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Contributions of CRISPR and Gene Editing Technologies to Plant Breeding and Biotechnology: Advances, Integrative Approaches and Future Directions

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Abstract

Crop improvement has been revolutionized by the development of genome editing technologies especially CRISPR/Cas systems via accurate, cost-effective and efficient editing of crop genomes. The paper describes the progression of conventional to current CRISPR enabled precision breeding and highlights the historical milestones and various genetic editing platforms. The paper covers recent advances: multiplexing for trait stacking, base/prime editing for precise nucleotide modifications, epigenetic editing, new Cas proteins and improvements in transport and efficiency. It covers the integration of genetic editing with other breeding approaches (genomic selection, speed breeding, high-throughput phenotyping) to speed up the generation of superior varieties. Crop case studies (Table 2) demonstrate effective edits giving disease resistance, stress tolerance and quality attributes in cereals, legumes and horticultural crops. We discuss technical barriers such as off target effects, efficiency of transformation, editing of polyploids and regulatory issues. Socio-ethical concerns and public acceptability difficulties are discussed. Finally, we highlight future research areas including improved HDR, novel delivery methods and AI-driven design and practical advice for breeders and industry. This study synthesizes existing information and gives an in-depth guide to the contribution of CRISPR to plant biotechnology and its future possibilities.

Keywords: Proteins, improvements, efficiency, polyploids, breeding

Introduction

Global agriculture is under pressure from a double whammy: to feed a rising population and to deal with climate change, new pests and diseases and less arable land. Traditional breeding has yielded numerous advancements, but it's very sluggish and not as exact [1]. Genome editing and in particular the CRISPR/Cas systems, provides a disruptive approach: targeted alterations to the DNA of plants that may add desired features without the addition of foreign DNA [2]. This has led plant biotechnology from transgenic techniques to a new age of precision breeding. With modern gene-editing techniques, plant scientists may swiftly develop new alleles or delete undesirable genes, supplementing and speeding up traditional procedures [3]. This article presents an in-depth analysis of the contribution of CRISPR and other genetic editing technologies to plant breeding and biotechnology. We summarize historical milestones, compare major editing systems and their features and discuss developments in approach [4, 5]. The discussion covers integration of genetic modification with fast breeding, genomic selection and phenotyping. Crop case studies show real-world results. Technical concerns (off-target mutations, delivery problems) and socio-ethical or regulatory barriers are discussed. Finally, future research needs and practical advice for breeders and developers are summarized [6].

Milestones in technology have marked advances in plant breeding and biotechnology (Fig. 1). For ages, breeders employed hybridization and mutagenesis (chemicals or radiation) to generate variety. Specific techniques in molecular biology in the 1980s and 1990s [7, 8]. The first site-specific nucleases were meganucleases, designed endonucleases that recognize

lengthy DNA sequences, used initially in maize in 2009 [9]. Shortly afterward, Zinc Finger Nucleases (ZFNs) were produced, proteins designed to have zinc finger DNA binding domains fused to a FokI nuclease to make double strand breaks (DSBs). Around 2009 ZFNs allowed the first targeted gene alterations in plants (e.g. in tobacco) showing precise deletion of genes [10]. Following the development of Transcription Activator-Like Effector Nucleases (TALENs), which use TAL effector proteins to bind to particular DNA regions, the first plant TALEN editing was described in 2012 (in rice). The design of TALENs was easier than ZFNs and was extensively used in early plant genome editing [11]. But then came the revolution of CRISPR / Cas9 adopted from bacterial immune systems in 2012. The first demonstration of CRISPR editing in plants was in 2013

(rice and tobacco) and since then it has been used rapidly because of its simplicity and adaptability [12]. Researchers quickly started using CRISPR/Cas9 to knock out genes in model and crop species, which resulted in things like as disease resistance and herbicide tolerance [13]. Shortly after the creation of CRISPR, the discovery and development of Base Editing (2016) and Prime Editing (2019) in mammalian systems took place and they were promptly transferred to plants in the period of 2017-2020. Base editors linked a deaminase enzyme to Cas9 nickase to enable direct, irreversible conversion of one target base into another without double-stranded breaks. Prime editors connected Cas9 nickase to a reverse transcriptase to provide a broader variety of targeted edits [14, 15].

