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REVIEW

Advancing vegetable genetics with gene editing: a pathway to food security and nutritional resilience in climate-shifted environments

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Abstract

As global populations grow and climate change increasingly disrupts agricultural systems, ensuring food security and nutritional resilience has become a critical challenge. In addition to grains and legumes, vegetables are very important for both human and animals because they contain vitamins, minerals, and fibre. Enhancing the ability of vegetables to withstand climate change threats is essential; however, traditional breeding methods face challenges due to the complexity of the genomic clonal multiplication process. In the postgenomic era, gene editing (GE) has emerged as a powerful tool for improving vegetables. GE can help to increase traits such as abiotic stress tolerance, herbicide tolerance, and disease resistance; improve agricultural productivity; and improve nutritional content and shelf-life by fine-tuning key genes. GE technologies such as Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR-Cas9) have revolutionized vegetable breeding by enabling specific gene modifications in the genome. This review highlights recent advances in CRISPR-mediated editing across various vegetable species, highlighting successful modifications that increase their resilience to climatic stressors. Additionally, it explores the potential of GE to address malnutrition by increasing the nutrient content of vegetable crops, thereby contributing to public health and food system sustainability. Additionally, it addresses the implementation of GE-guided breeding strategies in agriculture, considering regulatory, ethical, and public acceptance issues. Enhancing vegetable genetics via GE may provide a reliable and nutritious food supply for an expanding global population under more unpredictable environmental circumstances.

Keywords Breeding · CRISPR-Cas9 · Crop improvement · Environmental stress · Gene editing · Vegetables

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Abbreviations

Cas9	CRISPR-associated protein 9 nuclease
CRISPR	Clustered regularly interspaced short palindromic repeat
DSBs	Double-strand breaks
gRNA	Guide RNA
GE	Gene editing
HDR	Homology-directed repair
NHEJ	Nonhomologous end-joining
RBOHD	Respiratory burst oxidase homologue D
PAM	Protospacer adjacent motif
TALENs	Transcription activator-like effector nucleases
ZFNs	zinc-finger nucleases

Vegetable production and its challenges: how does climate change impact the vegetable industry?

Vegetable crops are plants cultivated primarily for their edible parts, such as leaves, stems, roots, flowers, or fruits, that are consumed as part of the human diet. These crops are rich in essential nutrients, vitamins, minerals, and dietary fibre, making them important components of a balanced and healthy diet. Vegetable crops are widely grown worldwide and are an integral part of global agriculture and food systems (Roychowdhury and Tah 2011). Approximately forty vegetable species are cultivated and consumed worldwide in both the summer and the winter (Kumari 2023). Most usable vegetable species belong to different families, such as Solanaceae, Cucurbitaceae, Leguminoceae, and Cruciferae, and are classified as leafy vegetables (amaranth, lettuce, spinach, kale, swiss chard), roots (carrots, potatoes, sweet potatoes, beets, radishes, turnips), stems (asparagus, celery, sago palm, florence fennel), inflorescences (broccoli, cauliflower, cabbage), or fruits (tomato, eggplant, peppers, cucurbits).

Vegetable production plays a critical role in global food security, nutrition, and economic livelihoods, but it faces many challenges that threaten its sustainability and effectiveness. Despite significant revenues, the irrigation process has caused major hurdles in vegetable production under climatic changes (Roychowdhury 2014; Roychowdhury et al. 2020). The effects of climate change on vegetable production can be multifaceted, affecting crop yield, quality, and distribution. There is an increased likelihood of experiencing poor outcomes: either immediate crop loss in the short term or prolonged decreases in yields over time due to variations in temperature and rainfall (Porter et al. 2019). An altered climate also promotes the growth of pathogens, pests and weeds (Jasper et al. 2020) and reduces vegetable production. Vegetable yields in South and Central Asia are expected to decline by up to 10% from 2020 onwards and will reach 30% by 2050, of which the estimated potential yield losses are 17% due to drought, 20% due to salinity, 40% due to high temperature and 15% due to low temperature (Rashid et al. 2020). Climate change will impose multiple stresses that should be addressed by a combination of improved varieties and management practices (Chakraborty et al. 2014). For most crops, tolerance to heat, drought, salinity, and other stresses is physiologically and genetically complex, complicating breeding programs (Hasanuzzaman et al. 2015; Anumalla et al. 2016). Even if stress-tolerant varieties are developed, farmer adoption will still be limited until the varieties meet the requirements of all the involved stakeholders, such as transporters, wholesalers, retailers, and consumers (Parajuli et al. 2019). The vegetable industry is intricately linked to market dynamics (Ma et al. 2024). Climate-related challenges can result in increased production costs, supply chain disruptions, and fluctuations in vegetable prices. Small-scale and subsistence farmers may face economic hardships due to climate-related uncertainties.

One of the most significant impacts of climate change on the vegetable industry is the alteration of growing seasons. Changes in precipitation patterns, including variations in rainfall frequency and intensity, can affect water for vegetable crops. This can lead to drought stress or waterlogging, which negatively impacts crop yields and quality. Inconsistent water availability also increases the likelihood of irrigation issues for farmers. Climate change exacerbates water scarcity, particularly in regions with constrained water resources. Since vegetables are water-intensive crops, they may face heightened competition for these resources. Farmers might need to modify their irrigation methods and implement water-efficient technologies to maintain vegetable production (Roychowdhury et al. 2020). Rising temperatures can lead to early flowering and fruiting, disrupting the synchronization between crop development and market demand. Additionally, changes in precipitation patterns and UV rays can result in water scarcity or excessive rainfall, which can negatively impact crop growth and survival (Hasanuzzaman et al. 2013; Chakraborty et al. 2014). Changes in temperature and humidity patterns contribute to the geographical redistribution of pests and diseases (Skendžić et al. 2021). New pests and diseases may emerge, and existing ones may proliferate. This requires adjustments in pest management strategies, including the development of resistant crop varieties and the use of integrated pest management practices (Skendžić et al. 2021). Climate change can disrupt traditional growing seasons, affecting the planting and harvesting times of vegetables. Warmer temperatures may lead to shifts in phenological events, influencing the timing of flowering, fruiting, and maturation. Farmers may need to adjust their planting calendars and explore alternative crop varieties suited to changing climatic conditions (Geissler et al. 2023). Changes in temperature and precipitation patterns can affect

