include editing OsERA1, the gene encoding the farnesyltransferase β-subunit that negatively regulates abscisic acid (ABA) signaling. Knockout of OsERA1 enhances the sensitivity of guard cells to endogenous ABA, promoting faster and more complete stomatal closure during the early phases of drought stress, while the ABA signaling cascade otherwise remains intact, providing a "primed" state rather than a permanently closed stomata phenotype.

3.2.2 Engineering Salinity Tolerance: Ion Exclusion and ROS Management

Salinity tolerance represents another critical pillar for climate-resilient agriculture, particularly in coastal lowlands and arid irrigated regions where secondary soil salinization affects over 20% of irrigated land worldwide. The OsHKT1;5 gene in rice encodes a class I high-affinity potassium transporter that, paradoxically, functions as a sodium transporter in planta. Localized specifically to the plasma membrane of root xylem parenchyma cells, OsHKT1;5 mediates the retrieval of Na^+ ions from the transpiration stream in the xylem, effectively sequestering the toxic ion in the root tissues and preventing its long-distance translocation to photosynthetic leaf mesophyll cells, where accumulation causes chlorophyll degradation, membrane damage, and premature senescence. Promoter editing to precisely upregulate OsHKT1;5 expression, either through inserting additional stress-responsive elements or disrupting repressor binding motifs, enhances this retrieval capacity, further reducing shoot Na^+ accumulation and significantly improving both biomass and grain yield under saline conditions. In a parallel achievement with profound field impact, Indian researchers have successfully edited the DST (drought and salt tolerance) gene in the highly popular MTU-1010 rice variety, which is grown across approximately 4 million hectares in southern, central, and eastern India. The DST gene encodes a zinc finger transcription factor that positively regulates ROS homeostasis and stomatal density; its targeted loss-of-function reduces water loss and enhances ROS scavenging, conferring enhanced tolerance to both drought and salinity simultaneously. These developments are particularly time-critical as climate model projections consistently indicate that rising sea levels, erratic monsoons, and increased irrigation dependence will progressively intensify both drought episodes and soil salinity over the coming decades.

3.3 Engineering Durable Biotic Stress Resistance

3.3.1 Susceptibility Gene Disruption: The OsSWEET14 Paradigm and Beyond

Pathogen resistance has been revolutionized through the precise targeting of plant susceptibility factors, a strategy that fundamentally disrupts host "S-genes" that compatible pathogens have evolved to exploit for their own proliferation. This approach uniquely generates broad-spectrum and genetically durable resistance because it targets conserved host machinery components that are indispensable for the pathogen's infection cycle, rather than directly recognizing highly mutable pathogen effector molecules. Any pathogen variant attempting to evade this resistance would need to fundamentally alter its core pathogenesis mechanism, a far more constrained evolutionary pathway than simple effector mutation. The classic and most compelling example is OsSWEET14 in rice, which encodes a sucrose transporter whose expression is pathologically induced by transcription activator-like (TAL) effectors secreted by Xanthomonas oryzae pv. oryzae (Xoo), the causative agent of bacterial blight, one of the most serious rice diseases globally. Specific TAL effectors (such as AvrXa7, PthXo3, and Tal5) bind directly to discrete effector-binding elements (EBEs) within the OsSWEET14 promoter, hijacking the plant's transcription machinery to

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dramatically upregulate sugar export into the apoplast, thereby feeding the invading bacterial colony. CRISPR-mediated precise disruption of these EBEs—typically small deletions of 5–20 base pairs within the conserved binding motifs—completely abrogates TAL effector binding and the resultant susceptibility, conferring broad-spectrum resistance to more than 95% of geographically diverse Xoo strains tested, while maintaining normal OsSWEET14 function under non-infected conditions and resulting in no measurable growth or yield penalty under multiseason field conditions.

3.3.2 Multiplex Genome Editing for Broad-Spectrum Resistance: Wheat MLO and Maize Gene-Stacking

In hexaploid bread wheat, durable powdery mildew resistance has been elegantly achieved by simultaneous multiplex editing of all three homoeologous Mildew resistance locus o (TaMLO) genes—TaMLO-A1, -B1, and -D1. The MLO protein is a critical negative regulator of defense responses, and its functional loss confers recessive, broad-spectrum resistance against powdery mildew blast [60]. Because a functional MLO is required on all three wheat subgenomes (A, B, and D) for susceptibility, complete resistance requires simultaneous knockout of all three homoeologs, a feat impossible through conventional mutagenesis but readily achievable with multiplexed CRISPR-Cas9 targeting conserved sequences [60]. Remarkably, research published in late 2025 uncovered an unexpected and agriculturally critical insight: the broad deletion of genomic segments surrounding the MLO loci, beyond the gene coding region itself, can trigger the activation of downstream yield-related genes, effectively compensating for the well-documented yield penalty that polyploid mlo mutants otherwise exhibit due to pleiotropic effects on leaf senescence and cellular homeostasis. This finding has provided an entirely new theoretical basis for the synergistic improvement of both powdery mildew resistance and high grain yield, guiding future editing strategies to include specific deletions of regulatory flanking regions [61] rather than being limited to coding sequence indels. In maize, the landmark VPM-0008 line developed by Pioneer Hi-Bred International (now part of Corteva Agriscience) and approved by the US FDA in February 2026 for food and feed use is a multi-disease resistance powerhouse. This elite inbred line carries six different native, conventionally identified disease-resistance genes—each previously mapped through quantitative trait locus (QTL) analysis and validated—precisely introduced via sequential CRISPR-Cas9-mediated targeted insertions into a single genetic background. The stacked disease package confers high-level resistance to northern leaf blight [21] (Exserohilum turcicum), gray leaf spot (Cercospora zeae-maydis), anthracnose stalk rot (Colletotrichum graminicola), and southern rust (Puccinia polysora), dramatically reducing the need for foliar fungicide applications and representing a new paradigm for quantitative disease resistance deployment [13].

Taking the QTL-stacking concept further, a parallel effort in maize has achieved simultaneous allele replacement at three separate disease-resistance QTL intervals in a single transformation cycle using a polycistronic tRNA-gRNA array, demonstrating that true multiplex HDR-mediated allele swapping is now feasible in a major cereal. In barley, multiplex editing of all three HvMLO homoeologs, combined with targeted insertion of the multi-pathogen resistance gene Ta-Lr34res at a defined intergenic safe-harbour locus, has yielded lines with durable resistance to powdery mildew, leaf rust, and spot blotch, without the senescence-associated yield drag historically linked to mlo mutations. These advances collectively signal a shift from single-gene resistancecassettes that mimic the quantitative, durable protection provided by polygenic resistance in natural germplasm, but assembled in a fraction of the time and without linkage drag. Figure 2 Showing Schematics of gene-editing strategies to confer stress tolerance. (A) Drought Tolerance: Knockout of the negative regulator OsERA1 enhances ABA signaling, leading to drought-induced stomatal closure without a yield penalty under normal conditions. (B) Salinity Tolerance: Promoter editing of OsHKT1;5 to upregulate its expression

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

in root xylem parenchyma, enhancing sodium retrieval from the xylem stream and protecting photosynthetic tissues. (C) Disease Resistance: Targeted disruption of the effector-binding element in the promoter of the susceptibility gene OsSWEET14, preventing Xanthomonas oryzae pv. oryzae TAL effector binding and conferring bacterial blight resistance [17,3].

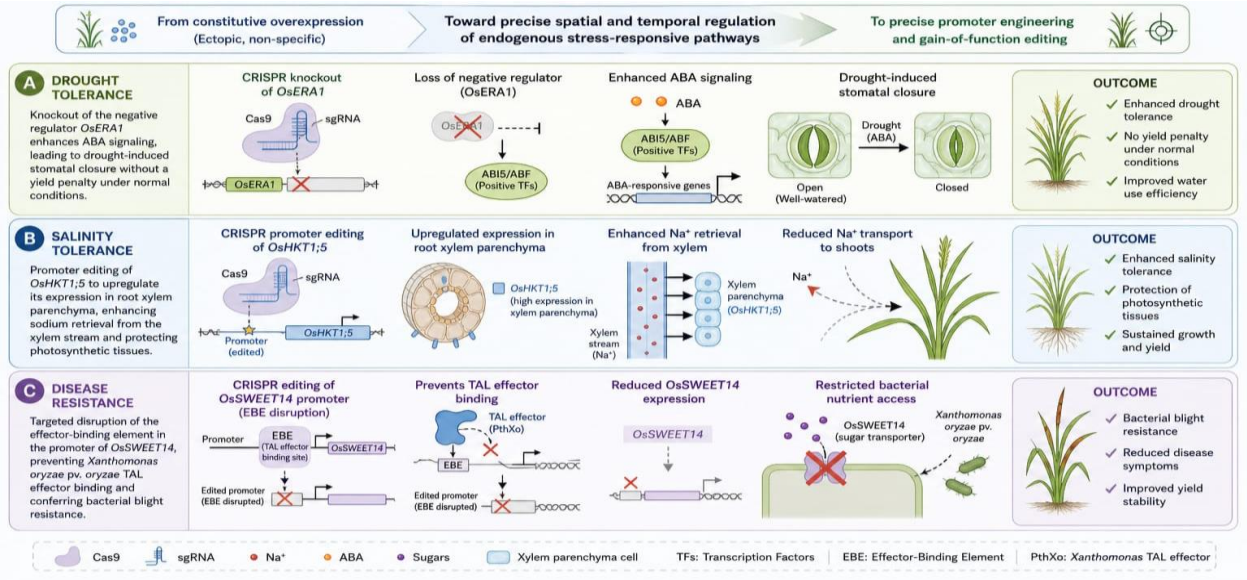


Figure 2: CRISPER Mediated Strategies for Stress Resilience

4. Harnessing the Phytobiome: The Microbial Dimension of Crop Strength

4.1 The Rhizosphere and Endosphere as Extended Plant Genomes

4.1.1 The Plant Holobiont: Reconceptualising the Crop as a Symbiotic Consortium

The conceptual framework of the plant "holobiont" has fundamentally reconfigured our understanding of crop biology, recognizing that a plant is not an autonomous organism but rather a complex, co-evolving symbiotic consortium whose phenotype is the product of the combined genetic potential of the plant host and its entire associated microbiota—bacteria, archaea, fungi, protists, and viruses [8]. The rhizosphere, the minuscule zone of soil directly influenced by root secretions and extending only 1–3 millimeters from the root surface, hosts microbial community densities orders of magnitude greater than bulk soil, functioning as a true "extended genome" that contributes vast metabolic capabilities the plant lacks [62,63] from its own evolutionary heritage. These capabilities include nitrogen fixation, phosphate and potassium solubilization from recalcitrant soil minerals, production of phytohormones (auxins, cytokinins, gibberellins) that shape root architecture, and the synthesis of a diverse arsenal of antimicrobial and volatile organic compounds. The holobiont concept has gained rigorous experimental support from large-scale metagenomic and metatranscriptomic surveys—such as the Earth Microbiome Project and the Global Crop Holobiont Initiative—which have revealed that the rhizosphere microbiome adds an estimated 10^3 to 10^4 unique gene families to the functional repertoire of the combined holobiont, vastly exceeding the genetic diversity present in the plant nuclear genome [64]. Importantly, the holobiont is now understood to be heritable to a significant degree: genome-wide association studies in maize, rice, and sorghum have identified specific plant loci that shape the abundance of individual microbial taxa, providing a mechanistic basis for the observation that particular rhizosphere community profiles can be selectively bred or edited through manipulation of host genes involved in root exudation, cell wall composition, and immune signalling. This insight is catalysing a

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ISSN: (e) 3007-1607 (p) 3007-1593

shift from viewing microbiome traits as purely environmental toward regarding them as extended, heritable phenotypes amenable toboth predictive modelling and deliberate genetic improvement [24].

