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CRISPR-Cas applications in vegetable crop improvement: Innovations, challenges and future prospects a review

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Abstract

Vegetable crops are essential components of global food security, human nutrition and agricultural economies. However, conventional breeding approaches are constrained by genetic bottlenecks, long generation cycles and the increasing pressure of biotic and abiotic stresses driven by climate change. The emergence of CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats CRISPR-associated protein) technology has catalyzed a paradigm shift in crop improvement by enabling precise, efficient and cost-effective genome editing. This review provides a comprehensive overview of CRISPR-Cas applications in vegetable crop improvement, encompassing a wide range of species including tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), eggplant (*Solanum melongena*), carrot (*Daucus carota*) and cabbage (*Brassica oleracea*). We discuss the molecular mechanisms of CRISPR-Cas systems, including Cas9, Cas12a, base editors and prime editors and their deployment in trait improvement such as disease resistance, yield enhancement, nutritional quality, shelf life and stress tolerance. Key innovations such as multiplexed editing, RNA virus-mediated delivery, ribonucleoprotein (RNP)-based transformation and in planta editing are highlighted. Regulatory landscapes across major jurisdictions, biosafety considerations, public perception challenges and ethical dimensions are critically evaluated. Finally, emerging opportunities including CRISPR-enabled smart crops, integration with artificial intelligence and the roadmap for translating laboratory discoveries into field applications are discussed. This review serves as a consolidated scientific resource for researchers, plant breeders, policymakers and biotechnology professionals working toward sustainable and resilient vegetable production systems.

Keywords: CRISPR-Cas9, genome editing, vegetable crops, disease resistance, nutritional quality, stress tolerance, plant biotechnology, food security