soil structure, nutrient availability, and microbial communities (Philippot et al. 2024). Extreme weather events such as floods and droughts can lead to soil erosion and nutrient leaching (Bogati and Walczak 2022). Maintaining soil health becomes crucial for sustaining vegetable production under changing climatic conditions. Climate change threatens the biodiversity of plant varieties, including the diversity of species. Certain specific heirloom or traditional vegetable varieties may become less viable in altered climates, leading to a loss of genetic diversity within crops (Muluneh 2021). Preserving and utilizing diverse germplasms has become essential for breeding climate-resilient vegetable varieties (Muluneh 2021). Climatic alternation can deteriorate soil health through diversification by various minerals and heavy metal components. The heavy metal response of different plant parts of *Solanum melongena* was analysed by Roychowdhury and Tah (2011). Adapting to the impacts of climate change in the vegetable industry requires a combination of resilient crop varieties, sustainable agricultural practices, improved water management, and the implementation of climate-smart technologies (Raza et al. 2019). Research, innovation, and collaboration across the agricultural value chain are essential to build climate-resilient vegetable production systems. These climatic instabilities until the harvest season can affect flowering, pollination, growth, and fruit development, leading to lower vegetable yields (Roychowdhury and Tah 2011; Roychowdhury et al. 2011). Such effects may vary across regions and crops, but several common trends and challenges are observed globally. Consequently, both traditional methods and modern plant molecular breeding techniques are being utilized to create vegetable varieties that offer improved nutritional value, increased resilience to biotic and abiotic stresses, and consistently improved yields (Abdallah et al. 2015).

Therefore, there is a need to develop next-generation crops that can withstand extreme environmental stresses. Traditional breeding techniques are commonly employed to increase agronomic performance and yield, although these methods are often lengthy, labor intensive, and time consuming (Zhang et al. 2018). Despite a few exceptions, mutation breeding has rarely been employed to improve vegetable crops, as most methods are not particularly potent (Roychowdhury and Tah 2013; Ray et al. 2012; Basu et al. 2012). Over the past few decades, numerous advancements in molecular biology techniques have emerged to increase crop yield and quality. Recently, site-directed nucleases—a genome editing technology that enables swift modification of crops and holds great promise for tailoring the specific genotypes of vegetable crops—have seen remarkable progress (Tian et al. 2021). These genome editing technologies allow for precise alterations of genes by deleting, replacing, or inserting specific sequences at targeted locations within the genome, resulting in the development of unique traits (Görücü Yilmaz 2021). First-generation tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have faced limitations because of their problematic mutagenesis, low editing efficiency, and the lengthy and labor-intensive processes involved in selection and screening (González et al. 2021). In contrast, CRISPR-Cas9 represents a second-generation genome editing technology that is more cost-effective and simpler to design and implement than earlier gene or genome editing (GE) tools. According to Wan et al. (2021), advancements in CRISPR−Cas9 technology for vegetable crops have rapidly enhanced GE capabilities, enabling the development of new genotypes with desirable phenotypic traits and base pair-level genomic modifications.

Gene editing tools and technology—mechanisms, variations and advancements

GE in vegetable crops has emerged as a groundbreaking and precise approach to enhance crop characteristics and agricultural productivity. Among all horticultural crops, tomatoes have gathered significantly more attention in GE, constituting nearly 42% of the studies, whereas potatoes account for approximately 13%. Although vegetable crops dominate GE in horticulture at 72%, successful applications have also been made in various leafy and underground vegetables. CRISPR-Cas9 and other GE tools have revolutionized plant breeding, enabling scientists to modify specific genes with unprecedented accuracy (Tuncel et al. 2023; Li et al. 2024). GE has various applications in vegetable crops, including improving disease resistance, enhancing abiotic stress tolerance, increasing nutritional content, and extending shelf-life. By targeting and modifying genes responsible for specific traits, researchers can develop vegetable varieties that are more resilient to environmental challenges, have improved nutritional value, and exhibit better postharvest characteristics. However, the responsible use of GE in vegetable crops requires careful consideration of ethical, regulatory, and environmental implications to ensure its safe and sustainable deployment in agriculture. In future agriculture, GE will continue to play a transformative role in vegetable breeding strategies, pushing the boundaries of crop improvement to new heights. Advancements in GE technologies will increase precision, efficiency, and versatility, enabling breeders to target more complex traits and regulatory elements within vegetable genomes (Tuncel et al. 2023). The ability to edit multiple genes simultaneously or to precisely control gene expression will unlock novel avenues for developing customized vegetable varieties tailored to specific environmental conditions and consumer preferences. As our understanding of the genetic basis of vegetable traits deepens, GE will facilitate the identification and manipulation of key genes. Moreover, GE has the potential to overcome breeding

barriers and accelerate the introgression of beneficial traits from wild relatives or distant species, enriching the genetic diversity of vegetable crops (Devi et al. 2022). With robust ethical frameworks and broad public acceptance, GE will empower vegetable breeders to address global challenges, such as food security, sustainability, and nutritional deficiencies, paving the way for more resilient and nourishing vegetable agriculture in the future (Atia et al. 2024).