4.1.2 Microbial Mediators of Stress Resilience: ISR, Nutrient Mobilisation, and Ethylene Modulation

The mechanisms underlying microbiome-mediated stress tolerance are exceptionally diverse and include the well-characterized phenomenon of induced systemic resistance (ISR), in which beneficial rhizobacteria (typically Pseudomonas, Bacillus, or Trichoderma spp.) prime the plant's innate immune system through jasmonic acid and ethylene signaling pathways, resulting in a faster, stronger, and more sustained defense response upon subsequent pathogen attack, without the continuous costly activation of immunity [65,22]. Nutrient solubilization is another critical service; phosphate-solubilizing bacteria release organic acids and phosphatases to liberate bound phosphate, while potassium-solubilizing microorganisms release organic acid anions that chelate mineral surfaces. Furthermore, bacteria expressing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzymatically degrade the ethylene precursor ACC exuded by roots under stress, lowering stress-induced ethylene peaks and alleviating the growth-inhibitory effects of salinity, drought, and flooding [66]. The composition and functional capacity of these microbial consortia are profoundly shaped by the plant's genotype, its dynamic profile of root exudates, the soil type and management history, and prevailing environmental conditions, yet their combined contribution is now understood to be absolutely essential for plant health, nutrient acquisition, and productivity.

Recent advances in synthetic biology have begun to harness these mechanisms in a targeted fashion: individual beneficial strains have been engineered to overexpress ACC deaminase, antimicrobial lipopeptides, or phosphate-mobilising enzymes, and then reintroduced into therhizosphere as designer probiotics with demonstrably improved stress-protective phenotypes in field-grown tomato, wheat, and rice [67]. Parallel efforts are now focusing on constructing simplified, defined synthetic microbial communities (SynComs) that recapitulate the core stress-protective functions of a natural rhizosphere consortiumwhile being reproducible and amenable to industrial formulation. Equally compelling, CRISPR-based editing of plant genes that control exudate composition—such as the ABCG37 transporter directing coumarin secretion or the F3'H flavonoid pathway genes—can predictably shift the relative abundance of beneficial versus pathogenic rhizosphere taxa,offering a host-mediated route to deliberate microbiome engineering. The convergence of holobiont theory, synthetic microbial biology, and host genome editing is giving rise to a new paradigm of "extended phenotype breeding" [80] in which the combined genetic resources of plant and microbiome are simultaneously optimised to achieve durable stress tolerance and nutrient efficiency in low-input agricultural systems.

4.2 Engineering the Microbiome: From Probiotics to Synthetic Communities (SynComs)

4.2.1 Single-Strain Inoculants: The Rhizobium Legacy and the Non-Legume Bottleneck

Direct inoculation with single-strain plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) has been commercialized for decades as biofertilizers, biostimulants, and biopesticides. The classic success story remains Rhizobium-based inoculants for legumes, which form highly specific root nodule symbioses fixing atmospheric nitrogen, reducing the need for synthetic nitrogen fertilizers in soybean, bean, alfalfa, and chickpea cultivation globally [23]. However, for the vast majority of non-leguminous crops.

The economic and environmental impact of rhizobial inoculants is substantial: in Brazil alone, the annual application of elite Bradyrhizobium strains to over 35 million hectares of soybean saves an estimated US$15–20 billion in nitrogen fertiliser costs [23] and avoids millions of tonnes of greenhouse gas emissions from industrial Haber Bosch nitrogen production. Building on this success, free-living nitrogen-fixing bacteria such as

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ISSN: (e) 3007-1607 (p) 3007-1593

Azospirillum brasilense and Azotobacter vinelandii have been developed as inoculants for non-leguminous cereals, with yield increases of 5–15% documented in maize and wheat under low-nitrogen conditions, though their contribution rarely matches the efficiency of symbiotic fixation.

4.2.2 Synthetic Microbial Communities (SynComs): Rational Design and Functional Resilience

Synthetic microbial communities (SynComs) represent a more sophisticated approach: rationally designed, defined, multispecies consortia of 520 functionally complementary microbes selected for functional robustness and predictable benefits [67]. SynComs offer functional redundancy (if one strain fails, another can perform the same function), greater resilience to environmental fluctuations, and the ability to occupy multiple ecological niches. For example, late blightsuppressive SynComs have been developed for potato, and saltstress protection has been achieved using SynComs combining Pseudomonas and Bacillus species [68]. However, SynComs face practical challenges: complex formulation, difficulties in scaling production, and limited understanding of strain invasion resistance (the ability of introduced strains to establish and persist in the native community).

Beyond these examples, drought-protective SynComs comprising carefully balanced Actinobacteria, Proteobacteria, and Firmicutes isolates have been shown to enhance root biomass and grain yield in wheat under terminal drought, while nitrogen-mobilising SynComs for maize, built from a core set of eight Klebsiella, Enterobacter, and Herbaspirillum strains, have achieved fertiliser replacement values of 30–50 kg N ha^{-1} in multi-location field trials. The design principles underlying these consortia have matured rapidly: high-throughput co-cultivation assays and metabolic network models now allow researchers to predict inter-strain interactions—cross-feeding, competition, and niche partitioning—before assembly, minimisingincompatibility and maximising functional synergy. Furthermore, genome-resolved metagenomics of natural disease-suppressive soils is being mined to identify the minimal keystone taxa sufficient to reconstitute suppressiveness in disease-conducive soils, providing an ecologically rational route to SynCom formulation [24].

4.3 Host Mediated Microbiome Engineering

4.3.1 Engineering Root Exudation to Selectively Recruit Beneficial Microbiomes

Host mediated microbiome engineering represents a frontier strategy that bypasses the need for repeated inoculation. By manipulating plant root exudate profiles through CRISPR edited transporter genes, it is possible to selectively recruit beneficial microbiome members—creating an artificially selected "cryforhelp" strategy [69]. For example, editing an ABC transporter to alter the secretion of specific coumarins or flavonoids can selectively feed beneficial microbes that have evolved to utilize those compounds as carbon sources. The clearest mechanistic demonstration comes from the ABCG37 transporter in Arabidopsis and its orthologues in crops: knockout or promoter-edited alleles of this gene drastically alter the profile of secreted coumarins, especially scopoletin and its derivatives, which are potent chemoattractants for specific Pseudomonas and Streptomycesstrains [69] with well-characterised antifungal properties. In rice, targeted editing of the OsABCG50 transporter has been shown to enrich Sphingomonas and Massilia spp. in the rhizosphere under salt stress, while in tomato, modification of the flavonoid-producing SlF3'H pathway shifts the root microbiome composition toward Bacillus spp. that induce systemic resistance against wilt pathogens [71]. The underlying principle is that plants naturally secrete blends of primary and secondary metabolites when under biotic or abiotic stress—a phenomenon termed the "cry-for-help" response—and that precise editing can amplify this signal, converting a transient stress-induced exudation into a of protective microbiota [70] without the need for external inoculation.

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4.3.2 Trade-Offs, Ecological Safety, and the Maize Benzoxazinoid Proof-of-Concept

This "selffertilizing" strategy requires no ongoing farmer investment and offers high specificity, as the recruitment mechanism is tailored to the target microbial taxa. However, metabolic tradeoffs may reduce yield if the synthesis of signaling compounds diverts resources from growth, and unintended effects on native microbial communities require careful ecological assessment. Editing maize benzoxazinoid pathways to shape the rhizosphere microbiome represents a proofofconcept for this strategy. Benzoxazinoids (BXs), such as DIMBOA and MBOA, are specialised defensive metabolites secreted by maize, wheat, and rye roots that profoundly shape the below-ground microbiota. CRISPR-mediated knockout of the key biosynthetic gene Bx1 (encoding indole-3-glycerol phosphate lyase) in maize completely abolishes BX production, resulting in a dramatically altered rhizosphere community with a sharp reduction in beneficial Methylophilaceae and an increase in pathogenic Fusarium, confirming the central role of BXs in assembling a protective microbiome. Critically, field trials with Bx1 CRISPR knockout lines have demonstrated that the loss ofBXs increases disease severity and reduces yield under pathogen pressure, but also that constitutively elevated BX secretion engineered through promoter edits can enhance beneficial microbial recruitment without the yield penalty observed when metabolites are fully blocked—a nuanced outcome that distinguishes pathway knockout from precision expression tuning.

Table 2: Comparative Analysis of Microbiome Engineering Strategies for Crop Resilience

Strategy	Mechanism	Advantages	Limitations	Example
Single-Strain Inoculant	Introducing a known PGPR or biocontrol agent (e.g., Bacillus subtilis)	Simple formulation; well-established regulatory pathway	Inconsistent field performance; often outcompeted by native microbiota	Rhizobium-based biofertilizers for legumes
Synthetic Community (SynCom)	Designed consortium of 5–20 functionally complementary microbes	Functional redundancy; greater resilience to environmental fluctuations; occupies multiple ecological niches	Complex formulation; difficult to scale; invasion resistance not fully understood	Late blight-suppressive SynCom for potato
Host-Mediated Recruitment	CRISPR-based editing of plant root exudates to selectively recruit beneficial microbes	"Self-fertilizing" system; no repeated inoculation required; high specificity	Possible metabolic trade-offs; may affect yield; unintended shifts in native microbiome	Editing maize benzoxazinoid pathways to shape rhizosphere microbiome
Microbiome Transplantation	Transfer of entire rhizosphere microbiome from stress-tolerant donor to susceptible plant	Transfers complex, co-evolved microbial networks; can provide multi-trait resilience	Risk of pathogen transfer; inconsistent results; challenge in donor selection	FMT-inspired approach applied to disease-suppressive soils

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5. OMICs-Driven Target Discovery for Next-Generation Traits

5.1 Genomics and Pan-Genomics for Allele Mining

5.1.1 Pan-Genome Assembly: Unlocking the Dispensable Genome for Stress-Resistance Allele Discovery

The transition from single, linear reference genomes to comprehensive pan-genome assemblies has fundamentally and irreversibly expanded the genetic diversity accessible for crop improvement, moving beyond the narrow slice captured in a single individual. A pan-genome systematically captures the full genetic repertoire of a species—including the "core genome" shared by all individuals and the "dispensable genome" comprising presence/absence variations (PAVs), copy number variations (CNVs), and structural variants that are absent from any single reference genome. Critically, many agronomically vital stress-resistance genes, particularly those encoding nucleotide-binding leucine-rich repeat (NLR) receptors and other defense-related proteins, reside in the dispensable genome and are subject to rapid birth-and-death evolution, making them invisible to traditional single-reference genotyping and GWAS approaches. The scale of the hidden genetic diversity revealed by pan-genomics is striking: in rice, a super pan-genome constructed from 3010 diverse accessions uncovered over 90,000 novel genes absent from the Nipponbare reference, including thousands of novel NLR and receptor-like kinase (RLK) alleles; in barley, 54,000 previously undescribed genes were identified across 20 genotypes; in tomato, a pan-genome constructed from 725 phylogenetically diverse accessions revealed a dispensable gene pool of over 4,000 genes enriched for disease-resistance and stress-tolerance functions. The enabling technology behind this explosion is the combination of long-read (PacBio HiFi, Oxford Nanopore) sequencing withchromosome-conformation capture (Hi-C), which together permit the assembly of gapless, phased, telomere-to-telomere pan-genomes, even for large and complex polyploid genomes.