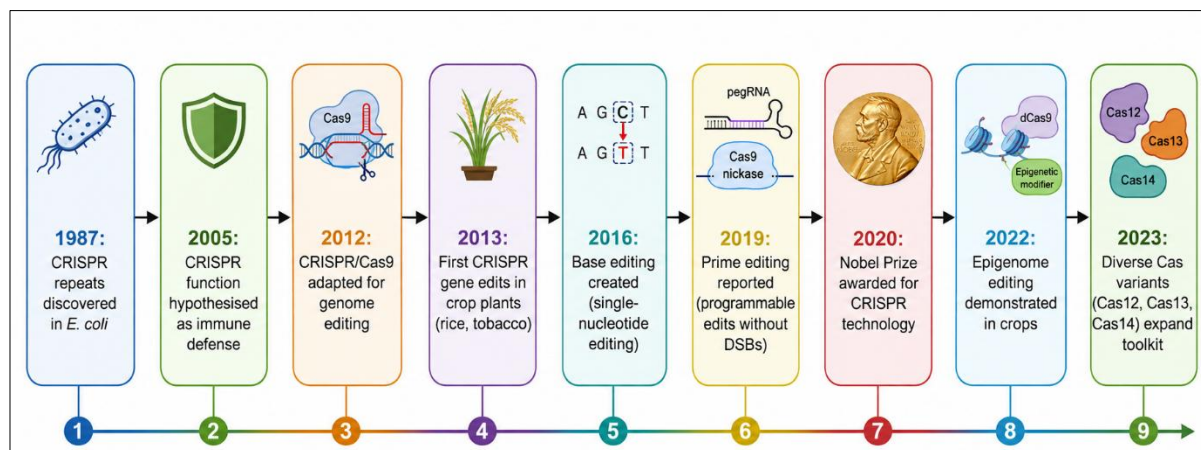


Fig 1: Timeline of key developments in genome editing for plant breeding.

Table 1: Comparison of Gene Editing Tools and Features [16, 17, 18, 19, 20, 21, 22, 23]

Tool/Technology	Recognition/Mechanism	Target Requirement	Off-Target Propensity	Delivery Formats
Meganucleases	Protein (homing endonuclease)	Long recognition sequence (~15-30 bp)	Low (high specificity)	Plasmid DNA
Zinc Finger Nucleases (ZFNs)	Engineered zinc-finger protein + FokI nuclease	Each zinc finger binds 3-6 bp (modular)	Medium-high (context-dependent)	Plasmid DNA
TALENs	Engineered TALE protein + FokI	One TALE module per base (very specific)	Low (more specific than ZFN)	Plasmid DNA, mRNA
CRISPR/Cas9	RNA-guided nuclease (Cas9)	PAM sequence NGG (SpCas9)	Moderate (many off-target detection methods exist)	DNA, RNA, RNP, viral vectors, nanoparticles
CRISPR/Cas12a (Cpf1)	RNA-guided nuclease (Cas12a)	PAM TTTV (T-rich); staggered DSB ends	Typically lower off-target than Cas9	DNA, RNP
CRISPR/Cas13	RNA-guided RNase (Cas13 variants)	No PAM (targets RNA sequence)	N/A for DNA; off-target can cleave other RNAs	RNA (gRNA + Cas13 protein)
Base Editors (CBE)	Cas9 nickase or dCas9 fused to cytosine deaminase	PAM-dependent (e.g. NGG for SpCas9), editing window	Off-target deamination possible; generally low	Plasmid DNA, RNP
Base Editors (ABE)	Cas9 nickase/dCas9 fused to adenosine deaminase	PAM-dependent (SpCas9 NGG, editing window)	Relatively specific; some off-target editing	Plasmid DNA, RNP
Prime Editors	Cas9 nickase + reverse transcriptase + pegRNA	Complex (requires PAM and primer binding sites)	Very high precision (no DSBs, few off-targets)	Plasmid DNA (delivers PE components)
dCas9-Fusion (Epigenetic Editor)	Catalytically dead Cas9 fused to effector (e.g. methylase)	PAM-dependent targeting	No DSBs (no off-target DSBs); off-target epigenetic modification possible	Plasmid DNA
CRISPRa/CRISPRi	dCas9 fused to activator (e.g. VP64) or repressor (KRAB)	PAM-dependent	No DSB; potential off-target gene regulation	Plasmid DNA

Advances in CRISPR and Gene Editing Technologies

The CRISPR era in plant science has rapidly evolved beyond simple gene knockouts. Several major advancements have expanded the toolkit:

- **Multiplex Genome Editing:** CRISPR may express several guide RNAs. This enables editing of numerous genes concurrently. tRNA or Csy4 processing systems may hold arrays of gRNAs in one construction.

Multiplexing allows stacking of features such as stacking several disease resistance genes or yield related loci in one generation. Recent research on crops like as tomato and wheat has used multiplex CRISPR to pyramid resistances and accelerate the process of complex trait modification [25, 26].