CRISPR-based gene editing and its variation

The recent advent of molecular biology has led to the development of modern agriculture with efficient GE technologies that enable the improvement of vegetable genomes with precise (nonrandom) manipulation. GE techniques include genome editing and engineering and allow the targeting and modification of specific DNA sequences (Devi et al. 2022). GE through the CRISPR−Cas9 system holds considerable potential for regulating genomic and epigenetic processes. The scaffold formed by the Cas9 and sgRNA components enables the targeting of specific DNA sites with various effectors or markers. This feature of CRISPR-Cas9 allows for the activation or repression of genes (known as CRISPRa and CRISPRi), thereby altering the transcriptional levels of genes (Ghavami and Pandi 2021). Although the double-strand break (DSB) method has proven to be a successful technique for genome editing, it also poses the risk of off-target GEs, which can result in unintended alterations. One such approach involves a dead Cas9 variant known as dCas9, in which the two catalytic domains are rendered inactive through point mutations. While dCas9 does not create double-strand breaks in the genome, it effectively binds to targeted sites. Owing to its unique characteristics, dCas9 is often combined with specific enzymes that can facilitate necessary genomic modifications. Furthermore, the CRISPRa and CRISPRi systems utilize this nuclease-deactivated Cas9, where the catalytic domains are inactivated and fused with transcriptional modulators (Jensen et al. 2021). GE introduces alterations in plant genomes in three common steps. An exogenous nuclease first recognizes the target DNA sequence; the nuclease then binds to the target DNA sequence and introduces DSBs; finally, which are repaired via endogenous nonhomologous end-joining (NHEJ) or homology-directed repair (HDR) pathways (Singh et al. 2020) (Fig. 1). In 2013, the first application of genome editing in a vegetable crop (*Brassica oleracea*) was achieved via TALEN technology. While ZFNs and TALENs are considered first-generation genome editing tools, CRISPR-Cas9 is the most recent and advanced tool. A FokI nuclease domain and a sequence-specific DNA binding module are found in ZFNs and TALENs. However, to become an active nuclease, the FokI nuclease domain must dimerize, which is both expensive and complicated (Das et al. 2023). The CRISPR−Cas9 system has stepped in to address this need and has emerged as a prominent GE tool because of its ease of use, low cost, and excellent efficiency (Roychowdhury et al. 2020).

The CRISPR-Cas9 system

The general mechanism of CRISPR involves three phases: interference, expression, and adaptability. Bacteria capture short, recognizable protospacer sequences from invading organisms and integrate them into their genome via adjacent CRISPR sites as a form of adaptation. These newly acquired sequences are referred to as CRISPR spacer sequences and are added to the CRISPR array to encode the invader's memory. After the protospacer from the invading organism is assimilated into the CRISPR locus, the subsequent phase is expression, during which the CRISPR locus is transcribed into precrRNA and subsequently processed into mature crRNAs. The final phase entails the creation of a complex between the RNA (crRNA) and the Cas protein. This complex subsequently aligns complementary bases with the invader's protospacer, enabling a second invasion. Ultimately, the Cas endonuclease protein aids in crRNA-directed cleavage of the DNA. The Cas9 protein, an RNA-dependent DNA endonuclease, and a guide RNA (gRNA) are the two main components of the CRISPR-Cas9 system (Fig. 2). The gRNA is a short RNA molecule that comprises 20 nucleotides that are complementary to target sequences and forms a complex with Cas9 to attract it to the target site

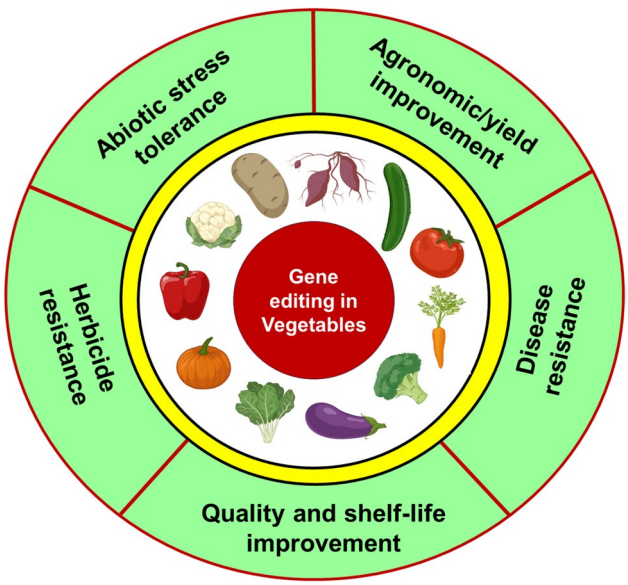

Fig. 1 Graphical abstract showing the successful implementation of gene editing tools to incorporate beneficial traits (e.g., abiotic stress tolerance, disease resistance, herbicide resistance, agronomic traits or yield improvement, quality, and shelf-life improvement) in vegetable crops

(Singh 2020). CRISPR-Cas9, therefore, depends on DNA−RNA interactions for target DNA sequence recognition, whereas other GE technologies, such as ZFNs and TALENs, rely on DNA−protein interactions. Recognizing the precise sequence via DNA−protein interactions necessitated designing and producing two distinct DNA-binding domains per target site, which was complicated and time-consuming. However, because CRISPR-Cas9 interacts with DNA via RNA, only an 18–20 bp oligonucleotide is needed. Cas9 and gRNA attach to a particular protospacer adjacent motif (PAM) sequence at the 3′ end of the target region to operate as genome editing tools. The CRISPR system was originally developed from *Streptococcus pyogenes* (SpCas9), but many different Cas9 orthologues with diverse properties have been identified. Within the Class I CRISPR−Cas system, various Cas protein modules form a complex known as the cr-RNA-binding complex, which is responsible for binding and processing the target. In contrast, the Class II CRISPR−Cas system features a single, multidomain cr-RNA-binding protein that functions similarly to the entirety of the Class I complex (Sharma et al. 2023). Class I encompasses Types I, III, and IV, whereas Class II consists of Types II, V, and VI (Makarova et al. 2020). A key consideration is the efficiency with which the CRISPR−Cas9 system is introduced into the target cell. This can be achieved through various forms, such as messenger RNA, ribonucleoproteins (RNPs), or plasmid DNA. A novel approach to genome editing known as RNP, which comprises a gRNA and the Cas9 protein, is also being