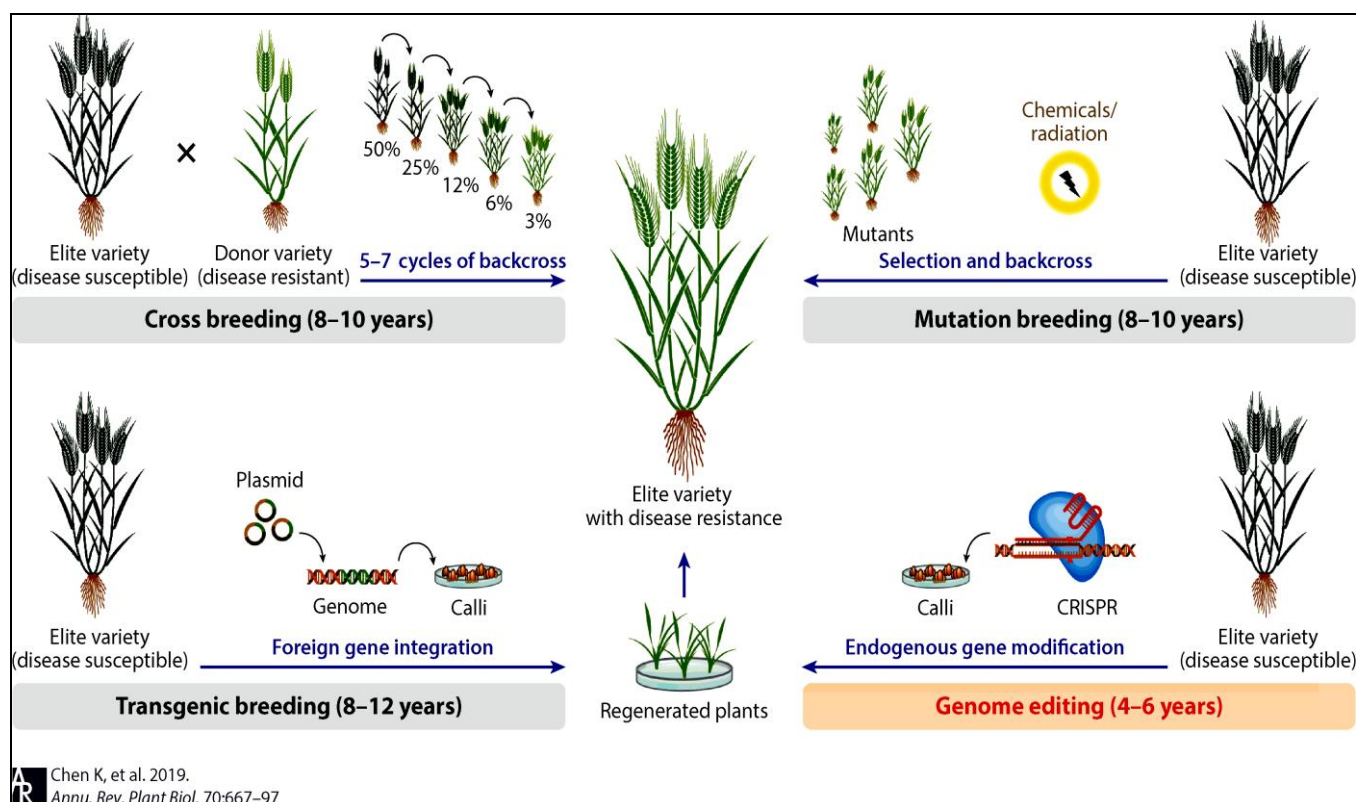
1. Introduction

Vegetables are an important part of the nutritional diet of humans, offering indispensable vitamins, minerals, dietary fibers, and phytonutrients that are vital for health. There has been an increase in the total production of vegetables worldwide over the last three decades, with an average of 1.1 billion metric tons produced annually, with Asia producing almost 75% of total vegetable production (FAO, 2023) ^[17]. Nevertheless, vegetables are highly susceptible to a wide range of risks, including fungal, bacterial, and viral diseases; insects; abiotic stresses such as drought, salinity, and temperature extremes; as well as post-harvest quality loss. Climate change is expected to increase these risks, resulting in a 10-25% decline in yields of important crops by 2050, as projected by the IPCC (2022) ^[25].

While the accomplishments of conventional plant breeding over the last century have been spectacular, its limitations have become more and more apparent. Plant breeding is a slow process, taking 8 to 12 years to produce a new variety. It is also limited by the genetic variation present in the species' gene pool and by the complex and unpredictable process of recombination. While the transgenic approach has been successful, it has faced significant hurdles, including consumer resistance, in many countries. This has spawned the urgent need for new and improved plant breeding technologies to rapidly and accurately manipulate the plant genome.

The discovery and application of the CRISPR-Cas system for genome editing in eukaryotes, as demonstrated by Jinek *et al.* (2012) [26], and later used in plants by Feng *et al.* (2013) [18], is arguably one of the most significant breakthroughs in biological technology. Unlike earlier technologies, including zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the CRISPR-Cas9 system is unrivaled with regard to simplicity, efficiency, versatility, and cost. CRISPR-Cas9 technology uses a small RNA molecule called a guide RNA (gRNA), which directs the Cas9 enzyme to a specific location in the genome where a double-strand break is

introduced, which is then repaired via non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms (Doudna & Charpentier, 2012) [15]. For vegetable crops, in particular, CRISPR-Cas technology has facilitated unprecedented progress in disease resistance, optimization of yield components, abiotic stress, nutritional content, and post-harvest qualities. The review aims to synthesize the current state of knowledge in this fast-developing area, identify key knowledge gaps, and examine the regulatory and societal aspects, and develop a vision for future research in vegetable crop improvement.



Chen K, et al. 2019.
Annu. Rev. Plant Biol. 70:667–97

Source: Chen *et al.*, 2019 [11]

2. Molecular mechanisms of CRISPR-Cas systems

2.1 CRISPR-Cas9: the foundational system

The CRISPR-Cas9 system, derived from *Streptococcus pyogenes* (SpCas9), remains the most widely used genome editing platform in plants. The system consists of two core components: the Cas9 nuclease and a single guide RNA (sgRNA) that combines the functions of the CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). The sgRNA contains a 20-nucleotide spacer sequence complementary to the target DNA and guides Cas9 to sites flanked by a 5'-NGG protospacer adjacent motif (PAM). Upon binding, Cas9 undergoes conformational changes and creates blunt-ended DSBs three base pairs upstream of the PAM site (Jinek *et al.*, 2012) [26].

Repair of DSBs proceeds predominantly via NHEJ in plant cells, resulting in small insertions or deletions (indels) that typically cause frameshift mutations and gene knockouts. HDR-mediated precise edits, while possible, are less frequent in somatic plant cells due to the predominance of NHEJ pathways. Several Cas9 variants have been developed to expand targeting range and improve specificity, including SpCas9-HF1, eSpCas9 and xCas9, which exhibit reduced off-target activity (Slaymaker *et al.*, 2016; Chen *et al.*,

2017) [10, 47].