5.1.2 From Discovery to Field: Pan-Genome-Informed Editing in Banana, Wheat, and Soybean

In banana, a crop with an extremely narrow cultivated gene pool due to millennia of vegetative propagation, pan-genome analysis comparing wild Musa acuminata and Musa balbisiana accessions with diverse cultivars identified multiple functional susceptibility genes whose products are hijacked by the soilborne fungus Fusarium oxysporum , Fusarium cubense tropical race 4 (TR4), the most devastating pathogen threatening global banana production. These susceptibility genes were found to be conserved across the pan-genome but exhibited flanking structural variation that could be exploited for editing. By targeting these genes through multiplex CRISPR-Cas9 knockout, researchers have successfully developed Cavendish banana lines that exhibit high-level, heritable resistance to TR4, a pathogen that has spread to over 20 countries and for which no effective chemical or biological control exists. These edited lines have now progressed to contained field trials in multiple locations, representing a potential lifeline for the global export banana industry. Similarly, in wheat, pan-genome analyses of diverse bread wheat and wild emmer accessions have revealed novel alleles of drought-responsive transcription factors and high-affinity nitrate transporters that were entirely absent from the Chinese Spring reference genome, opening new avenues for introgressing or de novo domestication of climate-resilient traits. In soybean, pan-genome analysis uncovered large structural variants near the Rhg1 locus conferring soybean cyst nematode resistance, with some haplotypes harboring tandem duplications of resistance genes that can be precisely introgressed using CRISPR-mediated targeted insertion. Beyond these single-crop examples, pan-genome-informed allele mining is now being systematised through the creation of “trait-associated dispensable gene” (TADG) databases that compile PAV-linked agronomic traits across multiple species, allowing breeders to query whether a phenotype of interest (e.g.,waterlogging tolerance, micronutrient density) is associated with dispensable genome

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variation in related germplasm. The integration of pan-genomics with high-throughput phenotyping platforms—drones, hyperspectral imaging, and automated greenhouse systems—has already accelerated the functional validation of candidate dispensable genes, reducing thegene-discovery-to-edited-plant cycle to under two years for some traits in rice.

5.2 Transcriptomics and Network Biology

5.2.1 Transcriptomics and Network Biology: From Co-Expression Modules to Master Regulatory Hubs

RNA sequencing (RNA-seq) has become a high-resolution, quantitative tool that, when integrated with sophisticated network biology algorithms such as weighted gene co-expression network analysis (WGCNA), identifies hub genes that act as high-level master regulators coordinating entire suites of downstream stress-responsive genes. Unlike single-gene knockout or overexpression, which often yields negligible or pleiotropic phenotypes due to network robustness, targeting these master regulatory hubs through precise promoter editing allows for the modular tuning of entire adaptive responses without completely abolishing basal functions. WGCNA constructs co-expression modules—clusters of genes with correlated expression patterns across hundreds of stress conditions and developmental stages—and identifies the central, most highly connected "hub" genes within each module, which are statistically most predictive of the module's overall behavior. These hub genes provide exceptionally high-priority targets for CRISPR-mediated intervention, as a single edit in a hub promoter can shift the expression trajectory of dozens to hundreds of downstream effectors simultaneously. A landmark example in maize drought tolerance is the transcription factor ARGOS8, which was identified through transcriptomic profiling of developing ears under water-deficit stress. ARGOS8 negatively regulates ethylene signaling, promoting cell division and sink strength under stress. However, its native promoter drives low-level expression. Using a homology-directed repair (HDR)-based promoter swap strategy, researchers replaced the native ARGOS8 promoter with the strong, moderately drought-responsive maize GOS2 promoter. The resulting edited maize lines exhibited elevated ARGOS8 expression specifically during reproductive development under drought, leading to increased grain yield of 5–10 bushels per acre [72] (~0.3–0.6 t/ha) under field drought stress conditions, without a detectable yield penalty under fully watered conditions. This case exemplifies how transcriptomics-guided targeting of regulatory hubs, combined with precise promoter engineering, can achieve tangible agronomic gains even for complex polygenic traits like drought tolerance. Extending this approach, single-cell RNA-seq (scRNA-seq) is now beginning to dissect stress responses at unprecedented cellular resolution in plants, identifying rare cell-type-specific regulators of root endodermal suberization, stomatal lineage decisions, and vascular differentiation that represent next-generation editing targets for abiotic stress resilience [9].

5.3 Metabolomics and Phenomics for Functional Validation

5.3.1 Metabolomics and Phenomics for Functional Validation: From Metabolite Effectors to Field-Ready Performance

High-throughput metabolic profiling using liquid chromatography-mass spectrometry (LC-MS) for semi-polar and polar metabolites, and gas chromatography-mass spectrometry (GC-MS) for volatile and primary metabolites, has become an indispensable functional validation layer in the omics pipeline. Metabolomics detects and quantifies the end products of gene expression and cellular regulation, directly identifying the key stress-responsive metabolites that serve as biochemical effectors of tolerance. These include osmolytes such as proline, glycine betaine, trehalose, and polyamines [73] that protect protein structures and maintain cellular turgor under osmotic stress; flavonoids and anthocyanins that act as radical-scavenging antioxidants and UV shields; and specific defense-related phytoalexins and benzoxazinoids that directly inhibit pathogen growth. By linking metabolite accumulation to genomic variation through metabolome genome-

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wide association studies (mGWAS), researchers can pinpoint the precise genes and regulatory variants controlling metabolite abundance. A seminal example is the identification of the SlGAD2 gene controlling γ-aminobutyric acid (GABA) accumulation in tomato fruit through mGWAS [4]. GABA is a health-promoting compound with blood-pressure-lowering effects, but its levels in commercial tomato cultivars are modest. mGWAS revealed that a specific C-terminal autoinhibitory domain in the glutamate decarboxylase SlGAD2 enzyme limits its activity. CRISPR-Cas9 was used to create a targeted gain-of-function deletion in this autoinhibitory domain, relieving autoinhibition without altering the gene's coding frame, and resulting in a 4–5-fold increase in fruit GABA content. This edited line was commercialized in Japan in 2021 as the "Sicilian Rouge High GABA" tomato, representing the world's first direct-to-consumer CRISPR-edited food [4]. Beyond individual metabolites, untargeted metabolomics is now being integrated with machine learning to predict whole-plant stress tolerance phenotypes from leaf metabolite fingerprints, accelerating the selection of edited lines with desirable metabolic profiles before resource-intensive field trials commence.

5.3.2 High-Throughput Phenomics: Bridging Genome Edits to Field Performance

Automated, high-throughput phenotyping platforms represent the critical bridge linking genomic edits to real-world performance. These platforms employ drone-based multispectral, hyperspectral, and thermal imaging to non-invasively quantify a wide array of physiological and growth parameters across thousands of small-plot entries under real field conditions. Measured parameters include the normalized difference vegetation index (NDVI) as a proxy for canopy greenness and photosynthetic capacity, canopy temperature depression as an indicator of stomatal conductance and water stress, and derived parameters such as water use efficiency (WUE), leaf area index, and early vigor. These systems can survey entire field trials in minutes, generating time-series data that capture the temporal dynamics of stress adaptation, rather than relying on single end-of-season destructive harvests. This dramatic acceleration of phenotyping throughput removes the phenotyping bottleneck that has historically constrained the rate of genetic gain [19], enabling the rapid screening and selection of the most promising edited lines from large populations of transformation events, and facilitating the direct correlation of genotype-edit combinations with yield stability under target environmental scenarios. The integration of phenomics with genomic and metabolomic data in multi-omic prediction models is now enabling the "genomic selection of edits," where the phenotypic consequences of a proposed edit are predicted in silico before any laboratory work begins [28].

Table 3: Landmark CRISPR-Engineered Crop Traits and Field Performance

Crop	Target Trait	Gene(s) Edited	Discovery Method	Strategy	Key Phenotypic / Field Data	Status
Rice	Bacterial blight resistance	OsSWEET14 (promoter)	TAL effector genomics	Pathogen susceptibility factor knockout	Broad-spectrum resistance to >95% of tested Xoo strains; no growth penalty	Field trials, pre-commercial
Wheat	Powdery mildew resistance	TaMLO-A1, B1, D1	Comparative genomics	Multiplex gene knockout	Complete and durable resistance; no negative	Greenhouse, field trials

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					pleiotropic effects	
Tomato	High GABA content	SlGAD2 (C-terminal domain)	Metabolomics / mGWAS	Gain-of-function deletion	4–5 fold increase in GABA levels; commercialized as Sicilian Rouge in Japan	Commercialized (2021)
Maize	Drought tolerance	ARGOS8 (promoter)	Transcriptomics (stress-responsive)	Promoter swap (HDR)	Increased grain yield under drought stress by 5–10 bushels/acre	Field trials (Phase 2)
Banana	Fusarium wilt (TR4) resistance	Multiple susceptibility genes	Pan-genome analysis	Multiplex gene knockout	Confirmed resistance to Panama disease TR4 strain in Cavendish	Contained field trials

6. Overcoming Bottlenecks: Delivery, Specificity, and Regeneration

6.1 The Tissue Culture and Genotype Dependency Barrier

6.1.1 The Genotype Dependency Barrier: Elite Cereal Recalcitrance to In Vitro Regeneration

The recalcitrance of elite commercial varieties, especially monocot cereals such as maize, wheat, sorghum, and barley, to in vitro regeneration remains the single most significant operational bottleneck limiting the application of genome editing across the full spectrum of crop diversity. The core problem is not the molecular editing event itself—which can be highly efficient, often exceeding 50–80% in protoplast or immature embryo systems—but rather the subsequent and indispensable need to regenerate intact, fertile whole plants from the small number of cells that have been successfully edited, a process that is profoundly genotype-dependent and often fails entirely in those very elite genetic backgrounds most urgently needed for commercial impact. The discovery and application of morphogenic regulators (MRs) has brought about a genuine paradigm shift in addressing this bottleneck. The ectopic co-expression of the maize developmental transcription factors BABY BOOM (BBM), an AP2/ERF family member, and WUSCHEL2 (WUS2), a homeodomain protein, induces a powerful somatic embryogenesis program from vegetative cells, effectively bypassing the stringent developmental requirements that normally restrict competence for regeneration to specific explant tissues and developmental windows. This system has enabled "genotype-independent" transformation in a widening array of previously recalcitrant species and elite inbred lines, drastically shortening the timeline from construct design to whole-plant recovery from often over 12 months to as little as 3–6 months [52].

6.1.2 Morphogenic Regulators and Transient Delivery: BBM/WUS2, GRF-GIF, and the Path to Tissue-Culture-Free Editing

Building on this foundation, the GRF-GIF chimera—a direct fusion protein combining the GROWTH-REGULATING FACTOR 4 (GRF4) with its essential transcriptional cofactor GRF-INTERACTING FACTOR 1 (GIF1)—has demonstrated further improved regeneration efficiency across multiple cereal crops, including maize, wheat, rice, and triticale. The GRF4-GIF1 chimera works through a distinct pathway from BBM/WUS2, promoting cell proliferation and shoot meristem specification, and can be deployed singly or synergistically with other MRs. Viral-

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mediated delivery of these morphogenic regulators, using deconstructed RNA viruses as transient expression vectors, has further enhanced transformation efficiency, achieving 75–79% embryonic calli formation in historically recalcitrant sorghum lines, all without the integration of any exogenous DNA. The ultimate frontier goal is to eliminate the need for sterile tissue culture entirely—delivering genome editing reagents directly to meristematic cells within the intact plant and recovering non-chimeric, edited shoots that can be clonally propagated or enter the breeding pipeline immediately [54]. Approaches under active investigation include nanoparticle-mediated delivery of RNPs to shoot apical meristems, pollen tube-mediated transformation, and in planta haploid induction coupled with genome editing, but achieving high efficiency and heritable edits without a tissue culture phase remains an ambitious, unsolved challenge.