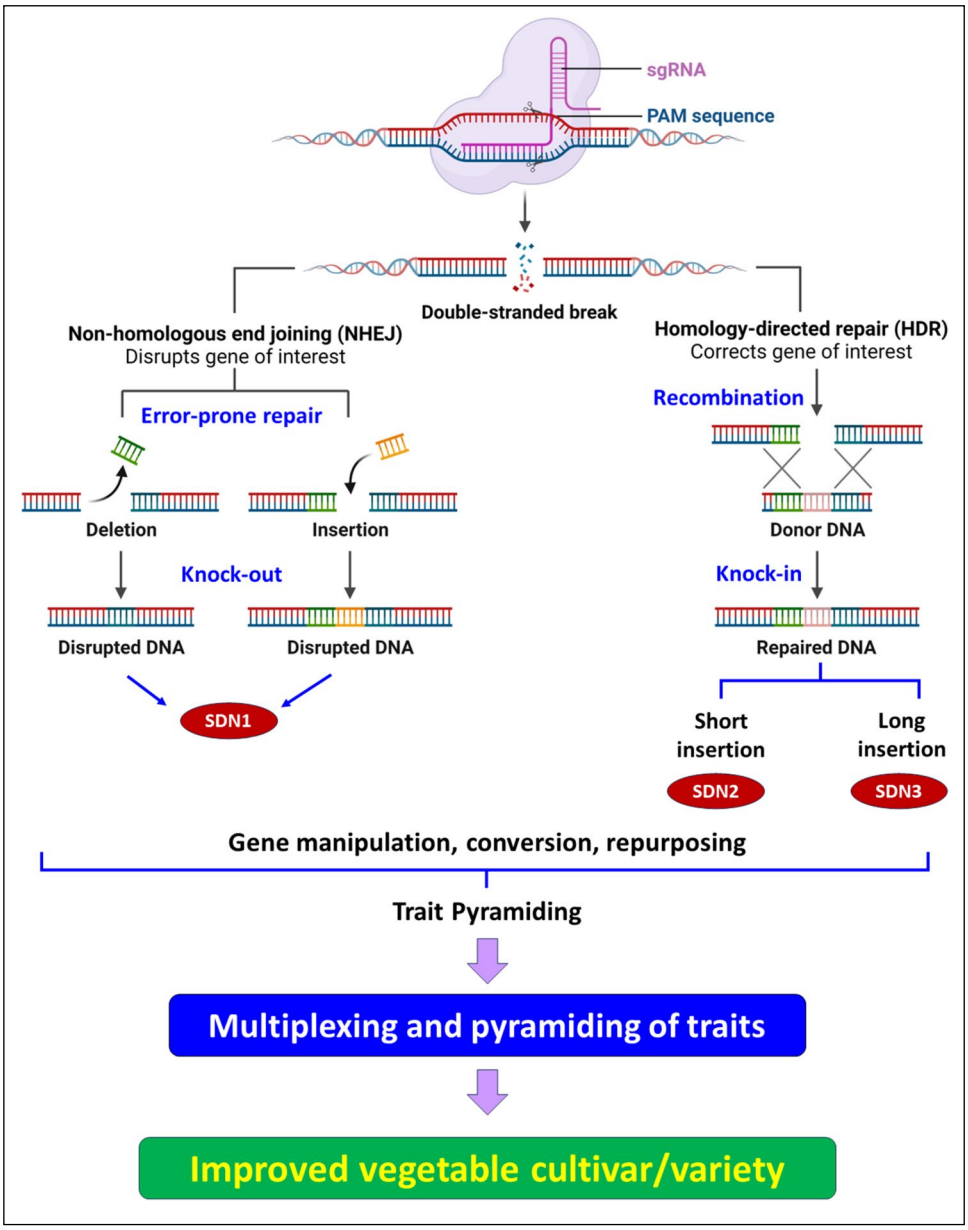

Fig. 2 Working model of CRISPR−Cas-mediated gene editing in vegetables and its involvement in breeding-mediated improvement

utilized. RNP-based genome editing boasts several significant advantages, such as being a DNA/transgene-free technique that minimizes off-target effects and reduces toxicity owing to its absence of DNA (Zhang et al. 2021).

Base editing (BE), prime-editing (PE), multiplex and epigenome editing

CRISPR modification in base editing modifies nucleotide bases with the help of sgRNA and the dCas9 protein, along with activation-induced deaminase (AID), such as adenine deaminase or cytidine deaminase base editors. This technique does not involve double-strand breaks, resulting in greater efficiency. Additionally, base editing has been applied for precise editing in plants. Base editors, specifically cytosine base editors (CBEs), facilitate the transition of cytosine to uracil through deamination, which is subsequently transformed into thymidine via DNA replication or repair mechanisms. These editors have gained significant traction for modifying the genomes of various plant species. Base editing employing the CRISPR-Cas9 system was utilized to alter gain-of-function mutations in *Arabidopsis* (Chen et al. 2017). Similarly, Azameti and Dauda (2021) accomplished targeted base editing for rice and tomato crops by using cytidine deaminase in conjunction with the Cas9 protein. In a related process, adenine deaminase (ABE) linked to dCas9 enables the conversion of adenine to inosine, allowing it to pair with cytosine. The guanine pairs of the newly synthesized strand with cytosine, altering the guiding DNA.