- **Base Editing:** Classic CRISPR relies on the cell's repair processes (NHEJ or HDR) to create modifications, which may be inefficient. So another option is base editing. Base editors avoid double strand breakage via chemical changes [27]. For the purpose of agriculture, cytosine base editors (CBE) and adenine base editors (ABE) have been employed to create point mutations for traits such as herbicide resistance (e.g., by modifying the target enzyme genes) or to specifically knock out genes [28]. In particular, CBEs have been used to insert stop codons into genes that influence grain quality, while ABEs have been used to alter genes that control flowering time. Base editing is limited to a small editing window and only certain base transitions (i.e. C→T or A→G). However, it is more accurate than other editing techniques, producing fewer random indels. They are creating new versions such as double base editors and RNA base editors [29].
- **Prime Editing:** Prime editing is an editing technique that in principle may make any little addition, deletion or base change. Prime editors have been successfully used to make single-base conversions in rice and wheat plants, however the efficiency is limited at now [30]. Continuous research in the fields of codon optimization and pegRNA design is constantly improving outcomes. Prime editing might potentially be used to generate precise alleles of complex traits like as nutrient profiles and genes associated with stress tolerance, which may need specific amino acid substitutions [31].
- **Epigenome Editing:** Editing the epigenome requires using dead Cas9 that has been attached to enzymes that add or remove epigenetic markers. This procedure is better than alteration of DNA sequence. For example, targeted DNA methylation has been used to mute genes in Arabidopsis (2019) and Cas12j fusions have been found to affect expression of rice genes [32]. Epigenetic editing may bring about heritable changes in gene activity without changing the DNA code and hence the possibility for reversible or tunable regulation of characteristics. It's a particularly attractive quality as it pertains to qualities mediated by gene regulatory networks, like blooming or ripening. Applications include the control of gene expression in developmental pathways or in response to stress. But that these marks have been perpetuated through so many generations is matter of significant investigation [33].
- **CRISPR Variants and Alternative Nucleases:** Besides CRISPR variants and alternative nucleases, a growing number of Cas nucleases are currently accessible. Cas12a (Cpf1) is widely utilized because it produces staggered cuts and has a different PAM requirement. Ultra-small Cas proteins (Cas12j and Cas14) have been introduced in plants for DNA editing, which might potentially reduce the amount of off-targets [34]. Or they might potentially be used to facilitate dispersion by viral vectors or single-vector constructs. Novel PAMs combined with new enzymes, such as CasY or CasX, allow editing of hitherto

inaccessible genomic regions. Furthermore, several systems may be used, allowing numerous independent alterations to be made in one cell employing orthogonal systems, which are CRISPRs from other species [35]. The fact that the various enzymes are from different species means that this type of variety not only increases the flexibility of where the enzymes may be used but might also reduce regulatory complications [36].

- **Improved Specificity and Engineered Enzymes:** Concerns about CRISPR off-target cleavage prompted the early creation of engineered high-fidelity variants of Cas9 (eSpCas9, HF1 and HypaCas9) that significantly reduce the amount of cuts that were not intended to be performed [37]. There are fewer off-target studies in plant systems than mammalian systems. Plant scientists often create many gRNAs and use variant Cas9 to minimize the amount of errors that occur. Further, temperature- or ligand-inducible approaches have been developed to control when and where CRISPR is active. This has served to minimize the off-target consequences associated with transient expression [38].
- **Transgene-free Editing Strategies:** One of the most important achievements in the area of transgene-free editing techniques is the delivery of CRISPR machinery as ribonucleoprotein complexes (pre-assembled Cas9 protein with guide RNA) or messenger RNA (mRNA) instead of DNA [39]. This approach enables gene modification without the need to insert foreign DNA into the genome of the plant, frequently easing the regulatory process since the resulting plant does not include any transgenic sequences. The employment of geminivirus replicons and mobile RNA systems has also allowed a high transient expression in the plant, resulting in an improvement in the editing efficiency and number of species [40].

Delivery Methods for Genome Editors

Effective delivery of gene editing tools into plant cells remains a major technical challenge, especially for crops with difficult tissue culture. Major delivery approaches include:

- **Agrobacterium-mediated Transformation:** This approach is frequently utilized, in which *Agrobacterium tumefaciens* transfers the gene editor DNA (T-DNA) into normal cells from plants. It is efficient in many dicots and some monocots, although it needs tissue culture and regeneration from changed cells. Frequently used in model crops (tomato, soybean) [41].
- **Biolistic (Gene Gun) Delivery:** Coating DNA or RNA onto microprojectiles (gold or tungsten) and shooting them into plant tissues. This approach is feasible for several monocots (rice, maize, wheat) more recalcitrant to *Agrobacterium*. It can deliver RNP complexes or plasmids. However, it may lead to many insertions or damage, therefore screening for clean modifications is required [42].
- **Protoplast Transfection:** Cells from plants without cell wall (protoplast) may be transfected with CRISPR DNA/RNA/protein by PEG or electroporation. This is rapid for assessing gRNA effectiveness, although regeneration of complete plants from protoplasts is usual only for few species. Protoplasts allow direct RNP administration, typically resulting in transgene-free plants [43].

- **Viral Vectors:** Plant viruses (such as geminiviruses or RNA viruses) may be used to deliver CRISPR components (usually gRNA or Cas12) to plants. These may infect numerous cells systemically. No tissue culture needed. For example, tobacco mosaic virus has been designed to transmit gRNAs for editing. By infecting meristematic tissue VIGE has been able to generate heritable alterations ^[44].
- **Nanoparticles:** New techniques include DNA/RNA loaded nanoparticles (lipid or polymeric) to cross the cell wall. Early demonstrations in plants are somewhat promising, particularly for transient delivery, although efficiency and scalability are still being developed ^[45].
- **Grafting and Cut-Dip Methods:** In woody or hard-to-edit species, altered rootstocks have been used to introduce editors by grafting or by dipping cuttings in enzyme solutions containing the CRISPR components. These new techniques are intended to be regeneration-free ^[46].