Table 4: Key CRISPR Toolbox Components and Their Applications in Crop Science

Tool / Component	Origin / Type	Mechanism	Primary Application in Crops	Limitations
SpCas9	Streptococcus pyogenes	DSB induction at NGG PAM sites	Standard gene knockouts, multiplex editing	PAM restriction; large size (4.1 kb) limits some viral vector delivery
Cas12a (Cpf1)	Acidaminococcus / Lachnospiraceae	DSB with staggered ends; T-rich PAM (TTTV)	Efficient editing in AT-rich genomes; multiplex editing via crRNA arrays	Less characterized than SpCas9; variable target efficiency
Base Editors (ABE/CBE)	Fusion protein: nCas9 + deaminase	C→T or A→G conversion without double-strand breaks	Gene knockout via stop codons; amino acid substitution for herbicide tolerance	Narrow editing window; bystander mutations; cannot perform transversions
Prime Editors (PE2/PE3)	Fusion: nCas9 + reverse transcriptase	Precise insertions, deletions, and all base substitutions	Precise allele replacement; regulatory motif editing in promoters	Low efficiency in many crops; complex pegRNA design
CRISPRa / CRISPRi	dCas9 + transcriptional activator/repressor	Epigenetic regulation without DNA cleavage	Activation of defense genes; study of essential genes without knockout	Transient effects; possible off-target transcriptional changes
Cas12f (CasΦ)	Hyperthermophilic archaea	Miniaturized nuclease; T-rich PAM	Potential for compact delivery systems;	Early-stage development; limited in planta optimization

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expands
targeting range

6.2 Efficient and DNA-Free Delivery Systems

6.2.1 Nanoparticle-Mediated RNP Delivery: Mesoporous Silica, Carbon Nanotubes, and the Promise of DNA-Free Editing

Nanotechnology offers revolutionary potential to bypass the tissue culture barrier entirely, enabling the direct delivery of genome editing cargo into intact plant cells through passive or facilitated penetration of the formidable plant cell wall. Mesoporous silica nanoparticles (MSNs), characterized by tunable pore sizes and surface chemistries, and carbon nanotubes (CNTs), with their high aspect ratios and ability to traverse lipid bilayers, have been demonstrated to passively deliver adsorbed or encapsulated ribonucleoprotein (RNP) complexes—pre-assembled Cas9 protein and sgRNA—through the plant cell wall and plasma membrane into the cytoplasm and nucleus [55], without the aid of biolistic bombardment or biological vectors. This RNP delivery modality offers multiple decisive advantages that align perfectly with both technical and regulatory objectives: absolutely no transgene integration into the host genome, the most rapid possible editing kinetics (as the pre-formed RNP complex is catalytically active immediately upon cytoplasmic or nuclear delivery, unencumbered by transcription and translation delays), and significantly reduced off-target editing due to the inherently transient intracellular lifetime of the protein and RNA components, which are degraded within hours by endogenous proteases and nucleases. However, the field is still in its early stages: the delivery efficiency of RNP nanoparticles into whole plant tissues, as opposed to protoplasts or isolated embryos, remains too low for routine practical use, and the vast majority of successful demonstrations are still confined to protoplast systems, which themselves require regeneration through tissue culture. Recent advances include the use of anionic polymers and specifically designed zwitterionic lipids to stabilize Cas9 RNP nanoparticles in physiological conditions, improving both the colloidal stability during delivery and the editing efficiencies obtained in protoplast and suspension cell systems, providing a proof-of-principle for further optimization in intact tissues.

6.2.2 Viral Vectors for Systemic sgRNA Delivery: Deconstructed RNA Viruses, High-Throughput Somatic Editing, and the Meristem Exclusion Challenge

In a complementary approach, plant RNA viruses have been engineered as highly efficient systemic delivery vectors for genome editing components, exploiting their natural ability to move through plasmodesmata and the phloem to infect entire plants. Deconstructed RNA viruses, including tobacco rattle virus (TRV) for dicots and foxtail mosaic virus (FoMV) for monocots, have been modified to carry sgRNA expression cassettes, which, upon systemic infection of transgenic Cas9-expressing plants, achieve somatic editing in newly developing leaves, stems, and roots without the need for stable transformation of each guide construct. This enables high-throughput, multiplexed gene editing screens directly in vegetative tissues, dramatically accelerating gene function validation and trait optimization. The approach can also achieve heritable edits if the viral replicon reaches meristematic cells, though meristem exclusion is a common viral restriction. However, a critical size limitation exists: viral capsids or movement proteins cannot accommodate the large Cas9 coding sequence (~4.2 kb) itself, meaning that this strategy inherently requires pre-existing transgenic Cas9 lines, which must be removed by backcrossing to achieve transgene-free status in the final product, adding time and complexity.

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6.3 Off-Target Mitigation and Specificity Validation

6.3.1 Engineering CRISPR Nucleases for Enhanced Fidelity and PAM Flexibility

Ensuring the absolute specificity of genome edits is paramount for both regulatory approval and public trust, driving extensive protein engineering efforts to improve CRISPR nuclease fidelity. Moving beyond wild-type SpCas9, researchers have developed highly versatile PAM-flexible variants, such as the near-PAMless SpRY nuclease, which can target almost any genomic sequence, thereby removing the major PAM constraint that previously limited access to many regulatory regions and small genes. Concurrently, a suite of high-fidelity variants—including HiFiCas9, eSpCas9 (1.1), and SpCas9-HF1—have been engineered through structure-guided substitutions (e.g., altering non-specific DNA contacts) to dramatically reduce off-target activity while maintaining or even improving on-target editing efficiency, achieving specificity enhancements of 10- to 100-fold compared to the wild-type enzyme. Beyond these SpCas9 derivatives, other high-fidelity platforms have emerged from alternative CRISPR systems: LbCas12a-HF and enAsCas12a-HF1 offer comparable fidelity improvements for T-rich PAM targeting, while the engineered Cas12f variants (e.g., Un1Cas12f1 and AsCas12f-HF) combine small size with enhanced specificity. A recent computational breakthrough, the development of deep-learning-based off-target prediction tools such as CRISPRspec and MOFF, now allows researchers to predict genome-wide off-target profiles with high accuracy for any Cas9 variant, enabling the selection of guide RNAs that minimise off-target risk in silico before any experiment begins. Moreover, new split-Cas9 systems, where the nuclease is divided into two inactive fragments that only become catalytically competent upon dimerisation at the target site, provide an additional layer of spatial and temporal specificity control, particularly valuable when editing is coupled to viral delivery or tissue-specific promoters.

6.3.2 Orthogonal Validation of Editing Specificity: Whole-Genome Sequencing and Enzymatic Off-Target Detection

For transgene-free edited lines, particularly those of clonally propagated crops where backcrossing is not an option, comprehensive whole-genome sequencing (WGS) at high coverage provides the most definitive and unbiased method for identifying any off-target mutations, including structural variants and large deletions. Complementarily, Digenome-seq, an in vitro method where purified genomic DNA is treated with Cas9-sgRNA RNP, then subjected to whole-genome sequencing to identify all cleavable sites genome-wide, offers a highly sensitive and genome-wide unbiased off-target nomination that can then be validate and selectively amplifying cleaved circles, routinely detecting off-target sites at frequencies as low as 0.1%, surpassing the sensitivity of targeted amplicon sequencing. SITE-Seq (selective enrichment and identification of tagged genomic ends by sequencing) employs biotin-tagged adapters to capture cleaved genomic fragments,providing a direct, quantitative readout of Cas9 cleavage activity at both on- and off-target sites without PCR amplification bias. In parallel, CHANGE-seq uses a streamlined, low-input protocol compatible with high-throughput screening of multiple guide RNAs.

7. Scientific Milestones from Lab to Field

7.1 Biofortification: Engineering Enhanced Nutrition

7.1.1 The High-GABA Tomato: A Landmark in CRISPR-Edited Commercial Food and Streamlined Regulation

The high-GABA tomato stands as a landmark achievement and a global bellwether, representing the world's first CRISPR-edited commercial food product to reach consumers. Japanese researchers led by Hiroshi Ezura at the University of Tsukuba developed the 'Sicilian Rouge High GABA' tomato using CRISPR-Cas9 to precisely delete the autoinhibitory domain of the SlGAD2 gene, which encodes glutamate decarboxylase—the key enzyme that catalyzes the irreversible conversion of glutamate to γ-aminobutyric acid (GABA). This targeted deletion removes the

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ISSN: (e) 3007-1607 (p) 3007-1593

calcium/calmodulin-binding autoinhibitory domain at the C-terminus of the enzyme, effectively relieving feedback inhibition and resulting in a 4–5-fold constitutive accumulation of GABA, a health-promoting compound with well-documented antihypertensive effects, stress reduction potential, and possible roles in improving sleep quality. The tomato received regulatory approval from Japan's Ministry of Health, Labour and Welfare in December 2020 and was subsequently commercialized by Sanatech Seed Co. in 2021, initially through direct-to-consumer online sales and later expanding to supermarkets, with clear labeling indicating it as a genome-edited food product. Crucially, Japan's regulatory framework treats genome-edited crops as conventional if the edit does not introduce foreign DNA, requiring only a notification to the government rather than the full, costly safety approval process mandated for genetically modified organisms. This streamlined regulatory pathway facilitated rapid commercialization—moving from lab to market in under five years—and has served as a powerful model for other jurisdictions considering how to regulate precision-bred products. The success of the GABA tomato has since inspired similar biofortification projects worldwide, including tomatoes with increased lycopene, anthocyanin-enriched purple tomatoes, and rice with elevated iron and zinc content through targeted editing of metal transporter genes, demonstrating the broad applicability of CRISPR for enhancing nutritional quality.

7.1.2 Low-Gluten Wheat for Celiac Disease: Multiplex Editing of α-Gliadin Families Without Sacrificing Baking Quality

Parallel progress in low-gluten wheat has tackled the challenge of celiac disease, an autoimmune disorder affecting approximately 1% of the global population. Wheat gluten contains hundreds of immunogenic peptide epitopes, primarily from the α-gliadin, γ-gliadin, and ω-gliadin protein families, which trigger the autoimmune response in genetically susceptible individuals. Through sophisticated multiplex editing of the α-gliadin gene families, which alone comprise over 100 gene copies distributed across the A, B, and D genomes of hexaploid bread wheat, researchers have produced wheat lines with dramatically reduced immunoreactivity—up to 85% reduction in the most reactive epitopes [74]—while carefully maintaining acceptable dough mixing properties, extensibility, and baking quality, which are intrinsically dependent on the gluten protein network. The technical challenge is immense: the high copy number and sequence similarity of gliadin genes require efficient targeting of conserved regions to simultaneously silence dozens of loci without inducing large-scale chromosomal rearrangements. Moreover, eliminating all gluten proteins would destroy breadmaking quality, so the strategy focuses on specifically removing the most immunodominant epitopes while preserving the structural gliadins and glutenins that confer viscoelasticity. This work is now progressing through advanced field trials and food-safety assessments, with the goal of producing bread, pasta, and other wheat products that are safe for celiac patients without requiring the complete elimination of wheat from their diets.