Matsoukas (2020) developed prime editing as an innovative tool for CRISPR-mediated genome editing. Prime editing involves copying and incorporating desired edits within guide RNA without the use of DSBs or donor repair templates (Anzalone et al. 2019). Although this technique has been reported in a wide variety of cereal crops owing to its advantage of encountering fewer bystander mutations, it has not been researched in vegetables (Fiaz et al. 2021). Thus, for prime editing in vegetable crops, the choice of suitable editing strategy should depend on the desired edit, availability of PAMs, editing efficiency, and chances of generating bystander mutations (Hao et al. 2021). The technique consists of three main components: a prime-editing guide RNA (pegRNA), a Cas9 nickase, and a reverse transcriptase enzyme-linked together. PegRNA is distinguished from other sgRNAs by its features, which include a primer binding site (PBS) at the 3′ end, a sequence that specifies the desired modifications adjacent to the PBS, and a sequence at the 5′ end that is complementary to the target site of the template DNA. The complementary guide sequence at the 5′ end of the pegRNA directs Cas9 to a specific DNA region, where it makes a nick in the PAM-containing DNA strand. When a nick occurs on the exposed 3′-OH group of the target DNA, the 3′ end of the nicked template strand binds with the PBS of the pegRNA, utilizing the RT template to create modified genetic material. This process results in either the formation of a 3′ flap containing the desired sequence or a 5′ flap that retains the original sequence, which is achieved through the hybridization of target DNA with RT (Wada et al. 2020). By selectively cutting the 5′ flaps, endonucleases generate a DNA duplex that includes both the original and edited strands with the intended modifications. This mismatch is rectified through cellular replication or a mismatch repair process, facilitating the stable incorporation of the desired sequence into the genome.

On the other hand, multiplex gene editing is a powerful biotechnological approach that allows simultaneous editing of multiple genes or genomic loci. In multiplex editing, sgRNAs are designed to target specific genomic loci. Each sgRNA directs the Cas nuclease to a distinct DNA sequence. sgRNAs, along with Cas proteins, are delivered into cells via vectors (e.g., plasmids, viral systems) or direct RNA−protein complexes. In this process, simultaneous editing saves time compared with sequential gene editing and enables the modification of traits controlled by multiple genes (Wu et al. 2024). In addition, advanced tools such as CRISPR-Cas12a and multiplexed HDR templates improve the targeting accuracy.

Another advanced gene editing tool is epigenome editing, which is essentially a cutting-edge biotechnological tool that allows precise modification of the epigenetic state of specific genomic loci without altering the underlying DNA sequence (Jogam et al. 2022). This technology focuses on altering epigenetic marks such as DNA methylation, histone modifications, and chromatin architecture to regulate gene expression. It comprises programmable DNA-binding domains (CRISPR-dCas9, i.e., with deactivated or dead Cas9), which are fused with epigenetic effectors to modulate the epigenome. The epigenetic effectors can be DNA modifiers [e.g., DNA methyltransferase (DNMT), TET proteins], histone modifiers [histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs)], and chromatic remodellers, which are essentially proteins that restructure chromatin to make it accessible. These systems have shown remarkable efficacy when used to plant RNA virus interference. Furthermore, they can bind RNA enough to permit A-to-G nucleotide editing when coupled with a dCas13 deaminase domain, enabling full-length transcript editing for harmful point mutations. Crop yields, resilience, and consumer preferences for meeting nutritional needs over genetically modified organisms (GMOs) have increased because of the rapid development of these adaptable modified CRISPR tools (Khan et al. 2024). The dCas9-SunTag system increases gene transcription by fusing dCas9 with VP64, hybrid VP64-p65-Rta (VPR) activators, and a transcription activation domain-like synergistic activation mediator (SAM). The dCas9-SunTag system is

a powerful transcriptional activator that comprises dCas9 linked to tandem GCN4 peptide repeats and a scFv GCN4 antibody coupled to sfGFP and VP64 (Papikian et al. 2019). Using dCas9 combined with DNMT or TET, researchers created an epigenetic regulator for plants. TET1 demethylates DNA by oxidizing 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). This approach revealed heritable DNA methylation at gene promoters and other off-target regions and thus can effectively alter epigenomes to modify plant traits. The dCas9–p300 acetyltransferase augments the acetylation of histone H3 lysine 27 (H3K27) in proximity to the promoter and enhancer regions, hence increasing gene expression.

Gene editing in vegetables enables the ability to cope with climate change effects

Vegetables are vital to the human diet because they contain cellulose, vitamins, trace elements, minerals, and other essential nutrients (Septembre-Malaterre et al. 2018). Therefore, scientists are continually attempting to improve vegetable varieties in terms of various yields and quality-related traits, such as increasing various dietary and agronomic values and tolerance to biotic and abiotic stresses (Abdallah et al. 2015). In this review, we address the application of genome editing, specifically CRISPR-Cas9, to vegetable crops to improve the traits enumerated in Table1.