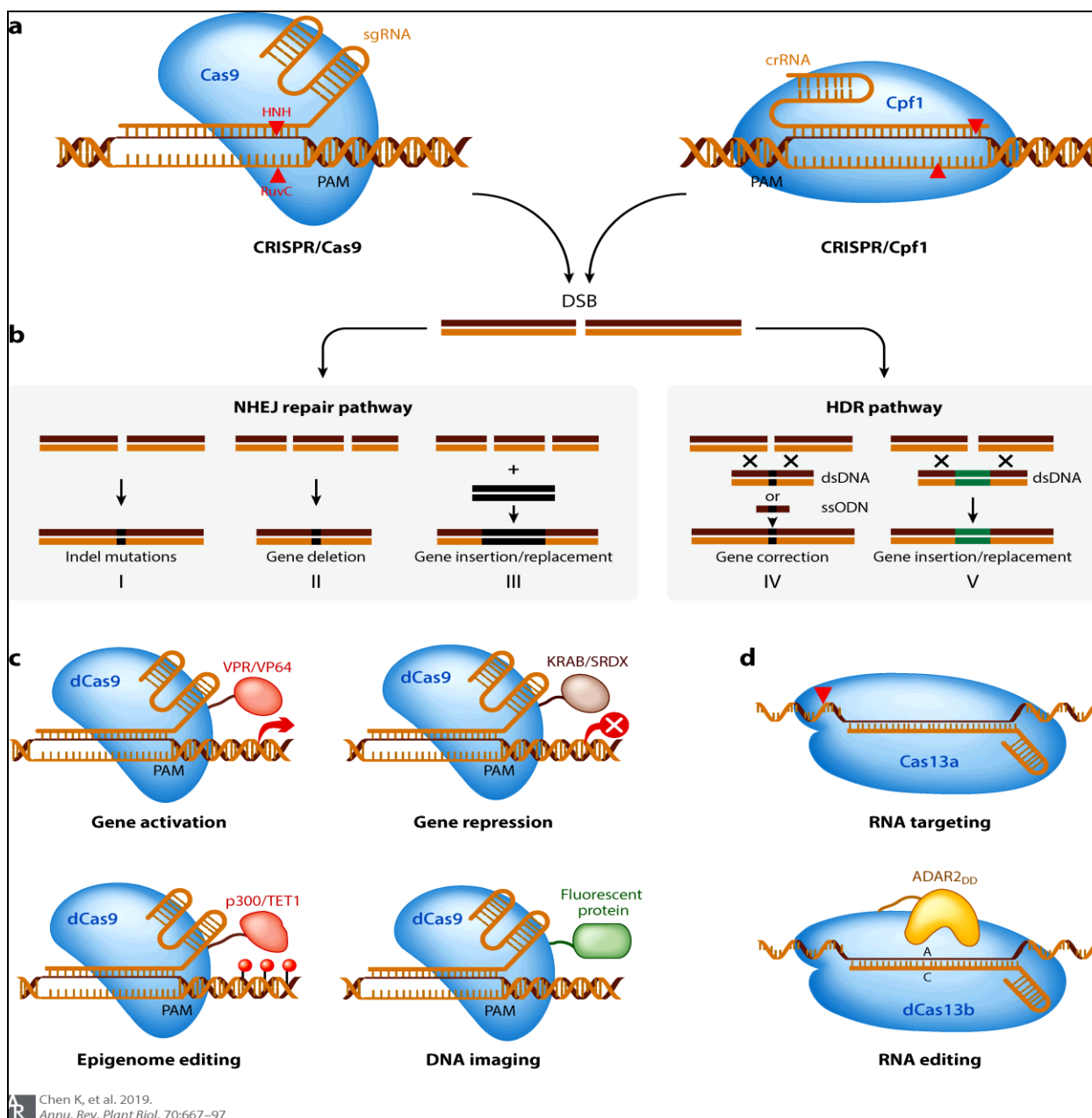
2.2 CRISPR-cas12a (Cpf1): Cas12a (formerly Cpf1), derived from *Acidaminococcus* sp. (AsCas12a) and *Lachnospiraceae* bacterium (LbCas12a), offers several advantages over Cas9 for plant applications. Cas12a uses a T-rich PAM (5'-TTTN-3'), generates staggered cuts distal to the PAM and processes its own crRNA array, enabling multiplexed editing with a single transcript (Zetsche *et al.*, 2015) [63]. These features have been exploited in tomato and other vegetables to simultaneously edit multiple target genes. Enhanced thermostable variants such as enAsCas12a have demonstrated superior editing efficiencies at room and elevated temperatures relevant to greenhouse production (Kleinstiver *et al.*, 2019) [29].

2.3 Base editing

Base editors enable direct, programmable conversion of one nucleotide to another without requiring DSBs or exogenous donor DNA templates. Cytosine base editors (CBEs) convert C to G to T to A base pairs, while adenine base editors (ABEs) convert A to T to G to C pairs (Komor *et al.*, 2016; Gaudelli *et al.*, 2017) [22, 31]. In plants, CBEs and

ABEs have been applied to introduce precise point mutations mimicking natural alleles associated with improved traits. Liu *et al.* (2020) ^[36] demonstrated efficient base editing in tomato using a plant-optimized ABE8e

achieving up to 80% editing efficiency. Base editing is particularly attractive in vegetable crops because many agronomically important traits are governed by single nucleotide polymorphisms (SNPs).



Source: Chen *et al.*, 2019 ^[11]

2.4 Prime editing

Prime editing, as developed by Anzalone *et al.* (2019) ^[3], is a flexible 'search and replace' genome editing tool that uses a prime editor fusion, PE2 or PE3, which is a Cas9 nickase and reverse transcriptase, with a pegRNA that encodes the target sequence and the edit. All 12 types of point mutations, as well as small insertions and small deletions, with minimal off-target effects, can be introduced with this tool. Tang *et al.* (2020) ^[51] were the first to use prime editing in rice and wheat, but this technology has since been harnessed to edit the tomato genome (Lin *et al.*, 2021) ^[35], as well as the potato genome.

2.5 Transcriptional and epigenetic modulation

In addition to nuclease-based gene editing, catalytically inactivated dCas9, when combined with transcriptional

activators (CRISPRa) or repressors (CRISPRi), allows for the modulation of gene expression. dCas9, when linked to epigenetic regulators such as histone acetyltransferases and DNA methyltransferases, expands the range of dCas9 applications. These tools are currently being tested in vegetable crops for the modulation of biosynthetic gene expression for flavor, color, and nutritional compounds (Lowder *et al.*, 2018) ^[38].