Off-Target Effects and Precision

- Ongoing technical issues include off-target editing, the process of creating undesired alterations to DNA at sites identical to the target sequence. In plants, careful guide design may help prevent off-target mutations. That means avoiding gRNAs with close-together homologous regions and using software methods that predict specificity. In empirical research, off-target rates in plant studies have typically been modest or even negligible ^[47].
- This may be because plants can tolerate some background mutation and outcrossing may purge unwanted changes. More often, high-fidelity Cas9 versions that are engineered to decrease off-target cleavage are being used where precision is critical. For example, altering of regulatory genes, where off-target disruption may affect several pathways potentially ^[48]. Methods adapted from animal systems (e.g. GUIDE-seq and Digenome-seq) are used to identify off-targets. Such procedures involve whole genome sequencing of the modified lines. Base editors have a very different profile: they do not create double-strand breaks (DSBs), but the deaminase enzymes might occasionally deaminate cytosines or adenines that are not the target of the enzyme ^[49].
- To limit the frequency of unwanted edits, researchers typically add other protein modules, such as UGI, which hinders base excision repair. Since multiplex editing increases the chance of off-targets via the use of many guides at once, each gRNA is rigorously tested ^[50]. Despite these concerns, there is a broad consensus that well engineered CRISPR modifications do not have undesirable off-target effects in plants. This is especially true when used with backcrossing or selfing to tidy the lines. However, safety measures and screening are highly recommended, especially as regards commercial cultivars ^[51].

Integrative Breeding Approaches and Workflows

Gene editing is most powerful when integrated into modern breeding pipelines. Below we describe key strategies that combine editing with other approaches to accelerate improvement:

- **Genomic Selection and Marker-Assisted Selection:** The traditional approach to marker-assisted selection (MAS) uses DNA markers linked to traits. With the CRISPR software, you may directly make the desired alleles you want and track them using markers. Genomic selection (GS) is the selection of lines in which prediction algorithms are used with genome-wide markers. Genetic sequencing and editing together make it feasible to design modifications based on predictive models. For example, modifications may be created by identifying key regions of the genome utilizing genetic sequencing for editing ^[52, 53].
- **Speed Breeding:** Longer light hours and suitable growth circumstances are techniques that reduce the generation time. More breeding cycles per year (usually four to six cycles) means more fish may be produced. That's called speed breeding. Editing mixed with that means that when an adjustment is made, it may be restored in lines via fast cycles. For example, a modified gene for drought tolerance in wheat was swiftly crossed into multiple backgrounds by the practice of speed breeding. This resulted in the generation of elite lines in just two years as compared to six years ^[54, 55].
- **Genomic Selection with Editing:** Another avenue is to use genomic prediction to identify deleterious alleles in a gene pool and then use CRISPR to make the superior allele in elite backgrounds. The process also includes modifying the genome. This "design and edit" cycle allows computer models to aid in the identification of changes before bench work. This is an example of a data-informed breeding approach ^[56].
- **Haploid Induction Combined with Editing:** New techniques are editing haploid inducer lines to generate doubled-haploid offspring with fixed modifications in a single crossing phase. This editing approach is called haploid induction plus editing. This approach circumvents the arduous backcrossing to delete the editing transgene ^[57].
- **Multiplex Editing for Trait Stacking:** If the genes match, they may be edited at the same time. This means you can stack many traits like disease resistance, yield and quality all in one go. This method is called multiplex editing for stacking of traits. Multiplex structures accelerate the trait pyramiding process, which would typically need a large number of offspring to be generated. Good varieties are immediately modified by breeders to achieve the attributes of following generation ^[58, 59].
- **High-Throughput Phenotyping Integration:** Fast phenotyping, which includes the use of drones, imaging platforms and sensors to gather data on changed plants at a massive scale is the last step of the approach. This allows breeders to quickly link alterations to the phenotypes that result, feeding back into the gene discovery and validation process ^[60].
- **Participatory Breeding:** Participatory breeding, for example, means that farmers and other local stakeholders are involved in determining the objectives. For example, modifying local landraces for traits that are desirable, yet maintaining adaptation to local conditions. This would make the landraces acceptable and significant ^[61].

- **Bioinformatics-Driven Pipelines:** Editing may be eased using bioinformatics-driven pipelines for computational operations. The procedures comprise gene identification, target selection, gRNA design, off-target analysis and phenotypic prediction. The integration of omics data, network analysis and machine learning into these procedures guaranties that the alterations will have a high likelihood of success^[62].
- **Systems Biology and Gene Network Editing:** In systems biology and gene network editing, mechanisms are emerging to alter many nodes in a metabolic or regulatory network. This is different from the usual way of altering just one gene. The bottlenecks in the pathway should be investigated thoroughly and editing, a frontier area in breeding, should be coordinated^[63].