Abiotic stress tolerance

Vegetable crops are subjected to numerous abiotic stresses, such as temperature, drought, salinity, and humidity, which negatively impact their production. It is estimated that drought stress has led to a yield reduction of 42% in soybeans and approximately 68% in cowpeas (Farooq et al. 2017). CRISPR-Cas9 allows the creation of more abiotic stress-tolerant vegetable varieties. In tomato, *bzr1* and *Slmapk3* mutants exhibit both heat and drought stress tolerance (Yu et al. 2019). In addition, tomato plants exhibit elevated leaf water content under drought conditions and have undergone *GID1*GE via CRISPR-Cas9 (Illouz-Eliaz et al. 2020). Moreover, Liu et al. (2020) demonstrated that CRISPR-mediated mutation of the *SlLBD40* gene significantly improved the drought resistance of tomatoes. Furthermore, Wang et al. (2017) identified *SlMAPK3* as a modulator of drought stress by using the CRISPR−Cas method to alter mitogen-activated protein kinases (MAPKs). Yin et al. (2018) reported the *Agrobacterium*-mediated heat stress tolerance target gene (*BZR1*) knockout from tomatoes. Knockout of the *SINPR1* gene resulted in the downregulation of drought-related genes and increased drought resistance (Li et al. 2019). In 2021, Wang et al. (2017) and Chen et al. (2017) described two diverse target genes, *FDM1* and *ARF4*, that are involved in the development of drought sensitivity and drought stress tolerance in tomato. Similarly, CRISPR-Cas9-mediated gene knockout of *9-cis-EPOXYCAROTENOID DIOXYGENASE4 (LsNCED4)* enabled high-temperature germination in lettuce (Bertier et al. 2018). Like excessive temperature, chilling stress hinders the development of certain vegetable crops, including tomato, eggplant, and pepper. GE techniques for the *CBF1* gene are being used to generate new cold-tolerant germplasms (Li et al. 2018e). Furthermore, these modified plants presented significant increases in indole acetic acid and hydrogen peroxide contents, contributing to the development of tomato plants capable of enduring chilling stress. To place *EPSPS* in chili under the control of glyphosate, CRISPR-Cas9 was employed to induce a mutation in the gene promoter region (Shimatani et al. 2017). The modified crops exhibited a degree of resistance to glyphosate, and subsequent studies indicated that selecting an alternative promoter might enable the development of chilli that are entirely resistant to glyphosate (Shimatani et al. 2017). Makhotenko et al. (2019) reported osmotic and salinity stress-tolerant potato cultivars through "*Colin*" knockout. Additionally, measures were taken to protect the plants from excessive UV-B exposure. UV-B photoreceptors in tomato were modified via CRISPR-Cas9 to generate *sluvr8* mutants with tolerance to high UV-B concentrations (Liu et al. 2020). High salt concentrations are also detrimental to plant survival (Petretto et al. 2019). *RBOHD* gene deletion through CRISPR-Cas9 confers K^+uptake and salinity tolerance in pumpkin (Huang et al. 2019). Similarly, salinity stress tolerance in sugar beets was reported by Pattanayak et al. (2023). Numerous gene types in sugar beets are associated with salt tolerance, including the recently discovered *BvbHLH93*gene, which features a helical structure linked to salt response or tolerance. Makhotenko et al. (2019) investigated CRISPR-Cas9 modification with RNP delivery via biolistic or vacuum infiltration methods for the salinity stress- and osmotic stress-responsive gene "*Colin*" in *Solanum tuberosum*.

Herbicide resistance

Weeds reduce vegetable yields because they compete together for space, light, water, and nutrients, and during cultivation, selective herbicides and pesticides are employed to suppress weed growth. The development of herbicide-resistant vegetables necessitates precise and straightforward GE, a capability provided by the CRISPR−Cas tool (Xie and Yang 2013). Two pathways led to the development of herbicide-resistant vegetable crops: the HDR pathway and the NHEJ pathway. Only the development of vegetable resistance to glyphosate, ACCase-inhibiting herbicides,

Table 1 Reports of CRISPR-Cas mediated gene editing evidences in different vegetable crops for trait improvement