7.2 Second-Generation Green Revolution Traits

7.2.1 Climate-Resilient Rice for India: Pusa DST Rice 1 and the OsCKX2 Editing Breakthrough

Climate-resilient rice edited for drought and salt tolerance has been released in India, marking a pioneering success in utilizing CRISPR-Cas9 for rapid, non-transgenic crop improvement in a developing-country context. Indian researchers identified the OsCKX2 gene, which encodes a cytokinin oxidase/dehydrogenase enzyme that degrades the plant hormone cytokinin, as a key regulator of grain number and stress tolerance. By editing the promoter and coding regions of OsCKX2 to modulate its expression, they generated a novel allele that increases grain yield by approximately 19%, allows earlier maturity by up to 20 days [10], and shows significantly better performance under low-fertilizer input and drought conditions. This variety, officially released as Pusa DST Rice 1, also exhibits improved tolerance to drought and salinity stress, a 20% reduction in greenhouse gas emissions due to

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ISSN: (e) 3007-1607 (p) 3007-1593

reduced fertilizer requirements and shorter flooding periods, and estimated national savings of 7.5 billion cubic meters of irrigation water annually when deployed across India's vast rice-growing area. The variety has shown moderate drought tolerance and sustained performance under low nitrogen and phosphorus inputs, attributable to a more robust and deeper root system architecture that enhances water and nutrient scavenging. It was developed using the popular MTU-1010 mega-variety as the recipient, ensuring that the edited line retained the grain quality, cooking characteristics, and regional adaptation that farmers already trust while adding stress resilience. This represents India's pioneering success in utilizing CRISPR-Cas9 for rapid, non-transgenic crop improvement, and has opened the floodgates for similar projects in chickpea, mustard, banana, and other crops of national importance managed by the Indian Council of Agricultural Research (ICAR) network.

7.2.2 Semi-Dwarfing Without Yield Drag: Engineering SLR1 Allelic Series for Tunable GA Response

Semi-dwarfing without the classic yield drag has been elegantly achieved through CRISPR-edited alleles of SLR1, which encodes a DELLA protein—a central repressor of gibberellic acid (GA) signaling in rice. The Green Revolution's sd1 allele (a defective GA20 oxidase) produces a constitutive reduction in bioactive GA, resulting in semi-dwarf stature but also pleiotropic effects on nitrogen responsiveness and grain filling. By directly editing the DELLA protein's GA-dependent degradation domain, researchers have created an allelic series with gradations of plant height effect [75]—from mild to severe dwarfing—without altering GA homeostasis itself. These novel SLR1 alleles confer improved lodging resistance, enhanced nitrogen use efficiency, and higher harvest index, and critically, the gradation of effects enables breeders to select the optimal balance of plant height and yield potential for specific environments, from high-input irrigated systems to rainfed lowland conditions. This demonstrates that gene editing can create precise allelic series that fine-tune quantitative traits, going far beyond simple gene knockouts to generate the genetic variation that conventional breeding has long relied upon but could never target with such precision.

7.3 De Novo Domestication of Orphan Crops

7.3.1 The De Novo Domestication Concept: Multiplex Editing of Domestication Genes in Orphan Species

De novo domestication of orphan crops represents a paradigm shift in plant breeding, using multiplex gene editing to rapidly transform wild, weedy, or semi-domesticated species into productive, modern crops within a few generations. The approach targets the small number of "domestication genes"—typically those controlling plant architecture, seed shattering, fruit size, day-length sensitivity, and germination—that distinguish wild progenitors from their cultivated descendants. In effect, the rational domestication pipeline condenses thousands of years of unconscious human selection into a handful of precise, simultaneous edits. The core gene toolkit first validated in the Solanaceae is now being systematically translated across diverse orphan species: loss-of-function edits of Shattering1 (Sh1) and qSH1 orthologues prevent seed dispersal in orphan cereals; targeted disruption of TFL1 homologues converts indeterminate to determinate growth; editing of FT and CEN family genes reduces photoperiod sensitivity; and disruption of GIGANTEA or Ppd-1 orthologues accelerates flowering time, enabling cultivation at higher latitudes. Crucially, the availability of chromosome-scale reference genomes constructed using long-read sequencing has now placed dozens of orphan species—including Digitaria exilis (fonio), Eragrostis tef (teff), Chenopodium quinoa (quinoa), Vigna unguiculata (cowpea), and Lablab purpureus (hyacinth bean)—within reach of de novo domestication. A landmark proof-of-concept was achieved in African rice (Oryza glaberrima),where editing just four domestication loci produced lines with erect architecture, non-shattering grain, and compact panicles in a single generation [5], retaining the species' inherent heat and drought tolerance.

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ISSN: (e) 3007-1607 (p) 3007-1593

7.3.2 Groundcherry as a Pioneer: Engineering Architecture, Photoperiod Insensitivity, and Fruit Size

Groundcherry (Physalis pruinosa), a Solanaceae species related to tomato and producing edible, sweet-tart fruit encased in a papery husk, was used as the pioneering test case. Despite its appealing flavor, groundcherry exhibits undesirable domestication traits: a sprawling, indeterminate growth habit, small fruit size, and strong day-length sensitivity. Researchers first developed comprehensive genomic resources, an annotated reference genome, and an efficient Agrobacterium-mediated transformation system for groundcherry, then used multiplex CRISPR-Cas9 to simultaneously edit the orthologs of well-characterized tomato domestication genes: SP (SELF-PRUNING) to confer determinate, compact growth habit; SP5G to confer photoperiod insensitivity, enabling flowering across a wider range of latitudes and seasons; and CLV1 (CLAVATA1) and its downstream effectors to increase fruit size by expanding the meristematic cell population. The resulting edited lines exhibited a dramatically transformed architecture: compact, bushy plants with synchronous fruit set, significantly larger fruit, and the ability to be cultivated in large-scale, mechanized agricultural production systems [76]. Beyond groundcherry, this de novo domestication approach has enormous potential for accelerating the domestication of climate-resilient "orphan" species that are locally important food sources but have historically lacked the investment of major commodity crops—candidates include teff, fonio, quinoa, cowpea, and various wild legumes and tubers that possess natural heat or drought tolerance but require only a handful of genetic changes to achieve the agronomic characteristics required for modern farming.

Building on this foundation, a second round of editing introduced mutations in the GGP1 (GDP-L-galactose phosphorylase) promoter to elevate ascorbic acid (vitamin C) content, and in MYB transcription factors to deepen anthocyanin pigmentation, enhancing both nutritional quality and visual appeal. A parallelresearch consortium in China has now achieved similar architectural conversion in another Physalis species, P. angulata, while a US-based team has extended the approach to Solanum ptycanthum (eastern black nightshade), an edible wild relative of tomato, producing determinate,day-length-neutral lines with fruit size increased three-fold. Field evaluation of edited groundcherry across multiple latitudes in North America confirmed stable photoperiod insensitivity and compact habit, while greenhouse cultivation trials demonstrated compatibility with high-density vertical farming systems.

8. Redesigning Plant Architecture for Next-Level Resource Use

8.1 Synthetic Biology for Enhanced Photosynthesis

8.1.1 Engineering Photorespiratory Bypasses to Recover Lost Carbon and Energy

Photorespiration—the energetically wasteful process by which the enzyme Rubisco oxygenates ribulose-1,5-bisphosphate (RuBP) instead of carboxylating it, generating the toxic intermediate 2-phosphoglycolate that must be recycled through a multi-organelle pathway—represents a significant and inherent inefficiency in C3 crops such as rice, wheat, and soybean. Under the high-temperature and drought conditions that are becoming increasingly prevalent with climate change, Rubisco's specificity for CO_2 over O_2 declines, and photorespiration can divert up to 30% or more of fixed carbon away from productive metabolism, representing a massive unrealized yield potential. Synthetic biology approaches have engineered novel photorespiratory bypass pathways that shortcut the native pathway, metabolizing the toxic glycolate entirely within the chloroplast or peroxisome by introducing a small set of heterologous enzymes from bacteria or algae, thereby recovering carbon dioxide and reducing energy (ATP and NADPH) loss. These engineered bypass pathways have achieved biomass increases of >40% in model systems [77] such as Nicotiana tabacum, a result widely hailed as a "holy grail" in plant synthetic biology. However, translating these remarkable laboratory results to stable, heritable yield increases under real field conditions in food crops remains immensely challenging: the

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ISSN: (e) 3007-1607 (p) 3007-1593

introduced pathways may impose metabolic burden, interact unpredictably with endogenous metabolism, or fail to perform optimally under fluctuating light, temperature, and water availability. Researchers are now using iterative design-build-test-learn cycles, guided by kinetic metabolic modeling and directed evolution of the introduced enzymes, to optimize the bypass pathway performance specifically in monocot and dicot crop backgrounds.

8.1.2 The C_4 Rice Moonshot: Anatomical and Biochemical Rewiring of Central Photosynthesis

Engineering the full C4 photosynthetic pathway into C3 rice is the long-term, ambitious moonshot goal of the international C4 Rice Consortium, involving laboratories across Asia, Australia, Europe, and North America. C4 crops—maize, sorghum, sugarcane, and millets—possess a biophysical CO_2-concentrating mechanism that compartmentalizes the initial carbon fixation step into mesophyll cells using phosphoenolpyruvate carboxylase (PEPC), which has no oxygenase activity, and subsequently concentrates CO_2 around Rubisco in the specialized bundle sheath chloroplasts, effectively suppressing photorespiration and enabling higher photosynthetic efficiency, especially under high temperature and water stress. Converting rice to a C4 plant would theoretically increase radiation-use efficiency by 30–50% [78] and improve water-use and nitrogen-use efficiencies simultaneously. Realizing this requires engineering two parallel and interdependent systems: anatomical changes, including the development of Kranz anatomy characterized by enlarged, chloroplast-rich bundle sheath cells intimately surrounded by mesophyll cells, and biochemical specialization, requiring cell-specific expression of the full C4 cycle enzyme suite (PEPC, PPDK, NADP-ME or NAD-ME, and associated metabolite transporters). While full C4 engineering remains beyond current capabilities, the consortium has made critical progress in identifying the genetic regulators of bundle sheath specification and chloroplast dimorphism through comparative transcriptomics of C3 and C4 leaves, developing computational models to predict the minimal genetic changes required for a functional C4 cycle, and building experimental pipelines to validate candidate regulators in rice and the C4 model grass Setaria viridis. Recent parallel work has also used CRISPR-Cas9 to precisely edit the promoters of OsNF-YC4, a gene involved in nitrogen signaling in rice, leading to increased grain protein content and decreased carbohydrate content, demonstrating that even subtle rewiring of central metabolism through genome editing can produce nutritionally significant trait shifts without requiring the massive architectural changes of full C4 conversion.