Crop Case Studies and Outcomes

Practical applications demonstrate CRISPR's impact on real crops. Table 2 summarises representative case studies across diverse species. Notable examples include:

- **Rice:** For rice, altering the gene OsSWEET14, which is a susceptibility gene to bacterial blight, has been a major achievement in developing resistant lines. Long-term disease resistance has been conferred by deletion of OsSWEET genes using CRISPR^[64]. Other rice improvements include increasing grain size (GS3 edits), enhancing submergence tolerance (changing SUB1A alleles) and modifying amylose content by editing starch synthesis genes for better nutrition and cooking quality. All of these changes impact rice^[65].
- **Wheat:** Hexaploidy makes wheat more challenging to edit, however multiplex CRISPR has been used to knock out all homeologs of genes such as TaGW2 to increase grain weight and inactivation of TaMLO to provide resistance to powdery mildew. Herbicide-resistant wheat was produced via TaALS base editing. Modifications introduced in TaQsd1 accelerated seed dormancy, increasing their resistance to sprouting^[66].
- **Maize (Corn):** The ratio of amylopectin/amylose has been changed via the use of CRISPR to edit maize starch genes such as WX or Waxy. It has also been used to reduce the production of mycotoxins and has been proven to promote resistance to diseases (for example ZmGln1;2 knock down for fusarium resistance)^[67]. Genetic modification of the roots of maize has improved the efficiency of nitrogen consumption via modifying the genes that control the nutrient transporters. Some attempts are also being made to produce particular features, including as drought tolerance, by targeting regulatory genes like ZmNAC111^[68].
- **Soybean:** Changes have been made to increase the quality of soybean oil. For example FAD2 genes have been removed to increase the quantity of oleic acid in the oil making a healthier oil. Modification of ALS or EPSPS genes has been shown to be a potential way of developing herbicide resistance. The blooming duration was modified using gene modification methods similar to CONSTANS to be adapted to different latitudes.
- **Resistance to cyst nematodes** is achieved via disease resistance changes such as knocking down GmSAGT1^[69].
- **Tomato:** The researchers used CRISPR to delete two genes, RIN and NOR, that control the ripening of tomatoes, producing tomatoes that stay firm and have a longer shelf life. Resistance against viruses has been achieved by changing the eIF4E genes. This has been used to block the replication of potyvirus. Deficiencies in ripening regulators also caused changes in fruit quality such as shelf life and sweetness, even in the absence of transgenes. Multiplex alterations are distinguished by stacking features such as early flowering and compact growth forms^[70, 71].
- **Potato:** CRISPR editing of VInv (vacuolar invertase) led in low-acrylamide potato by the reduction of sugar accumulation in the plant. Resistance to the ring-spot virus has been developed by concentrating on genes that cause vulnerability. At current work is being carried out to improve the vitamin content of tubers and to raise the quantity of amylopectin in waxy potatoes^[72].
- **Cassava:** Cassava brown streak disease is a serious disease. CRISPR knockouts of viral susceptibility genes (eg eIF4E isoforms) have generated plants with less virus load. Now, modifications that would minimize the quantity of cyanogenic glycosides are being tested to enhance food safety^[73].
- **Banana:** Transient editing of genes associated with resistance to bacterial wilt has shown encouraging outcomes in bananas. However, because bananas are sterile, the only way to replicate them is by micropropagation. The storage life has been extended by targeting editing, ripening genes^[74].
- **Barley:** Genetically engineered barley to remove male sterility (knockout of MS1) to facilitate hybrid seed production. Genes related for malting quality and disease resistance were also targeted^[75].
- **Sorghum:** alterations to improve sugar content in stalks, leading to greater grain yield under drought circumstances by modifications to key stress-responsive transcription factors. These alterations are for biofuel application^[76].
- **Rapeseed (Canola):** BnFAD2 genes were modified using CRISPR, resulting in a decrease of glucosinolate content and improved oil quality. Also the genes for adaptation of the flowering period were addressed^[77].
- **Cotton:** The quality of the fiber was improved by altering regulatory genes controlling the development of the fiber. EPSPS was edited to provide herbicide tolerance^[78].
- **Apple and Grapevine (Fruits):** CRISPR has been used to remove genes in apple that lead to browning or susceptibility to fire blight. One of the editing aims for grapes is susceptibility to powdery mildew. The development is slow but steady, due to the obstacles presented by these perennial crops (long cycle, regeneration)^[79].