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
Sweet potato	*Ipomoea batatas* (L.) Lam.	Convolvulaceae	2n = 6x = 90	*IbGBSSI*, *IbSBEII*	Starch biosynthesis pathway genes encoding granule-bound starch synthase I (*IbGBSSI*) and encoding starch branching enzyme II (*IbSBEII*)	InDel knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Wang et al. (2019a)
Cassava	*Manihot esculenta* Crantz	Euphorbiaceae	2n = 2x = 36	*ncbp-1*, *ncbp-2*	Host eukaryotic translation initiation factor 4E (eIF4E) isoforms (novel cap-binding protein-1 and 2) responsible for the + sense RNA virus mediated Cassava brown streak disease (CBSD)	InDel knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Gomez et al. (2019)
Chicory	*Cichorium intybus* L.	Asteraceae	2n = 2x = 18; 2n = 4x = 36	*CiPDS*	Chicory phytoene desaturase gene in chlorophyll and carotenoid biosynthetic pathway for depigmentation	InDel knockout	*Agrobacterium rhizogenes*-mediated and protoplast transfection	CRISPR-Cas9	Bernard et al. (2019)
Carrot	*Daucus carota* subsp. *carota* L.	Apiaceae	2n = 2x = 18	*F3H*	Flavanone-3-hydroxylase gene to change in the anthocyanin biosynthesis pathway	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Klimek-Chodacka et al. (2018)
				DcPDS, *DcMYB113*	Depigmentation phenotype through chlorophyll and anthocyanin biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Xu et al. (2019)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
Lettuce	*Lactuca sativa*	Asteraceae	2n = 2x = 18, 2n = 4x = 36	9-cis-EPOXY-CAROTENOID DIOXY-GENASE4 (*LsNCED4*)	Seed germination inhibition in high temperature	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Bertier et al. (2018)
Pumpkin	*Cucurbita moschata*	Cucurbitaceae	2n = 2x = 40	Respiratory burst oxidase homolog D (*RBOHD*)	Salt sensitivity through the expression of K^+ transport genes (*GRF12*, *AHA1* and *HAK5*)	Knockout	*Agrobacterium rhizogenes* mediated	CRISPR-Cas9	Huang et al. (2019)
Cucumber	*Cucumis sativus* L.	Cucurbitaceae	2n = 2x = 14	Eukaryotic translation initiation factor 4E (*elF4E*)	Broad-sense resistance against viral diseses	Loss of function	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Chandrasekaran et al. (2016)
				CsWIP1	Inhibiting carpel development	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Hu et al. (2017)
				CsaMLO	Powdery mildew resistance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Tek et al. (2022)
				elF4E	Viral disease resistance (WMV, ZYMV, PRSV)	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Fidan et al. (2023)
Ethiopian mustard	*Brassica carinata*	Brassicaceae	2n = 4x = 34	Fasciclin-like arabinogalactan protein 1 (*BcFLA1*)	Hairy root phenotype	Site-directed deletion	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Kirchner et al. (2017)
				BcPHT1	Transporter aided inorganic phosphate (Pi) uptake	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Kirchner et al. (2018)
Chinese cabbage	*Brassica rapa* L.	Brassicaceae	2n = 2x = 20	Phytoene desaturase (*PDS*), FRIGIDA (*FRI*)	Carotenoid biosynthesis pathway and vernalization determinant	Site-directed InDel mutagenesis	PEG 4000 mediated protoplast transfection	CRISPR-Cas9 ribonucleoprotein (RNP) complex	Murovec et al. (2018)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				FLOWERING LOCUS C (*BraFLC2*, *BraFLC3)*	Vernalization independent early flowering	Knockout	PEG 4000 mediated protoplast transfection	CRISPR-Cas9 RNP	Jeong et al. (2019)
	Brassica rapa spp. *pekinensis* L.			*BrLEAFY* (*BrLFY*)	Delay in bolting time	Knockdown	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Shin and Park (2022)
Chinese kale	*B. oleracea* var. *alboglabra*	Brassicaceae	2n = 2x = 18	*BaPDS1*, *BaPDS2*	Albino phenotype through blocking chlorophyll biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Sun et al. (2018a)
				BoaCRTISO	Carotenoid isomerase for carotenoid biosynthesis	Knockdown	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Sun et al. (2020)
				BocPDS1, *BocPDS2*	Albino phenotype through blocking chlorophyll biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Wang et al. (2024)
				BoMYB28	Glucosinolates biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Neequaye et al. (2021)
Camelina	*Camelina sativa* (L.) Crantz	Brassicaceae	2n = 6x =	*CsDGAT1*, *CsPDAT1*	Seed oil (triacylglycerol) synthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Aznar-Moreno and Durrett (2017)
				CsFAD2	Fatty acid biosynthesis and composition	Knockout	Floral dip	CRISPR-Cas9	Jiang et al. (2017)
				Fatty Acid Elongase1 (*FAE1*)	Fatty acid composition	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ozseyhan et al. (2018)
Cabbage	*Brassica oleracea* var. *capitata*	Brassicaceae	2n = 2x = 18	*BolC.GA4.a*	Dwarfism	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Lawrenson et al. (2015)
				BoPDS	Albino phenotype through blocking chlorophyll biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ma et al. (2019)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				GIGANTEA (GI)	Regulation of flowering time	Knockout	PEG - mediated protoplast electro-transfection	CRISPR-Cas9 RNP	Park et al. (2019)
				BoPDS1	Albino phenotype through blocking chlorophyll biosynthesis	Knockout	PEG 4000 mediated protoplast electro-transfection	CRISPR-Cas9	Lee et al. (2020)
Rapeseed	*Brassica napus* L.	Brassicaceae	2n = 4x = 38	*ALCATRAZ (ALC)*	Seed shattering and dispersal	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Braatz et al. (2017)
				Histone lysine methyltransferases (*BnaSDG8.A*, *BnaSDG8.C*)	Floral transition	Knockout, Knockdown	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Jiang et al. (2018)
				SEED FATTY ACID REDUCER (BnSFAR4, *BnSFAR5)*	Fatty acid composition	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Karunarathna et al. (2020)
				BnEOD3	Seed size and numbers	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Khan et al. (2021)
				Fatty acid desaturase 2 (*BnFAD2*)	Desaturation of oleic acid	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Okuzaki et al. (2018)
				BnITPK	Reduction in phytic acid content	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Sashidhar et al. (2020)
				BnWRKY11, *BnWRKY70*	Resistance to the fungal pathogen *Sclerotinia sclerotiorum*	Knockout	*Agrobacterium tumefaciens* mediated	Multiplexing CRISPR-Cas9	Sun et al. (2018a, b)
				BnaA6.RGA , *BnaDA2*, *BnaFUL*	DELLA protein for drought tolerance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Yang et al. (2017); Wu et al. (2020)
				CLAVATA3 (*BnCLV3*)	Fruit (silique) development	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Yang et al. (2018)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				BnTT8	Yellow seed coat colour	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Zhai et al. (2020)
				BnaMAX1	Plant architecture and high-yield	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Zheng et al. (2020)
				BnSVP	Flowering time	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ahmar et al. (2022)
				BnALS	Herbicide resistance	Point mutation	*Agrobacterium tumefaciens* mediated	A3A-Cytidine deaminase base editor	Cheng et al. (2021)
				BnFAE	Low Erucic acid	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Cheng et al. (2023)
				BnTTG1	Oil content	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Cheng et al. (2023)
Brinjal	*Solanum melongena*	Solanaceae	2n = 2x = 24	*SmelPPO4*, *SmelPPO5*, *SmelPPO6*	Polyphenol oxidases (PPO) mediated reduced level of flesh browning	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Maioli et al. (2020)