3. CRISPR-cas delivery methods in vegetable crops

3.1 Agrobacterium-mediated transformation

Agrobacterium tumefaciens-mediated transformation remains the dominant delivery system for CRISPR constructs in dicotyledonous vegetables such as tomato, pepper, eggplant and potato. T-DNA vectors carrying Cas9 and sgRNA expression cassettes are integrated into the plant

genome, enabling stable, heritable editing. A critical consideration is the generation of transgene-free edited plants in subsequent generations by segregation. This approach has been successfully applied to tomato (Brooks *et al.*, 2014) ^[7], potato (Butler *et al.*, 2015) ^[8] and pepper (Hunziker *et al.*, 2020) ^[24].

3.2 Biolistic (particle bombardment) delivery

Biolistic transformation involves coating gold or tungsten particles with CRISPR constructs and accelerating them into plant cells using pressurized helium or electrical discharge. This method is species-agnostic and has been applied to recalcitrant species including carrot (Klimek-Chodacka *et al.*, 2018) ^[30] and onion. Delivery of RNPs pre-assembled complexes of Cas9 protein and synthetic sgRNA via biolistics produces transient editing without stable transgene integration, facilitating the generation of regulatory-compliant edited crops (Liang *et al.*, 2017) ^[34].

3.3 Protoplast transformation

This method allows for highly efficient transient transfection with DNA, RNA, or RNPs. It is particularly useful for the delivery of RNPs since the protein-RNA complex is rapidly degraded after the editing event, leaving no residual non-native nucleic acid. This method has been used to transform tomato (Yoo *et al.*, 2007) ^[61], lettuce (Woo *et al.*, 2015) ^[58] and potato (Andersson *et al.*, 2018) ^[2]. However, the difficulty of regeneration from protoplasts is a major constraint for the application of this method in many vegetable crops.

3.4 RNA virus-based delivery

Tobacco Rattle Virus (TRV), Cucumber Mosaic Virus (CMV), and other RNA viruses from plants have also been engineered to introduce sgRNAs in transgenic plants that constitutively express Cas9. Ellison *et al.* (2020) ^[16] used a system of virus-induced genome editing (VIGE) in tomato plants to carry out targeted mutagenesis in meristematic tissues that are involved in heritability in the next generation. This system has greatly streamlined the process and has also been used in potato and other solanaceous vegetables.

3.5 Nanoparticle-mediated delivery

The new nanocarrier technologies, such as carbon nanotubes, mesoporous silica nanoparticles, lipid nanoparticles, and peptide delivery systems, provide the possibility of using non-transgenic approaches for the delivery of CRISPR-Cas in plants. Liu *et al.* (2023) showed that using pollen-mediated delivery of lipid nanoparticles could introduce heritable changes in various vegetable crops without the need for tissue culture or the bacterium *Agrobacterium*. Although in its infancy, the application of nanoparticles in the delivery of CRISPR-Cas in plants is likely to revolutionize the process in various vegetable crops with diverse genomes.

4. CRISPR-cas applications in specific vegetable crops

4.1 Tomato (*Solanum lycopersicum*)

Tomato is the most extensively studied vegetable crop for CRISPR-Cas applications, serving as both a crop improvement target and a model system for Solanaceae. Key achievements include:

- **Disease Resistance:** SIMlo1, an ortholog of the barley

mlo gene conferring powdery mildew susceptibility, was knocked out in tomato by Nekrasov *et al.* (2017) ^[42], producing plants with broad-spectrum resistance to *Oidium neolycopersici* without yield penalty. Similarly, targeting SIJAZ2 truncation alleles enhanced resistance to *Pseudomonas syringae* (Ortigosa *et al.*, 2019) ^[43].

- **Fruit Quality and Shelf Life:** Disruption of the polygalacturonase gene (SLPG) delayed fruit softening and extended shelf life by over 30% compared to wild type (Wang *et al.*, 2019) ^[55]. Multiplex editing of ethylene pathway genes (ACS2, ACS4, ACO1) further extended post-harvest shelf life by modulating ethylene biosynthesis (Li *et al.*, 2018) ^[33].
- **Nutritional Enhancement:** CRISPR-mediated knockout of SGR1 (stay-green 1) and LCY-E (lycopene epsilon cyclase) increased lycopene content by 5.1-fold and beta-carotene by 10-fold, respectively (Zhu *et al.*, 2018; D'Ambrosio *et al.*, 2018) ^[14, 66]. Editing GABA-T increased gamma-aminobutyric acid (GABA) levels five-fold, enhancing the nutraceutical value of tomato fruits (Takayama *et al.*, 2015) ^[50].
- **Yield and Architecture:** Targeted editing of CLV3 and WUS homeostatic pathway genes expanded inflorescence meristem size and increased fruit number by 2.5-fold (Rodriguez-Leal *et al.*, 2017) ^[46]. Fine-tuning of SELF-PRUNING (SP) and SP5G genes using CRISPR allele series generated novel plant architectures suitable for greenhouse or field cultivation (Soyk *et al.*, 2017) ^[48].