Table 2: Crop Case Studies: Edited Traits and Outcomes [80, 81, 82, 83, 84, 85, 86]

Crop	Edited Gene(s)/Target	Trait/Outcome	Benefits	Deployment Status/Notes
Rice	OsSWEET14 (bacterial blight S gene)	Increased disease resistance	Durable blight resistance	Field trials complete; breeding in progress
	OsSUB1A (submergence tolerance gene)	Improved flood tolerance	Maintains yield during floods	Advanced breeding lines
	GS3 (grain size gene)	Larger grain size (higher yield)	Increased grain yield	Under evaluation in breeding programs
	Wx (starch synthesis)	Altered amylose/amylopectin ratio (quality)	Modified cooking/eating quality	Research stage
	ART1 pathway genes	Enhanced aluminium tolerance	Soils with low pH arable	Early research stage
Wheat	TaGW2 (yield regulator) (all homoeologs)	Increased grain weight (higher yield)	More wheat per plant	Field trials ongoing
	TaMLO (powdery mildew susceptibility)	Powdery mildew resistance	Reduced fungicide need	Developing mildew-resistant varieties
	TaALS (acetolactate synthase)	Herbicide resistance (IMI herbicides)	Weed control trait	Approved in some jurisdictions (US)
	TaQsd1 (seed dormancy gene)	Faster germination (reduced dormancy)	Uniform seedling emergence	Breeding evaluation
	TaGW7 (grain width/shape)	Modified grain size/shape	Yield/quality tuning	Research stage
Maize	ZmWX (waxy gene)	Altered starch (waxy corn)	Specialty corn with 100% amylopectin	Commercial varieties possible
	ZmFAD2 (oil desaturase)	Altered oil composition	Higher oleic oil content	Research/proof-of-concept
	ZmLOX (lipoxygenase)	Reduced undesirable compounds	Improved storage quality	Early trials
	ZmNAC111 (drought regulator)	Enhanced drought tolerance	More stable yield under water stress	Under investigation
	Bt gene clusters insertion (transgene)	Pest resistance (corn borer, via editing insertion)	High yield under pest pressure	Currently GM lines (future CRISPR HDR)
Soybean	GmFAD2-1A/1B (fatty acid desaturase)	Increased oleic acid content (healthier oil)	Healthier edible oil	Commercial pipeline
	GmALS (herbicide target)	Herbicide (IMI) tolerance	Simplified weed management	Research stage
	GmSACPD-C (stearoyl-ACP desaturase)	Altered oil saturation	Tailored oil profiles	Testing
	E1/E2 flowering genes	Modified flowering time	Adaptation to regions, yield stability	Proof-of-concept
	GmSNAP (nematode resistance gene)	Soybean cyst nematode resistance	Reduced nematode damage	Field evaluation
Tomato	RIN, NOR (ripening regulators)	Extended shelf life, firmness	Longer shelf life, reduced waste	Some edited lines tested
	SleIF4E (viral susceptibility)	Virus resistance (Pepino mosaic virus, etc.)	Reduced viral disease	Greenhouse tests
	SELF PRUNING (growth habit)	Compact growth, determinate vines	Easier management, higher density planting	Promising variety prototypes
	Multiple fruit size/shape genes	Enhanced fruit yield/size	Increased marketable yield	Research stage
Potato	VInv (vacuolar invertase)	Low-reducing-sugar (low acrylamide potato)	Healthier fried potato (less acrylamide)	Prototypes in development
	GBSS (waxy gene)	Zero amylose (waxy potato starch)	Specialty starch for industry	Field trials
	eIF4E (PVY virus)	Virus resistance (Potato virus Y)	Reduced virus loss	Lab plants achieved resistance
Cassava	eIF4E isoforms	Cassava brown streak virus tolerance	Reduced CBSV symptoms	Field evaluation
	CYP79D1/D2 (cyanide production)	Lower cyanide level	Safer cassava for consumption	Field trials ongoing
Banana	BLB susceptibility genes	Bacterial wilt resistance	Improved banana yields	Early experimental; vegetative propagation
	Polyphenol oxidase (browning)	Reduced browning	Improved fruit quality	Research stage
Barley	MS1 (male fertility gene)	Male sterility (for hybrid seed production)	Enables hybrid barley production	Under breeding trials
	Barley MLO (mildew resistance)	Powdery mildew resistance	Reduced fungicide use	Field testing
Rapeseed (Canola)	BnFAD2 (desaturase)	Higher oleic acid (oil quality)	Healthier canola oil	Candidate varieties
	BnTT8 (seed coat color)	Yellow-seeded canola (less fiber)	Improved oil content	Breeding development
Cotton	GhDPP1 (phosphoinositide)	Fiber strength/length improvement	Better fiber quality	Preliminary data
	GhMYB25 (fiber development)	Altered fiber traits	Improved cotton yield and quality	Early trials
Apple	PPO (polyphenol oxidase)	Reduced browning	Better fresh-cut quality	Research/experimental
	MdTFL1 (flowering time)	Modified flowering and bloom	Adapted growth cycles	Studies in orchard trials

Technical Challenges and Limitations

Despite its promise, several technical challenges remain in plant genome editing:

- **Transformation Efficiency:** Difficult to change or regenerate many crops (especially perennials and certain grains and legumes). Some species are not easily changed because of restricted tissue culture.

Genotype-independent transformation (e.g. developmental regulators to induce embryogenesis) and direct editing methods (e.g. in planta CRISPR using floral dip, albeit most of this is now in Arabidopsis) are under investigation^[87].