8.2 Optimizing Plant Architecture for High-Density Farming

8.2.1 Smart Canopy Ideotype: Erect Leaves and Enhanced Light Interception

The "smart canopy" ideotype is a conceptual plant architecture designed to maximize light interception and whole-canopy photosynthetic efficiency under the high-density planting systems that are increasingly necessary as arable land shrinks. CRISPR editing of genes controlling leaf angle—specifically LIGULELESS1 (LG1) and its interacting partners in maize—creates erectophile leaves with an upright, vertical orientation. Erect leaves allow incident sunlight to penetrate deeper into the lower layers of the canopy, reducing the shading of lower leaves and distributing light more evenly across the entire photosynthetic surface. This improves whole-canopy carbon assimilation because the upper leaves, receiving saturating light, can no longer increase photosynthesis, while the previously shaded lower leaves transition from the light-limited to the light-saturated regime. Erect leaf architecture also facilitates closer row spacing and higher planting densities—potentially increasing plant populations per hectare by 30–50%—thereby increasing total yield per unit land area without altering per-plant yield. Recent field evaluations of CRISPR-edited LG1 maize lines have confirmed yield advantages of 8–12% at densities of 90,000–100,000 plants ha [79]$^{-1}$, compared to the standard 65,000–70,000 plants ha^{-1}, with no lodging penalty. The erect leaf trait has now been successfully introduced into diverse heterotic groups using edits in regulatory regions of LG1 that modulate ligule developmentwithout

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abolishing it entirely, preserving mechanical support while achieving a 15–25° reduction in leaf angle. In rice, parallel editing of OsLG1 and the OsSPL14 (IPA1) locus has combined erect leaves with improved panicle architecture, yielding a "dual-purpose" ideotype suited for ultra-high-density transplantation.

8.2.2 Tassel Reduction and Root Architecture for Harvest Index and Resource Efficiency

Beyond leaf angle, tassel branching genes such as RAMOSA1 have been edited to reduce tassel size and branch number, reallocating photosynthate and nutrients away from excessive pollen production and toward grain-filling, increasing harvest index without compromising the pollen supply needed for successful fertilization. Additional architectural targets include genes controlling root angle and depth, enabling the development of "steep, cheap, and deep" root ideotypes that access water and mobile nutrients from deeper soil horizons, conferring both drought and nitrogen-use efficiency. Editing RAMOSA1 and the related RAMOSA2 and RAMOSA3 genes in elite maize inbreds has produced tassels with 40–60% fewer branches while maintaining pollen viability above the threshold required for full seed set in hybrid production, translating to a harvest index increase of 3–5 percentage points. This reduction in tassel mass also improves lightpenetration to the upper leaves of the canopy during anthesis, further contributing to grain yield. For root architecture, precise editing of DRO1 (DEEPER ROOTING 1) orthologues in maize and wheat has successfully steepened root angles by 10–15° relative to the soil surface, while editing of OsNAC5 in rice has increased root diameter and xylem vessel area, improvinghydraulic conductance under drought. A particularly exciting advance is the development of "steep, cheap, and deep" ideotypes through multiplex editing: combining a steep root angle allele with reduced root cortical aerenchyma (via RCA gene editing, reducing metabolic cost per unit root length) and enhanced root hair density (via RSL gene promoter edits) has produced maize lines with superior nitrogen capture from deep soil layers and a 20% reduction in optimal nitrogen fertiliser requirement in field trials. The integration of these above-ground and below-ground architectural edits into a single genetic background—a "complete ideotype" line—has now been demonstrated in rice, with the simultaneous presence of erect leaves, reduced tassel equivalent (panicle branching optimised for grain number), and deeper roots yielding a 25% increase in grain yield under combined high-density planting and moderate drought stress. These results underscore that the rational, multi-target editing of plant architecture genes is one of the most tractable and high-impact applications of CRISPR for closing the yield gap in major cereals.

8.3 Speed Breeding and Gene Editing Synergy

8.3.1 Speed Breeding and CRISPR: Accelerating Generation Cycles and Trait Stacking in Polyploid Crops

Speed breeding—the use of precisely controlled environment conditions including extended photoperiods of 20–22 hours, optimized light spectra with high red-to-far-red ratios, precise temperature regimes, and high-density planting—dramatically accelerates plant life cycles, slashing generation times to as little as 6–8 weeks for wheat and barley, enabling 4–6 complete generations per year, compared to just 1–2 generations per year in field conditions or 2–3 in traditional heated greenhouses. When integrated with CRISPR genome editing, speed breeding creates a powerful synergy that dramatically accelerates the entire trait development pipeline. After an initial editing event, speed breeding enables the rapid fixation of the edited allele through self-fertilization or backcrossing, the rapid trait stacking of multiple independent edits into a single elite line through marker-assisted rapid-cycle crossing, and the early evaluation of the introgressed edited trait in homogeneous, homozygous genetic backgrounds for robust phenotyping.

8.3.2 Democratizing Speed Breeding: Overcoming Capital Barriers for Public and Developing-Country Programs

This synergy is particularly transformative for polyploid crops like hexaploid wheat and

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tetraploid potato, where conventional breeding is inherently slow due to complex genetics, the need to fix alleles across multiple subgenomes, and lengthy seed-to-seed cycles. For instance, a trait requiring three independent edits on different subgenomes, each followed by a backcross into an elite background, could take 15–20 years conventionally but can be compressed to 4–6 years using speed breeding coupled with multiplex editing. However, speed breeding demands significant capital investment in controlled environment facilities with LED lighting, precise climate control, and automation, creating an accessibility gap that currently limits the adoption of this technology by public breeding programs in developing countries and smaller private entities. Efforts are underway to establish low-cost speed breeding protocols using locally fabricated growth chambers and solar-powered lighting to democratize the technology.

9. The Global Regulatory and Ethical Landscape

9.1 The Product-vs-Process Regulatory Divide

9.1.1 The United States and Canada: Null Segregant Logic and Product-Based Exemptions

The United States' "null segregant" approach—treating SDN-1 and SDN-2 edits as equivalent to conventionally bred crops when the final product contains no foreign DNA and the trait could theoretically be achieved through natural or induced mutation—represents the most permissive and product-focused regulatory model. Under the USDA's SECURE rule (Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient), a genome-edited crop is exempt from biotechnology regulations if (1) no recombinant or foreign DNA remains in the final plant product, and (2) the introduced modification could otherwise have been achieved through conventional breeding or mutagenesis. This product-based, case-by-case approach has enabled the commercialization of several CRISPR-edited crops, including high-oleic soybeans and Calyno high-oleic canola, without the extensive, multi-year regulatory burden and associated costs of transgenic deregulation. Canada has officially declared through Health Canada that targeted edits to a plant's endogenous genome, creating no novel combination of genetic material, do not create a "novel trait" and are therefore not subject to the pre-market notification and safety assessment regulations that apply to GMOs. However, Canada's approach notably differs from the US in affirming that the initial transient presence of recombinant DNA (such as the Cas9 transgene) during the editing process does not automatically trigger regulation, as long as the final commercial product contains no foreign DNA.

9.1.2 Latin America: Argentina's Pioneering Resolution 173/2015 and Regional Harmonization

In the Americas, Argentina pioneered a product-based regulatory approach through Resolution 173/2015, which established a framework that assesses the final product's characteristics and the nature of the genetic change rather than the method used to create it—a model subsequently adopted by Brazil, Chile, Colombia, Costa Rica, Guatemala, and several other Latin American countries under the umbrella of the Southern Agricultural Council (CAS). Argentina's system allows developers to consult early in the product design phase, receiving a regulatory classification confirmation based on a dossier describing the edit, requiring only confirmation that no foreign DNA is present in the final product and that the edit does not generate a new combination of genetic material. Remarkably, Brazil and Argentina mutually recognize each other's regulatory assessments, effectively creating a regional common market for genome-edited products and dramatically shortening commercialization timelines across Latin America. By 2025, Argentina had evaluated 28 geneedited microbes for agricultural use and approved 86 transgenic events across five major crops.

9.1.3 The European Union and United Kingdom: The Two-Tier NGT Agreement and the Precision Breeding Act

The European Union has historically been the most restrictive jurisdiction. However, in December 2025, EU lawmakers reached a provisional trilogue agreement on plants developed using new genomic techniques (NGTs).

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The agreement, endorsed by member states on December 19, 2025, and by the Environment and Food Safety Committee on January 28, 2026, establishes a twotier system: NGT1 plants (those with modifications comparable to those achievable through conventional breeding) would face reduced regulatory burdens, while NGT2 plants (those with more complex modifications) would remain subject to existing GMO regulations. The agreement also confirms that NGT plants and plant material can be patented, preserving the patentability essential for industry investment and commercialization. The full regulation is expected to enter into force in 2028 [14]. The UK, which departed from the EU regulatory framework, implemented the Precision Breeding Act, which came into effect on November 13, 2025. The first crop to receive a marketing notice confirmation under this pathway was a CRISPRedited highlipid barley developed at Rothamsted Research, approved on April 13, 2026—the first such approval in a major European economy [12].

9.2 Ethical Stewardship and Global Equitability

9.2.1 Intellectual Property, Patent Thickets, and Emerging Access Models

Intellectual property and patent thickets remain significant barriers to equitable access. The foundational CRISPR/Cas9 patent (IN397884) is held by ERS/CVC, and licensing terms have been contentious. However, alternative governance models are emerging: ICAR's 2025 patent for TnpBbased plant genome editing—a smaller, potentially more easily delivered editing system—represents a new indigenous editing platform for Indian agriculture. Proposals for technology licensing frameworks aim to ensure equitable access [27] for wheat breeding and other crops of importance to global food security, reducing inequalities in agricultural innovation. Beyond India, several other developing nations are actively building their own CRISPR platforms to circumvent restrictive patent landscapes: China's numerous patents on Cas12a and Cas12b variants, and the Ethiopian Biotechnology Institute's work on teff-specific editing tools, exemplify a broader trend of "innovative sovereignty."

9.2.2 Societal License, Consumer Benefits, and Transparent Communication

The societal license to operate for genomeedited crops depends on transparent communication of consumerrelevant benefits. The Sicilian Rouge High GABA tomato succeeded in the Japanese market because it delivered a clear, tangible health benefit (stress reduction and blood pressure management) that consumers valued. In contrast, crops edited solely for agronomic traits (herbicide tolerance, yield) have been less visible to consumers and may not generate the same public acceptance. Transparency in regulatory processes and public communication remains essential.: Consumer research across multiple countries has consistently shown that acceptance increases significantly when gene-edited products provide direct health or environmental benefits—such as reduced mycotoxin contamination, enhanced vitamin content, or lower allergenic potential—rather than solely farmer-centric advantages.

In this context, the development of "consumer-facing" edited products, including hypoallergenic peanuts, reduced-acrylamide potatoes, and high-fiber white bread wheat, is strategically critical for building a reservoir of public trust. Equally important is the role of transparent labelling: countries with mandatory but factual labelling (e.g., Japan's "genome-edited" label) haveexperienced less consumer backlash than those with ambiguous or alarmist labelling frameworks.

9.3 Environmental Risk Assessment and Post Market Monitoring

9.3.1 Gene Flow, Biocontainment, and Wild Relative Interactions

Evaluating potential gene flow from edited traits to wild relatives is a critical component of environmental risk assessment. For crops with wild relatives in the same geographic region—such as rice, where wild Oryza species are native to Asia and Africa—the escape of alleles conferring herbicide tolerance or stress resistance could alter the ecological dynamics of wild populations. Strategies for biocontainment include plastid transformation (since plastids are generally maternally inherited), male sterility, and the use of

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genetic use restriction technologies (GURTs), though the latter have been controversial. More recently, "transgene-delete" systems and autonomous excision cassettes have been developed that remove the editing machinery and any linked selectable markers before flowering, effectively leaving only the intended edit in the reproductive tissues and drastically reducing the probability oftransgene flow. For gene-edited traits themselves, the risk assessment must evaluate whether the edited allele, if introgressed into a wild gene pool, confers a selective advantage that could increase invasiveness; early data from weedy rice populations edited for stress tolerance suggest that while some alleles (e.g., submergence tolerance) couldtheoretically enhance fitness in flood-prone habitats, most yield-optimising edits carry trade-offs that reduce competitiveness in unmanaged environments.