4.2 Potato (*Solanum tuberosum*)

Potato presents unique challenges for CRISPR editing due to its tetrasomic polyploidy, which requires simultaneous editing of four alleles for complete trait modification. Key achievements include:

- **Cold-Induced Sweetening:** Knockout of the vacuolar invertase gene (VInv) in tetraploid potato by Clasen *et al.* (2016) ^[12] prevented cold-induced sweetening a major quality defect leading to carcinogenic acrylamide formation during frying. All four VInv alleles were successfully disrupted and the resulting plants showed dramatically reduced reducing sugar accumulation in cold storage.
- **Bruising Reduction:** PPO (polyphenol oxidase) gene family members (StPPO2) were disrupted using CRISPR, reducing enzymatic browning upon mechanical damage by 70-80% (Gonzalez *et al.*, 2020) ^[23]. This trait has direct implications for post-harvest quality and consumer acceptance.
- **Late Blight Resistance:** CRISPR-mediated disruption of susceptibility genes DMR6 (downy mildew resistant 6) orthologs in potato conferred enhanced resistance to *Phytophthora infestans* without compromising yield or plant fitness (Kieu *et al.*, 2021) ^[27]. Stacking this edit with existing R-gene loci using CRISPR offers a durable resistance strategy.

4.3 Pepper (*Capsicum annuum*)

Improvement of pepper using CRISPR technology has also involved disease resistance, capsaicin biosynthesis, and fruit quality. The CaMLO2 gene was knocked out to improve pepper resistance to powdery mildew caused by *Leveillula taurica* (Wan *et al.*, 2020) ^[54]. The capsaicin synthase gene (CS) knockout was used to develop sweet peppers from

pungent peppers (Hunziker *et al.*, 2020) [24]. The CaERF28 transcription factor was also used in pepper improvement through CRISPR technology to delay pepper ripening by about 15 days through modulation of the ethylene signal.

4.4 Cucumber (*Cucumis sativus*)

The CRISPR-Cas9 gene editing tool in cucumber research so far has been used for disease resistance, sex determination, and fruit quality. The knockout of CsMLO8 and CsMLO11 genes provided long-lasting resistance to powdery mildew (*Sphaerotheca cucurbitacearum*) in a study conducted by Wheatley *et al.* (2021) [57]. The editing of CsACS genes, which regulate sex expression, facilitated the manipulation of both monoecious and gynoeious lines, particularly in hybrid seed production (Lu *et al.*, 2018) [39]. The simultaneous knockout of CsPDS and CsChlI genes showed the efficiency of multiplex editing and served as a proof of concept for high-level trait stacking in cucumber (Li *et al.*, 2016) [32].

4.5 Lettuce (*Lactuca sativa*)

Lettuce is an emerging model for CRISPR-Cas in leafy vegetables, due to its relatively short generation time and amenability to transformation. Woo *et al.* (2015) [58] pioneered RNP-mediated CRISPR editing in lettuce protoplasts, achieving transgene-free knockouts of BIN2. More recently, editing of LsBBX (B-box zinc finger proteins) improved lettuce tolerance to high-temperature bolting, a critical agronomic trait for summer production (Zhang *et al.*, 2022) [64]. Reduction of oxalic acid content via

oxalate oxidase gene editing is under investigation to improve nutritional safety.

4.6 Eggplant (*Solanum melongena*)

Eggplant CRISPR research has addressed fruit browning and disease resistance. Knockout of SmAP1 (acyl-protein thioesterase 1) reduced anthracnose susceptibility by modulating jasmonate-mediated immunity (Zhu *et al.*, 2022) [65]. Disruption of polyphenol oxidase genes reduced enzymatic browning of cut eggplant flesh, improving processing quality. Virus-induced CRISPR editing using TRV has been validated as a scalable approach for this crop (Bharat *et al.*, 2020) [5].

4.7 Other vegetable crops

CRISPR-Cas editing has been extended to an increasingly broad repertoire of vegetables. In watermelon (*Citrullus lanatus*), knockout of CIPDS and CIWRKY genes demonstrated the feasibility of editing (Tian *et al.*, 2017) [52]. In carrot (*Daucus carota*), biolistic delivery of CRISPR constructs enabled targeted mutagenesis of DcPDS and carotenoid biosynthesis genes (Klimek-Chodacka *et al.*, 2018) [30]. In cabbage and other Brassica species, CRISPR has been used to modify fatty acid composition, glucosinolate profiles and self-incompatibility genes (Sun *et al.*, 2018). Onion and garlic, among the most recalcitrant species for transformation, represent emerging targets where nanoparticle and RNP delivery may unlock CRISPR potential (Mao *et al.*, 2022) [41].