- **Polyploidy and Redundancy:** Many crop plants like wheat (hexaploid), canola (tetraploid) and potato (tetraploid) have multiple copies of genes. Screening is difficult and the overall efficiency is decreased since all homeologs need to be altered to get a visible phenotype. Multiplex guidelines are helpful, but often a hassle^[88].
- **Homology-Directed Repair (HDR) Efficiency:** Donor templates for precise insertion or replacement (SDN-2/3) are notoriously inefficient in plants because cells favor error-prone non-homologous end joining (NHEJ). Approaches to increase HDR (such as cell-cycle synchronization, geminivirus replicons, drugs) have had modest success, but large DNA insertions as a routine remain difficult^[89].
- **Off Target and Mosaicism:** Off target mutations are possible, although often modest as mentioned. Another concern is the issue of mosaicism: altering early zygotic cells may give rise to chimera plants with a combination of changed and unedited cells. Screening of progeny is necessary to find fully changed lineages. Procedures such as RNP or tissue culture diminish the mosaicism^[90].
- **Trait complexity:** Many important traits (yield, drought tolerance) are quantitatively inherited, controlled by many genes and environment interaction. Editing particular genes could give small improvements. Using CRISPR successfully for such traits could need altering networks of genes or a combination of editing and conventional breeding for polygenic gains^[91].
- **Detection of Unintended implications:** Correct alterations may have downstream implications on gene networks. Negative pleiotropy has to be ruled out by broad phenotyping. Edited plants should be monitored for growth performance, yield and off-target features throughout generations^[92].
- **Why Scalability:** While editing tens to hundreds of plants may be appropriate for laboratories, commercial breeding requires thousands of lines. Active development areas include automation (e.g. robotic transformation or *in vitro* propagation) and phenotyping scale-up^[93].
- **Regulatory and IP hurdles:** In certain countries, patents or regulations on the technology restrict the use of CRISPR tools. Researchers must also navigate freedom-to-operate landscapes and comply with biosafety regulations for the release of modified plants^[94].

Phenotyping and Bioinformatics Integration

- Editing genes does not take place in a vacuum, but must be combined with data driven crop assessment. Need high throughput phenotyping. Now, edited plants may be systematically scored for attributes like biomass, canopy temperature and growth rate utilizing drones, satellite photography and automated greenhouse systems^[95]. This phenomic data facilitates quick

selection of top performing altered lines and understanding of complicated trait effects. For instance, thermal cameras might be used to identify cooler canopies (i.e. improved water consumption) in CRISPR-edited wheat drought tolerance field experiments over hundreds of plots. Incorporating such data with genotypes speeds up the comprehension of genotype-phenotype links^[96].

- **Bioinformatics-based editing techniques. Every stage is guided by software tools:** gRNA design tools anticipate guide sequences that are efficient and specific. Off-target analysis software finds probable trouble spots. Genome browsers and databases facilitate the identification of target genes and variations in crop genome sequences^[97]. Genomic selection models in breeding programs may also be re-estimated to incorporate known altered alleles and therefore increase predicted accuracy. Machine learning and network analysis are useful for target identification. For instance, omics data may be mined to predict which transcription factor knockouts would increase stress tolerance^[98]. Large genomic and phenomic data sets are utilized to improve editing targets and to forecast unexpected interactions. Moreover, digital pipelines enable regulatory compliance: risk evaluations for gene flow or allergenicity may be conducted *in silico*. On the issue of public transparency, we are looking at traceability and blockchain tools to be able to trace the gene-edited varieties through the supply chain.^[99]

Future Directions and Research Gaps

Looking ahead, several frontiers and gaps should be addressed to fully harness gene editing in agriculture:

- **Improving High-Definition Replication (HDR) and Insertion Techniques:** At the moment, inserting or replacing large DNA segments by homology-directed repair (SDN-2/3 edits) is an inefficient process^[100]. It is necessary to develop new tactics, such as improving the distribution of donor templates (for example, viral replicons and single-stranded donors) and synchronizing edits with cell cycle stages that favour high dynamic range (HDR images). If this endeavor were to be successful, it would make it possible to do accurate gene swapping and the insertion of complete gene cassettes without the need for conversion^[101].
- **Extending coverage to Orphan and Underutilized Crops:** A significant amount of research has been conducted on main grains and vegetables. There is a lack of progress in the use of gene editing to orphan crops, including as millet, teff and pigeon pea, which are essential for ensuring food security in developing nations. It is important for programs to make investments in protocols for these crops (transformation, regeneration and genomic data) in order to develop climate-resilient varieties in areas where standard breeding strategies are sluggish^[102, 103].
- **Polyploid and Complex Genome Editing:** In addition to wheat, other crops such as sugarcane and strawberry contain complex polyploid genomes. This problem might be solved by developing new editing tools or techniques that are capable of effectively targeting numerous alleles. For instance, innovative base editors that are able to change several nucleotides at the same time would be an example. Also, information from the

whole genome might be used to direct editing to certain subgenomes^[104, 105].