9.3.2 Ecological Impact of Microbiome Engineering: SynComs and Soil Biodiversity

Longterm ecological studies on the impact of microbiome engineering strategies on native soil biodiversity are largely lacking. The introduction of SynComs, even rationally designed consortia, may displace native microbial communities or alter biogeochemical cycles in ways not captured by shortterm studies. Standardized protocols for assessing microbiome impacts, including metagenomic sequencing and functional assays of soil processes, are needed to establish safety guidelines. To address this gap, several long-term field observatories—such as the European Ecotron facilities and the US National Ecological Observatory Network (NEON)—have begun multi-year trials tracking SynCom persistence, horizontal gene transfer to native microbiota, and shifts in nutrient cycling rates following repeated inoculationEarly results indicate that while most introduced strains decline to below detection within one growing season, certain SynCom members can persist at low abundance for multiple years, particularly in the rhizoplane, and that repeated season-on-season application produces cumulative shifts in the relative abundance of specific native bacterial phyla, includingAcidobacteria and Verrucomicrobia.Figure 3 showing that A comparative overview of genetic modification strategies across three eras of plant biotechnology. (Panel A) Transgenesis: A vector (e.g., plasmid) carrying a gene of interest from a distantly related or unrelated species, a selectable marker, and regulatory elements is integrated randomly into the plant genome via Agrobacterium; screened for single-copy, high-expression events. (Panel B) Cisgenesis/Intragenesis: The introduced gene cassette is sourced entirely from the plant's own or a cross-compatible gene pool; while the insertion event is still random, the genetic boundaries are respected. (Panel C) CRISPR-Cas9 Precision Breeding: A ribonucleoprotein (RNP) complex or DNA construct is delivered transiently. The endogenous gene is targeted with nucleotide precision, creating a small deletion or SNP that recapitulates a naturally occurring beneficial allele. The final selected product can be "null-segregant"—carrying the desired edit but completely free of any foreign DNA.

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ISSN: (e) 3007-1607 (p) 3007-1593

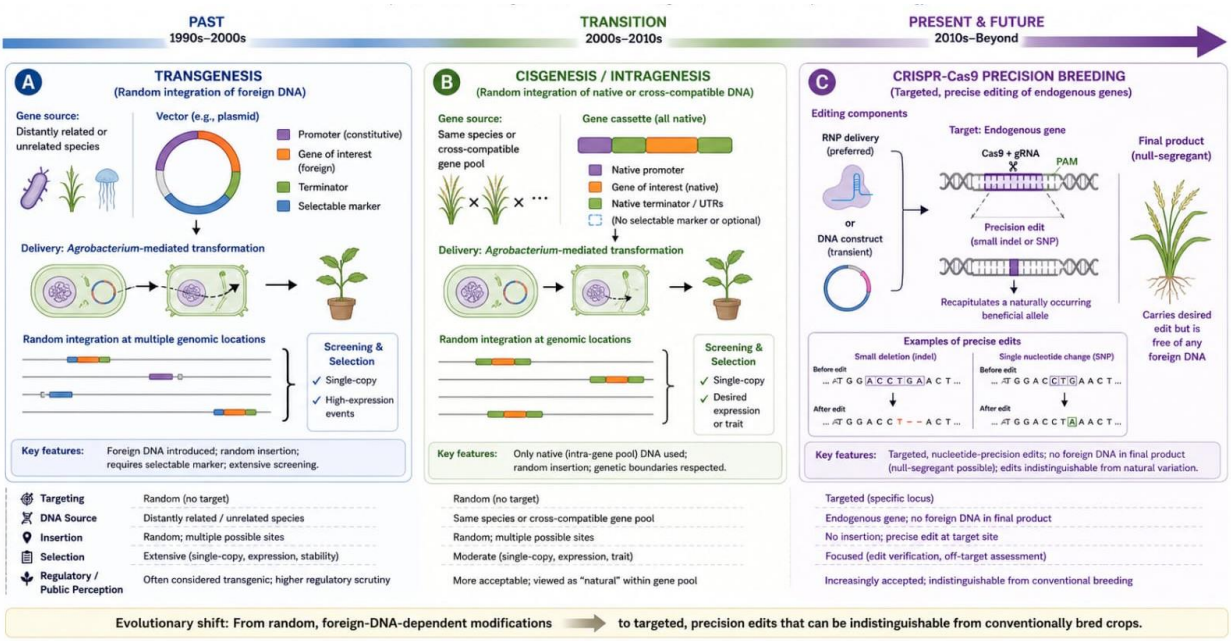


Figure 3: The Evaluation of Plant Genetic Modification from Transgenic to precision Editing

10. Challenges, Future Frontiers, and the Path to 2050

10.1 Technical Grand Challenges

10.1.1 Multiplex Editing of Polygenic QTLs: Efficiency, Fidelity, and Epistatic Complexity

The transition from single-gene knockouts to the rational multiplex editing of polygenic quantitative trait loci (QTLs) that control complex yield and resilience traits remains one of the foremost technical frontiers in crop biotechnology. Most agronomically critical characteristics—grain yield under drought, nitrogen use efficiency, heat tolerance during reproductive development, and broad-spectrum disease resistance—are governed by dozens to hundreds of small-effect QTLs, each contributing a modest fraction of the overall phenotypic variance. While editing multiple loci simultaneously is now technically feasible using polycistronic tRNA-gRNA arrays, Csy4 ribonuclease systems, or viral vector-delivered guide RNA libraries, the overall efficiency and fidelity decline sharply as the number of simultaneous targets increases, due to competition for the Cas nuclease, increased likelihood of off-target cleavage, and the difficulty of delivering all components at effective concentrations to the same cell. Moreover, the combinatorial interactions among edited alleles in polygenic backgrounds are inherently unpredictable; favourable alleles at individual loci often exhibit non-additive epistatic effects, and stacking them blindly can result in negative phenotypic outcomes such as reduced vigour or unintended developmental defects. Addressing this challenge requires not only technical improvements in multiplex editing efficiency but also the development of predictive genetic models—potentially trained on large-scale genome-to-phenotype datasets—that can simulate the combined effect of multiple edited alleles in silico before any laboratory work begins, thereby prioritizing the most promising allele combinations for empirical testing [7].

10.1.2 mRNA Stability and In Vivo Delivery for Transgene-Free Editing Across Diverse Crops

RNA stability and in vivo delivery for mRNA-based editing systems constitute another formidable barrier to achieving efficient, transgene-free genome editing across diverse crop species. Messenger RNA is inherently labile in biological environments due to ubiquitous RNases, and its large size and negative charge

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ISSN: (e) 3007-1607 (p) 3007-1593

present substantial obstacles to cellular uptake through the rigid polysaccharide plant cell wall. Although synthetic mRNAs incorporating modified nucleosides such as pseudouridine and 5-methylcytidine to increase stability and reduce immunogenicity, along with nanoparticle encapsulation in lipid or polymer carriers to facilitate wall penetration, have shown promise in animal systems and isolated plant protoplasts, translation of these approaches to whole-plant tissues remains extremely difficult. The additional barrier of the nuclear envelope for delivery of mRNA that must be translated in the cytoplasm and then imported as protein or re-imported as RNP into the nucleus compounds the challenge. While some progress has been made using cell-penetrating peptides and carbon nanotube carriers, the field still lacks a robust, broadly applicable method for mRNA-based transient editing that avoids both tissue culture and transgene integration entirely—a capability that would dramatically accelerate breeding timelines.

10.1.3 Orphan Crop Investment: Genomic Resources and De Novo Domestication for Food Security

Orphan crop investment requires a deliberate and sustained shift in research and development funding priorities, away from the small number of major commodity crops (maize, rice, soybean, wheat) that currently dominate both public and private sector breeding investment, and toward the diverse array of climate-resilient orphan crops—including finger millet, teff, cassava, yam, cowpea, pigeonpea, groundcherry, fonio, and various neglected legume and tuber species—that are essential for regional food security, nutritional diversity, and livelihood resilience in developing countries, particularly across sub-Saharan Africa and South Asia. These crops have historically lacked even basic genomic resources, let alone optimized transformation and regeneration protocols, making them inaccessible to modern breeding technologies. The de novo domestication approach, which uses multiplex CRISPR editing to simultaneously modify a relatively small number of key domestication genes controlling shattering, growth habit, photoperiod sensitivity, and organ size, holds exceptional promise for rapidly transforming wild or semi-domesticated orphan species into productive, high-value crops without the centuries-long domestication bottlenecks faced by ancient farmers. However, fully realizing this promise demands substantial upfront investment in reference-grade genome assemblies, pan-genomes, transformation systems, speed breeding infrastructure, and locally led breeding networks that are currently absent for almost all orphan crops [19]. International research consortia, development agencies, and national agricultural research systems will need to coordinate these investments to ensure that the benefits of precision breeding reach the crops and communities that need them most. Figure is showing that A holistic workflow for developing and deploying a novel CRISPR-edited crop variety. The pipeline commences with Target Gene Identification via bioinformatics (GWAS, pan-genomes). The Design & Validation phase involves sgRNA construction, protoplast transient assays, and whole-plant transformation. At T0, edited events are screened, and transgene-lines are selected. Field Trials span confined environmental release (T2-T3) to multi-location agronomic performance testing (T4-T5). The endpoint highlights the final stages of Deregulation & Seed Multiplication, leading to the Distribution of a market-ready, "stronger" product. Crucially, the diagram shows a feedback loop from field performance data back to the design phase for iterative improvement.