Table 1: CRISPR-Cas applications in vegetable crop improvement

Crop	Trait category	Target gene (s)	CRISPR tool	Outcome / improvement
Tomato	Disease resistance	SIMLO1	Cas9	Broad-spectrum resistance to powdery mildew (<i>Oidium neolycopersici</i>) by disrupting the susceptibility gene.
Tomato	Quality & nutrition	ANT1, SGF714	Cas9	Increased anthocyanin accumulation, producing purple tomatoes with enhanced antioxidant content.
Tomato	Yield & growth	CLV3, WUS, SP	Cas9	Multiplex editing of fruit size and plant architecture genes to increase yield per plant.
Tomato	Abiotic stress	SIARF4	Cas9	Improved drought and salt tolerance through modulation of auxin signaling pathways.
Tomato	Development	RIN, NOR, CNR	Cas9	Extended shelf life by targeting ripening regulators, reducing post-harvest losses.
Potato	Disease resistance	StDMR6-1	Cas9	Enhanced resistance to late blight (<i>Phytophthora infestans</i>) without yield penalty.
Potato	Quality & nutrition	StGBSS, StSBE	Cas9	Modified starch composition (high-amylose or waxy starch) for industrial and food applications.
Potato	Abiotic stress	StVIF2	Cas9	Reduced cold-induced sweetening for improved cold storage and lower acrylamide in fried products.
Potato	Pest resistance	StAOS, StLOX3	Cas9	Enhanced resistance to Colorado potato beetle via jasmonate pathway modification.
Lettuce	Quality & nutrition	LsBBX29	Cas9	Elevated carotenoid levels and improved nutritional value of leaves.
Lettuce	Abiotic stress	LsNPF6.3	Cas9	Reduced nitrate accumulation in leaves, improving food safety for fresh consumption.
Pepper	Quality & nutrition	CaKR1	Cas9	Knockout of capsaicin biosynthesis genes to produce mild or non-pungent pepper varieties.
Pepper	Disease resistance	CaPDS	Cas9	Established transformation system; gene silencing confirms function in disease pathways.
Carrot	Quality & nutrition	DcLCYB1	Cas9	Altered carotenoid pathway to increase β -carotene (pro-vitamin A) in storage roots.
Cucumber	Yield & growth	CsLFY	Cas9	Accelerated flowering and modified plant architecture to enhance fruit yield in greenhouses.
Cucumber	Disease resistance	CsMLO	Cas9	Resistance to powdery mildew via mlo-based susceptibility gene disruption, similar to barley.
Watermelon	Development	CICG01G089020	Cas9	Seedless fruit development by targeting genes controlling seed coat and endosperm formation.
Eggplant	Quality & nutrition	SmCHS1, SmCHS2	Cas9	Reduced anthocyanin in skin for novel purple-free varieties with consumer preference potential.
Spinach	Abiotic stress	SoNHX1	Cas12a	Improved salinity tolerance through enhanced vacuolar Na ⁺ compartmentalization.
Cabbage	Pest resistance	BolTPS	Cas9	Increased glucosinolate content by editing terpene synthase loci, deterring aphid infestations.

5. Targeted traits and innovations in CRISPR-mediated vegetable improvement

5.1 Disease and pest resistance

Pathogens account for 10-40% of global vegetable crop losses annually (Strange and Scott, 2005) ^[49]. CRISPR-based resistance strategies primarily exploit susceptibility (S) gene knockouts, which disrupt host factors exploited by pathogens without introducing exogenous resistance genes. MLO family members, eIF4E translation initiation factors and SWEET sugar transporter genes are prominent S-gene targets across multiple vegetable crops. Knockout of eIF4E1 in cucumber conferred resistance to Cucumber vein yellowing virus, Zucchini yellow mosaic virus and Papaya ringspot mosaic virus-W (Chandrasekaran *et al.*, 2016) ^[9]. This milestone demonstrated that CRISPR can replicate natural resistance alleles found in wild germplasm.

Multiplexed editing of multiple susceptibility loci enables stacking of resistance to different pathogen races, addressing the limitation of single-gene resistance breakdown. CRISPR-mediated pyramiding of resistance mechanisms combining S-gene knockout with enhanced immunity gene activation via dCas9-VP64 activators represents a particularly promising strategy for durable broad-spectrum resistance (Peng *et al.*, 2017) ^[44].

5.2 Abiotic stress tolerance

Climate change-driven abiotic stresses principally drought, salinity, heat and cold represent escalating threats to vegetable production. CRISPR has been used to modulate stress response pathways by targeting negative regulators. In tomato, editing of SlARF4 (auxin response factor 4) improved drought tolerance by modulating stomatal conductance and root architecture (Bouzroud *et al.*, 2020) ^[6]. Knockout of SlHyPRP1 (hybrid proline-rich protein 1) enhanced salt stress tolerance through reduced ion accumulation (Tran *et al.*, 2021) ^[53].

In potato, CRISPR editing of StDREB transcription factors modulated cold acclimation pathways, reducing chilling injury during storage and transport. Targeting ACC deaminase-related genes in pepper demonstrated potential for heat stress mitigation. As CRISPR techniques become more efficient in polyploid vegetables, simultaneous editing of all allelic copies of stress-responsive genes will enable more substantial improvements in abiotic stress tolerance.

5.3 Nutritional quality enhancement

CRISPR has emerged as a powerful tool to redesign the nutritional profiles of vegetable crops, addressing both micronutrient deficiencies prevalent in developing countries and the demand for functional foods in affluent markets. Key achievements include increased lycopene and beta-carotene in tomato, reduced solanine content in potato, elevated anthocyanin levels in purple lettuce (via MYB activator editing) and modified glucosinolate profiles in Brassica vegetables (Gao *et al.*, 2021) ^[21].