- **Multiplex Trait Engineering:** The stacking of quantitative trait loci will be advanced by tools that ease multiplex editing (for example, huge gRNA arrays and synthetic polycistronic constructs). When combined with synthetic biology circuits, CRISPR has the potential to provide dynamic control. For example, an inducible gene circuit that only triggers an edit when stress is present might be accomplished using this combination^[106].
- **Integration with Artificial Intelligence:** Machine learning has the potential to enhance every step, including the prediction of the most effective gRNAs, the modelling of off-target probability, the forecasting of phenotypic consequences of particular changes and the analysis of phenomics data. Through the mining of massive datasets, AI-guided design may be able to discover gene targets that are not obvious. The research should concentrate on developing efficient AI pipelines that can be evaluated in actual breeding operations^[107, 108].
- **Innovations in Delivery:** Development of non-transformative delivery continues to be an area of ongoing research and development. New developments, like as carbon nanotubes or nano-lipid carriers, which are able to infiltrate plants without the need for tissue culture, have the potential to make editing more accessible to species that were previously inaccessible. There is potential for advancement in the electroporation of germline cells or pollen, with the latter being shown in *Arabidopsis*^[109].
- **Regulatory Science:** There is a need for rules that are the result of scientific research and that evaluate the danger of gene-edited crops on an individual basis. It would be helpful to have worldwide rules, standards for traceability of modifications and comparative risk assessment approaches. Policy may be improved with the provision of empirical facts on gene flow, ecology and the long-term field performance of altered plants, which can be obtained through research. The integration of social scientific findings into future work should be a priority for public engagement and social science. It is possible to guarantee that technology is in line with the requirements of society by gaining an understanding of consumer preferences, resolving ethical problems (such as the role that genome editing plays in sustainable agriculture) and developing participatory techniques^[110, 111].
- **Safety Mechanisms:** The development of "kill switches" or gene lockout systems for altered crops (for instance, inserting a self-deleting element after trait adoption or molecular barcodes to identify modified plants) might alleviate worries about the spread of the disease and reduce the cost of regulatory oversight^[112].
- **Integration of Omics:** As multi-omics (genomics, transcriptomics and metabolomics) becomes more commonplace, gene editing may make use of the data that is generated by these technologies. Metabolomic editing that is specifically targeted, for instance, has the potential to fine-tune the nutritional or phytochemical profiles of crops. The mapping of whole-plant metabolic networks should be a focus of future study in

order to uncover novel editing targets for the purpose of enhancing nutrition or lowering aspects that are detrimental to nutrition^[113].

- **Adaptation to Climate Change:** In the face of harsh weather, it is essential to edit for stress tolerance due to factors like as heat, drought and salt. Editing genes that are responsible for stress sensors or transcription factors that control numerous pathways might be required for this. Additionally, it necessitates evaluating the altered lines under settings of many stresses in the field. It is important to note that modifying symbiotic pathways, such as the nitrogen fixation process in cereals, is a task that carries a high risk but also a great potential benefit^[114].

Conclusion

Editing genes, especially using CRISPR/Cas systems, is now the backbone of contemporary plant biotechnology, providing accuracy and speed not seen before. This study summarizes the contributions of CRISPR and related technologies to plant breeding, with emphasis on important advancements, various applications and integrative tactics. CRISPR has allowed targeted increases in yield, quality and resilience in several crops. Further advances like base and prime editing extended the editing repertoire to single base precision, while multiplex editing and epigenetic editing unlocked new possibilities for complex trait engineering. These molecular techniques are most potent when paired with modern breeding procedures, including as speed breeding, genomic selection and high-throughput phenotyping, which may reduce the journey from gene discovery to better variety. Engineered nucleases, new delivery vectors and stringent screening are gradually overcoming technical hurdles like as delivery techniques and off-target problems. The regulatory landscape is shifting internationally, with some governments accepting gene modification as a normal progression of breeding, while others are implementing tighter GMO-like regulations. Part of the deployment problem is learning to deal with these discrepancies. There are also socio-ethical difficulties, but history shows that gene-edited crops particularly those with obvious consumer or environmental advantages may acquire public support via open communication and collaborative development. Going ahead, research should focus on improving editing tools (better HDR, increasing the CRISPR toolset), applying them to all crop species and incorporating data-driven and AI-enabled design. The ultimate aim is a totally seamless breeding pipeline: genetic targets discovered by omics and modelling, swiftly confirmed by precise editing and rapidly bred into elite lines by accelerated breeding procedures. For breeders and biotech companies, the practical lesson is clear: invest in genome editing capabilities and incorporate it early in breeding programs for characteristics that provide significant benefits. Be mindful of developing regulatory and IP landscapes and engage stakeholders to ensure technology is utilized properly. When used responsibly, CRISPR and genetic modification might have a major role to play in sustainable agriculture, helping to secure food security and fulfil the needs of a changing climate. We are moving toward a time of precision breeding, promising better, quicker, more customized agricultural development for the twenty-first century.

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