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

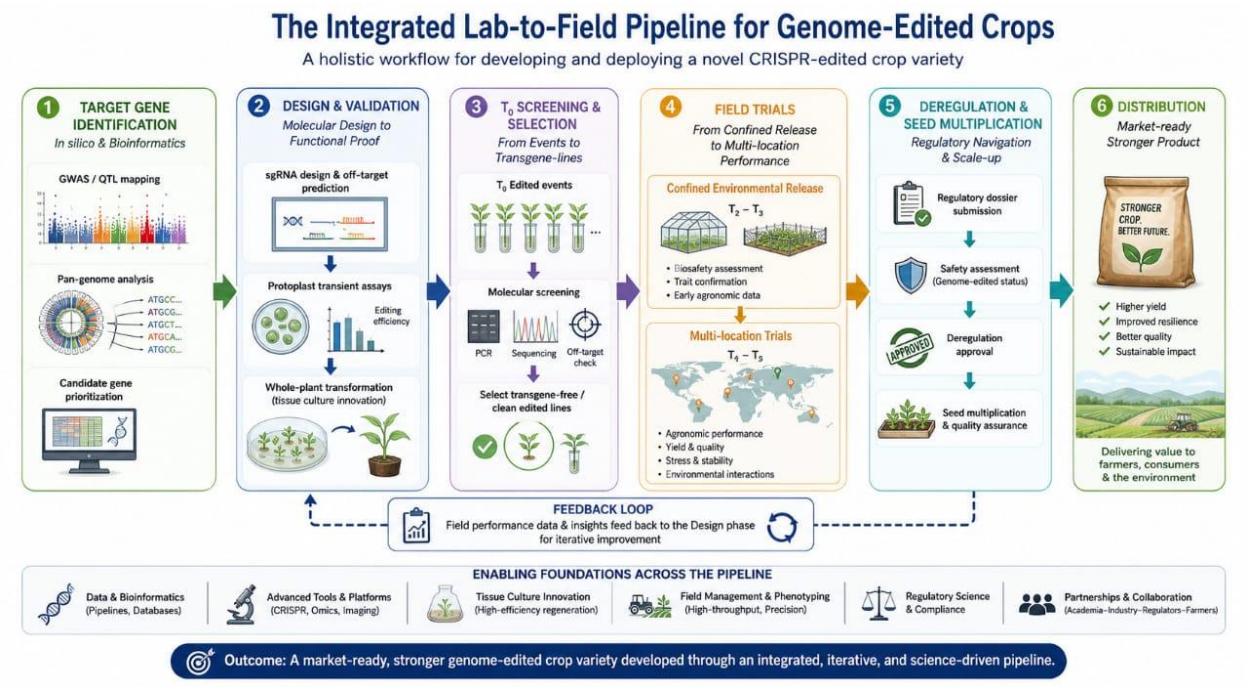


Figure 4. The integrated Lab to Field Pipeline for Genome Edited Crops

10.2 Convergence Technologies: AI and Synthetic Biology

10.2.1 AI-Driven De Novo Design of Next-Generation CRISPR Nucleases for Plant-Specific Applications.

Artificial intelligence-driven protein design is now fundamentally transforming the development of next-generation CRISPR nucleases, moving beyond the natural diversity of known systems into the de novo creation of tailor-made enzymes with properties optimized for plant-specific applications. Deep learning models such as AlphaFold3, RFdiffusion, and ProteinMPNN can now generate completely novel protein folds and active sites, designing Cas-like nucleases from scratch that are smaller, PAM-free, and exhibit orders-of-magnitude higher specificity [18] than any naturally occurring CRISPR effector. These AI-designed nucleases can be optimized for the unique cellular environments of different crop species—accounting for intracellular pH, ion concentrations, and the chromatin landscape—and can be engineered to operate with minimal off-target activity even in large, repetitive genomes. Additionally, machine learning models trained on massive datasets of experimentally determined on- and off-target cleavage sites can now predict off-target risk profiles for any given sgRNA with accuracy approaching that of empirical validation, dramatically reducing the screening burden and accelerating regulatory dossier preparation. However, the translation from a computationally designed protein sequence to a functional, stable, efficiently expressed enzyme in a plant cell remains a significant gap, as current protein design tools are largely trained on bacterial and mammalian expression data, and validation in plant systems with codon optimization, subcellular targeting, and developmental toxicity assessment lags considerably behind the rapid pace of computational innovation.

10.2.2 Digital Twins of Crops: Integrating Genomic Prediction, Crop Simulation, and Environmental Data for In Silico Pre-Screening

The concept of "digital twins" of crops—comprehensive, dynamic computational models that integrate genome-scale metabolic models, structural plant models, and environmental response functions to simulate the growth,

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ISSN: (e) 3007-1607 (p) 3007-1593

development, and yield of a specific genotype in a specific location under projected climate conditions—promises to fundamentally reshape the breeding pipeline. These in silico crop models would predict the optimal combination of genome edits, agronomic practices, and even microbiome augmentation for a given environment, entirely before a single seed is planted. Advanced digital twin platforms for maize and soybean are already under active development, integrating genomic prediction models, process-based crop simulation models such as APSIM and DSSAT, and high-resolution soil and weather data layers. The goal is to compress the variety development timeline from the current 8–10 field seasons to just 3–4, by pre-screening millions of possible edit combinations in silico and advancing only the most promising virtual candidates to greenhouse and field validation. However, the immense complexity of genotype-by-environment-by-management interactions, the incomplete understanding of many fundamental physiological processes at the molecular level, and the current lack of high-quality, open-access phenotype datasets linking specific allelic states to field performance across diverse environments remain substantial barriers. Addressing these gaps will require large-scale, collaborative data collection efforts, standardized phenotyping protocols, and continued investment in the fundamental plant biology that underpins model parameterization.

10.3 The Vision: Climate-Smart Agriculture as a Service

10.3.1 Seed as a Service: Integrating Genome-Edited Germplasm with Season-Specific SynCom Coatings

The future paradigm of "seed as a service" envisions a highly integrated agricultural input system in which elite, genome-edited germplasm is delivered to farmers together with a customized, season-specific coating of synthetic microbial communities (SynComs) that are precisely formulated based on the predicted environmental challenges of the coming growing season. In this model, a farmer in a drought-prone district would receive seeds of a drought-optimized edited variety, whose surface carries a rationally designed consortium of ACC deaminase-producing bacteria for ethylene stress mitigation, phosphate-solubilizing fungi for improved nutrient acquisition under dry soil conditions, and a protective layer of biofilm-forming bacteria that prime systemic immunity against the specific fungal pathogens forecasted for that region. The seed company would assemble this optimized genotype-plus-microbiome package annually, integrating seasonal climate forecasts (now reliable 4–6 weeks ahead), high-resolution soil microbiome maps, and local disease surveillance data. This approach would shift crop management from reactive measures—applying irrigation or fungicides when stress is already apparent—to proactive, pre-deployed resilience, maximizing the probability of a stable yield under increasingly unpredictable conditions.

Pilot demonstrations of this concept are already emerging: in Kenya, a public-private partnership is testing drought-tolerant edited maize varieties coated with a defined SynCom of desiccation-tolerant Bacillus and Pseudomonas strains, with the coating formulation adjusted monthly based on sub-seasonal rainfall forecasts.In Australia, predictive models combining soil moisture sensors, El Niño–Southern Oscillation (ENSO) forecasts, and field-level disease risk maps are being used to determine the optimal microbial consortia for coating canola and wheat seed, with early results showing a 10–15% reduction in yield variability under erratic rainfall.

10.3.2 Realizing the Vision: Interdisciplinary Coordination, Technological Timelines, and Governance

Realizing this vision demands an unprecedented degree of coordination across disciplines: the integration of sub-seasonal-to-seasonal climate forecasting with soil metagenomics, microbial ecology, plant breeding, genome editing, seed coating technology, and supply chain logistics. While the enabling technologies are advancing rapidly, and the increasing precision of probabilistic seasonal climate forecasts makes a 2040–2050 timeframe plausible for prototype deployment, critical governance questions

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regarding data ownership, equitable access, and the potential for corporate consolidation of the seed-microbiome interface will need to be addressed proactively to ensure that this next agricultural revolution benefits all farmers, not just those in high-income markets. A central governance challenge concerns the ownership and control of the massive datasets—soil microbiome profiles, farm-level disease incidence, and local weather—required to formulate the SynCom coatings. If these data are held exclusively by a small number of multinational seed companies, the "seed as a service" model could create a new dependency on proprietary inputs that is even more binding than the current seed-chemical package. To counter this, proposals for farmer-owned data cooperatives and open-access, publicly curated soil health databases are gaining traction in policy discussions at the FAO and the African Union's Seed and Biotechnology Programme. Another emerging concern is the potential for SynCom formulations to be patented as trade secrets, limiting the ability of national research systems to adapt coatings to local conditions; some experts advocate for a "mandatory deposit" model in which commercial SynCom consortia must be catalogued in a public biorepository for independent safety and efficacy testing. Equally important is the development of decentralized, low-cost formulation infrastructure—portable lyophilisation units and locally produced carrier substrates—so that low-income countries are not dependent on imported coated seed but can produce their own season-specific inoculants using regionally adapted microbial strains. The future of climate-smart agriculture as a service thus hinges not only on scientific breakthroughs but on the deliberate construction of inclusive governance architectures that embed equity, transparency, and local capacity-building into the very fabric of the emerging seed-microbiome delivery system.

Table 5: Current Technological Limitations and Emerging Solutions in Crop Gene Editing

Limitation	Description	Emerging Solution
Regeneration Bottleneck	Most elite crop varieties are recalcitrant to tissue culture, preventing efficient gene delivery and recovery of edited plants	Use of morphogenic regulators (GRF–GIF chimeras, WUS2/BBM) enabling selection-free and genotype-flexible plant regeneration
HDR Efficiency	Precise knock-in via homologous recombination is extremely inefficient in somatic plant cells	Use of geminiviral replicons (GVRs) to increase donor template copy number; Cas9–Rad51 fusion to enhance HDR over NHEJ
Multiplexing Capacity	Engineering complex multi-gene traits is difficult due to limited simultaneous editing capacity	tRNA–gRNA polycistronic systems and viral vector-delivered gRNA arrays enabling simultaneous targeting of up to ~10 loci
Transgene Elimination	Stable integration of Cas9 raises regulatory concerns and increases off-target risks	DNA-free editing using RNP or AuNP complexes; transient Cas9 expression during embryogenesis followed by genetic segregation

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Polyploidy	Editing all homoeoalleles in complex genomes (e.g., wheat, potato) is challenging	High-activity, PAM-flexible editors targeting conserved sequences; optimized sgRNA design for multi-allele recognition
Microbiome Consortia Stability	Synthetic microbial communities (SynComs) often fail in field conditions due to competition with native soil microbiota	Encapsulation in biodegradable nanocarriers and creation of protected nutrient niches for introduced microbes

11. Conclusion

The journey from the rudimentary transgenesis of the 1990s to a holistic, systems-level approach that now seamlessly integrates precision gene editing, multi-OMICs-informed target discovery, and rational microbiome engineering has fundamentally transformed plant biotechnology over the past two decades. Cisgenic blight-resistant potatoes, CRISPR-edited high-GABA tomatoes, and multiplex-edited disease-resistant maize have set critical commercial precedents, demonstrating that precision-bred crops can successfully navigate regulatory pathways, reach consumer markets, and deliver tangible health and environmental benefits. Yet these products represent only the first wave of what is now possible. The next frontier lies in harnessing the full complexity of the phytobiome as an extended and engineerable genome, in constructing entirely synthetic metabolic pathways for enhanced photosynthesis and nitrogen fixation, and in deploying artificial intelligence to design novel biological parts, regulatory circuits, and even whole-plant ideotypes de novo. The landmark regulatory approvals achieved in the 2025–2026 period—including India's release of genome-edited drought-tolerant rice varieties, the United Kingdom's first Marketing Notice for CRISPR-edited high-lipid barley under the Precision Breeding Act, the EU's provisional agreement on a differentiated regulatory framework for New Genomic Techniques, and the US FDA's clearance of multi-disease-resistant VPM-0008 maize—collectively signal a regulatory environment that is becoming demonstrably more permissive, predictable, and science-based, providing the commercial confidence required for sustained investment in plant biotechnology innovation.

Achieving the imperative of global food security by 2050, against a backdrop of accelerating climate change, degrading soils, and increasing pressure on water and land resources, will depend not only on the accelerating power of our molecular and computational tools but also, and perhaps more critically, on the establishment of harmonized global regulations that enable innovation without compromising safety, on the promotion of open-access innovation models that democratize enabling technologies, and on a profound commitment to the ethical and equitable distribution of these transformative technologies across all nations and farming communities. Access for developing countries and smallholder farmers to the full suite of enabling platforms—CRISPR tools, genotype-independent transformation systems, speed breeding facilities, and the genomic resources for orphan crops—is every bit as critical as the technologies themselves, and failing to ensure this access would risk exacerbating existing inequalities in agricultural productivity and food sovereignty. The convergence of CRISPR precision breeding, synthetic biology-driven photosynthetic enhancement, rationally designed and ecologically informed microbial consortia, and AI-powered target discovery and phenotype prediction offers a tangible, scientifically grounded pathway to a form of climate-smart agriculture that can sustainably nourish a global population approaching 10 billion without exhausting the planetary resources upon which all agriculture ultimately depends. The defining challenge of the coming decade is not whether these technologies will work—their efficacy has already been convincingly demonstrated in laboratories and field trials around the world—but whether the political will, economic frameworks, and ethical governance

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structures can evolve with sufficient speed and foresight to keep pace with the relentless advance of the science itself.

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