CRISPR-mediated reduction of anti-nutritional factors represents a parallel priority. Reduction of solanine alkaloids in potato, oxalate in spinach and lectins in leguminous vegetables can improve bioavailability of minerals and protein and reduce adverse health effects. These edits are particularly valuable when combined with positive nutritional traits, creating multi-trait improved varieties through multiplexed CRISPR editing (Ye *et al.*, 2020) ^[60].

5.4 Post-harvest quality and shelf life

Post-harvest losses in vegetables can exceed 40-50% in developing countries due to rapid senescence, mechanical damage and pathogen infection. CRISPR provides precise tools to address these losses by targeting ethylene biosynthesis, cell wall metabolism and browning enzymes. Beyond the tomato and potato examples cited above, CRISPR-mediated editing of cell-wall-loosening enzymes (expansins, polygalacturonases, pectate lyases) in pepper and cucumber has extended shelf life by 20-40% in controlled storage trials (Yu *et al.*, 2022) ^[62].

5.5 Yield and plant architecture

Domestication-associated traits including determinate growth, reduced branching and increased fruit size arose from natural mutations in key developmental genes. CRISPR enables re-capitulation of beneficial domestication alleles or introduction of novel quantitative trait modifications. Beyond tomato meristem expansion, CRISPR editing of tomato CLAVATA pathway genes in wild *Solanum pimpinellifolium* enabled rapid *de novo* domestication, simultaneously modifying fruit size, number and plant architecture (Zsogon *et al.*, 2018). Similar approaches are being explored for eggplant and pepper to accelerate domestication of wild relatives harboring disease resistance or stress tolerance.

6. Challenges and limitations

6.1 Off-target editing

Despite the high specificity of CRISPR-Cas systems, off-target editing remains a significant concern, particularly in crops with complex polyploid genomes where sgRNAs may hybridize to homeologous sequences. Whole-genome sequencing and bioinformatic tools such as CRISPOR, Cas-Offinder and GT-Scan are essential for sgRNA design and off-target prediction (Concordet and Haeussler, 2018) ^[13]. Use of high-fidelity Cas9 variants, paired nickase strategies and RNP delivery (which minimizes Cas9 persistence in cells) substantially reduces off-target frequencies. Nevertheless, comprehensive off-target assessment remains a prerequisite for regulatory submission and commercial deployment.

6.2 Delivery and transformation efficiency

Efficient delivery of CRISPR components and subsequent plant regeneration represent bottlenecks for many vegetable species. Recalcitrant regeneration from callus or protoplasts is a major constraint in species such as carrot, onion, garlic and leafy greens. Morphogenic regulator-assisted transformation (using BABY BOOM, WUS2, or GRF-GIF overexpression) has demonstrated substantial improvements in transformation efficiency across multiple recalcitrant species (Lowe *et al.*, 2016) ^[37]. Species-specific optimization of tissue culture protocols, *Agrobacterium* strains and selection conditions remains a major research priority.

6.3 Polyploidy and allelic complexity

Many economically important vegetables are polyploid (tetraploid potato, hexaploid wheat, allotetraploid Brassica species), requiring simultaneous editing of multiple alleles to achieve homozygous phenotypes. While CRISPR can successfully edit all alleles in polyploids as demonstrated for VInv in tetraploid potato editing efficiency and the

probability of achieving full allele disruption decrease with increasing ploidy. Advanced editing strategies using multiple sgRNAs, optimized Cas protein concentrations and selection of multiplex-edited events via molecular screening are being developed to address this challenge.

6.4 Regulatory frameworks and compliance

The regulatory treatment of CRISPR-edited crops varies substantially across jurisdictions and profoundly influences the pace of commercialization. The United States generally does not regulate CRISPR-edited crops lacking inserted DNA as GMOs under the USDA SECURE rule (2020). The European Court of Justice (2018) ruled that most new genomic techniques, including CRISPR, fall under the existing GMO Directive 2001/18/EC, though proposed revisions under the New Genomic Techniques (NGT) regulation (2023) seek to differentiate SDN-1 (small deletions/substitutions) edits from transgenesis. China, Japan, Australia, Canada and Brazil have established or are developing more permissive frameworks for DNA-free or transgene-free CRISPR products. This regulatory fragmentation creates market access challenges for developers targeting global markets (Whelan and Lema, 2015) ^[56].

6.5 Intellectual property and access

The CRISPR-Cas9 patent landscape is complex, with foundational patents held by the Broad Institute, UC Berkeley and affiliated institutions in various jurisdictions. Patent licensing costs and freedom-to-operate uncertainty can restrict access for public sector breeders and researchers in developing countries. Open licensing initiatives, patent pools and humanitarian use exceptions are important mechanisms for ensuring equitable access to CRISPR tools for crop improvement in food-insecure regions (Fudge *et al.*, 2021) ^[20].

6.6 Public perception and consumer acceptance

Consumer acceptance of CRISPR-edited vegetables varies significantly by geography, cultural background and communication framing. Studies indicate higher acceptance when edits mimic natural variation, reduce agrochemical use, or confer consumer-facing benefits such as enhanced nutrition or extended shelf life. Transparent communication of the precision and safety of CRISPR editing, engagement with civil society organizations and participatory approaches involving farmers and consumers in trait prioritization can improve public trust. The designation of CRISPR-edited crops as 'non-GMO' in some jurisdictions while classifying them as GMO in others creates consumer confusion that requires harmonized global science communication strategies (Lucht, 2015) ^[40].

7. Future prospects

7.1 Multiplex editing and trait stacking

The ability to simultaneously edit multiple genomic loci enables the introduction of several desirable traits in a single transformation event, dramatically accelerating the breeding cycle. Advances in multiplex sgRNA expression systems, including tRNA-sgRNA arrays and Csy4-mediated processing, allow editing of 6-10 targets simultaneously (Xie *et al.*, 2015) ^[59]. Future vegetable varieties will likely incorporate stacks of disease resistance, stress tolerance, nutritional enhancement and yield improvements achieved

through single-round multiplex editing rather than sequential introgression.

7.2 Precise gene replacement via HDR and prime editing

While NHEJ-based knockouts dominate current CRISPR applications, the ability to introduce specific, predetermined sequence changes via HDR or prime editing will unlock a much broader range of improvements. Prime editing in particular offers transgene-free, precise allele replacement that closely mimics classical breeding outcomes, potentially satisfying regulatory criteria for 'conventional' breeding products in many jurisdictions. As prime editing efficiency in plants improves through optimization of pegRNA architecture and PE protein variants, applications in vegetable crops are expected to expand rapidly (Anzalone *et al.*, 2020) ^[4].

7.3 CRISPR in de novo domestication

The concept of de novo domestication using CRISPR to rapidly introduce domestication syndrome traits into wild relatives harboring superior stress tolerance, disease resistance, or nutritional profiles represents a transformative approach to vegetable improvement (Fernie and Yan, 2019) ^[19]. Wild tomato relatives, wild eggplant and wild Brassica species harbor traits absent from elite germplasm. CRISPR-mediated introduction of yield-enhancing and quality traits into these wild backgrounds could generate novel crop types within 3-5 years rather than the decades required by conventional introgression breeding.

7.4 Integration with genomics and artificial intelligence

The power of CRISPR editing is amplified when integrated with high-resolution genomic data, genome-wide association studies (GWAS) and machine learning algorithms that identify high-value editing targets. AI-driven sgRNA design tools now predict cutting efficiency and off-target risk with high accuracy (Kim *et al.*, 2019) ^[28]. Pangenome analyses of vegetable species enable identification of species-wide functional variation, guiding target selection for broad-spectrum improvements. The convergence of genomics, phenomics and CRISPR editing within precision breeding pipelines represents the future of scientifically driven vegetable improvement.

7.5 CRISPR-enabled climate resilience

Climate change adaptation represents one of the most urgent applications of CRISPR in vegetable crops. Engineering heat-tolerant flowering phenology, drought-adapted root architectures, salinity-tolerant ion homeostasis and modified carbon fixation pathways are among the targets under active investigation. CRISPR-edited vegetable varieties tolerant to 2-4 °C temperature increases and capable of productive growth under 20-30% reduced water availability are feasible within the next decade, providing critical insurance against projected climate impacts on food systems (Abdelrahman *et al.*, 2021) ^[1].

7.6 Regulatory harmonization and global governance

However, to realize the full potential of CRISPR technology for the improvement of vegetable crops, there needs to be a move towards a more convergent approach to the regulation of the technology internationally, emphasizing a science-based and proportionate approach to risk assessment. The European Union's proposed NGT regulation and the

OECD's New Approaches to Risk Assessment project are significant developments toward the internationalization of the regulation of the technology. Product registries and tiered systems of approval, depending on the nature of the edit, and the formation of international scientific committees could aid the technology and consumer confidence (Purnhagen *et al.*, 2021) ^[45].

8. Conclusions

CRISPR-Cas genome editing has proven to be a revolutionary innovation in vegetable crop improvement, with unprecedented precision, efficiency, and versatility in the entire range of vegetable species/crops. From powdery mildew-resistant tomatoes and cucumbers to bruise-free potatoes, long shelf-life peppers, and nutrition-enhanced lettuces, the range of applications already demonstrated with the new technology underscores the immense opportunities it has to transform vegetable production for sustainable and nutritious food systems.

Significant advances are already being made in base editing, prime editing, multiplexing, de novo domestication, and AI-assisted precision breeding with CRISPR-Cas systems. These advances are expected to revolutionize the development of climate-resilient, high-quality vegetable crops in much shorter periods than the decades it has taken traditionally.

However, there are several issues to be addressed with the new technology, including regulatory hurdles, intellectual property rights, transformation recalcitrance in certain species, and perception issues, which will require concerted efforts from the scientific community, policymakers, and communicators to address.

With continued innovation, cooperation, and governance, it has the potential to support the development of vegetable crops that are more resilient, nutritious, productive, and sustainable, which will make significant contributions to global food security in the coming decades.

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