Potato	*Solanum tuberosum*	Solanaceae	2n = 2x = 24, 2n = 3x = 36, 2n = 4x = 48	*ACETOLACTATE SYNTHASE (StALS1*, *StALS2)*	Herbicide resistance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9, Gemini virus mediated CRISPR-Cas9	Butler et al. (2015, 2016)
				StIAA2	Auxin (IAA) synthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Wang et al. (2015)
				StMYB44	Phosphate transport	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Zhou et al. (2017)
				Granule-bound starch synthase (*GBSS*)	Starch biosynthesis	Knockout	PEG-mediated protoplast-transfection	CRISPR-Cas9, CRISPR-Cas-RNP	Andersson et al. (2017, 2018)
				GBSS1	Starch biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Kusano et al. (2018)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				St16DOX	Steroidal glycoalkaloid (SGA) biosynthesis	Knockout	*Agrobacterium rhizogenes* mediated	CRISPR-Cas9	Nakayasu et al. (2018)
				S-RNase	Controlling self-incompatibility	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ye et al. (2018)
				S-locus RNase (*S-RNase*)	Gametophytic self-incompatibility	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Enciso-Rodriguez et al. (2019)
				StPPO2	Polyphenol oxidases (PPO) mediated reduced level of tuber browning	Knockout	PEG-mediated protoplast-transfection	CRISPR-Cas9-RNPs	González et al. (2020)
				Coilin	Abiotic (osmotic and salinity) and biotic (potato virus Y) stress tolerance	Knockout	*E. coli* mediated	CRISPR-Cas9-RNPs	Makhotenko et al. (2019)
				Starch-branching enzymes (*SBE1*, *SBE2*)	Starch biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated and PEG mediated protoplast transfection	CRISPR-Cas9	Tuncel et al. (2019)
				StDND1, *StCHL1*, *StDMR6-1*	Late blight resistance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Kieu et al. (2021)
				Inv_T1, *VInv_T2*, *VInv_T3*	Improve cold storage, extend postharvest shelf life.	Knockout	PEG 4000 mediated protoplast electro-transfection	CRISPR-Cas9	Clasen et al. (2016)
				StGBSSI	tetraploid potato	Knockout	PEG 4000 mediated protoplast electro-transfection	CRISPR-Cas9	Veillet et al. (2019)
				SSR2	Biosynthesis of cholesterol	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Sawai et al. (2014)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				St SSR2	glycoalkaloid synthesis	Knockout	PEG-mediated protoplast-transfection	CRISPR-Cas9	Nicolia et al. (2015)
Tomato	*Solanum lycopersicum*	Solanaceae	2n = 2x = 24	*ABORTED MICROSPORES* (*SlAMS*)	Pollen viability	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Bao et al. (2022)
				CAROTENOID CLEAVAGE DIOXYGENASE 8 (*CCD8*), *MORE AXILLARY GROWTH 1 (MAX1)*	Resistance against *Phelipanche aegytiaca* parasitic week	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Bari et al. (2019, 2021)
				ARGONAUTE7 (*SlAGO7*)	Leaf architecture	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Brooks et al. (2014)
				ANT1	Purple plant and fruit	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Čermák et al. (2015)
				SlMPK20	Sugar and auxin metabolism	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Chen et al. (2018)
				DECREASE IN DNA METHYLATION 1 (*Slddm1a*, *Slddm1b*)	DNA methylation and chromatin remodeling	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Corem et al. (2018)
				Carotenoid isomerase (*CRTISO*)	Carotenoid biosynthesis	Knock-in	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Dahan-Meir et al. (2018)
				Acetolactate synthase gene (*ALS1*, *ALS2*, *ALS3*) or *acetohydroxy acid synthase* (*AHAS*)	Sulfonylurea herbicide resistance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Danilo et al. (2019)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				SlMYB12	Development of pink fruit due to flavonoid accumulation	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Deng et al. (2018)
				Sedoheptulose-1,7-bisphosphatase (*SlSBPASE*)	Photosynthetic carbon fixation	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ding et al. (2018)
				NAD Kinase 2 A (*SlNADK2A*)	Oxidative stress	Knockout	*Agrobacterium tumefaciens* mediated	Multiplexing CRISPR-Cas9	Hashimoto et al. (2018)
				PHYTOENE SYNTHASE (PSY1)	Carotenoid biosynthesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Filler et al. (2017)
				SlEIN2, *SlARF2B*, *SlERFE1*	Ethylene signaling	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Hu et al. (2019)
				RIN	Fruit ripening	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ito et al. (2015); Li et al. (2018a, 2020)
				SlAGAMOUS-LIKE 6 (SlAGL6)	Parthenocarpy and fruit development	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Klap et al. (2017)
				SlCBF1	Cold tolerance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Li et al. (2018b)
				lncRNA1459	Fruit ripening	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Li et al. (2018a)
				Y-aminobutyric acid (*GABA-TP1*, *GABA-TP2*, *GABA-TP3*)	Metabolic regulation	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Li et al. (2018c)
				SGR1, *LCY-E*, *LCY-B1*, *LCY-B2*	Lycopene content	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Li et al. (2018d)
				UV RESISTANCE LOCUS 8 (*SlUVR8*)	Photomorphogenesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Liu et al. (2020)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				Powdery Mildew Resistance 4 (*PMR4*)	Powdery mildew disease resistance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	SantillánMartínez et al. (2020)
				ClMlo1	Powdery mildew disease resistance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Nekrasov et al. (2017)
				Glutamate decarboxylase (*SlGAD2*, *SlGAD3*), *SlyGABA-TP1*, *SlyGABA-TP2*, *SlyGABA-TP3*	Increase of ɣ-aminobutyric acid (GABA)	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Nonaka et al. (2017)
				SlJAZ2	Bacterial speck resistant	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ortigosa et al. (2019)
				SlPDS, *SlPIF4*	To test targeted mutagenesis	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Pan et al. (2016)
				Ty-5 (SlPelo), *Mildew resistance locus o 1 (SlMlo1)*	Resistance to Tomato Yellow Leaf Curl Virus (TYLCV) and Powdery Mildew (Pm)	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Pramanik et al. (2021)
				SlCLV3, *SlWUS*	Floral organ number and fruit size	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Rodríguez-Leal et al. (2017)
				Solyc12g038510	Jointless-2 (J-2) phenotype	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Roldan et al. (2017)
				SHORT-ROOT, *SCARECROW*	Hairy root	Knockout	*Agrobacterium rhizogenes* mediated	CRISPR-Cas9	Ron et al. (2014)
				SELF-PRUNING 5G (SP5G).	Day length sensitivity	Knockdown	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Soyk et al. (2016)
				ARF7	Parthenocarpic fruit development	Knock down, knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Hu et al. (2018)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
				DNA demethylases (SlDML2)	Control of fruit ripening	Loss of function	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Lang et al. (2017)
				SlIAA9	Parthenocarpic fruit development	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Ueta et al. (2017)
				ANT2	Purple-pigmented tomato	Loss of function	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Filler et al. (2017)
				CcLOL1, *CaGLK2*, *and CcAPRR2*	Development of Chloroplast	Knockout	*Agrobacterium tumefaciens* mediated	Agrobacterium tumefaciens-mediated	Borovsky et al. (2019)
				Alcobaca (SLALC)	Enhancement of shelf-life	Base editing	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Yu et al. (2017)
				SBPASE	Senescence of leaves	Knockout	*Agrobacterium tumefaciens* mediated	Agrobacterium tumefaciens-mediated	Ding et al. (2018)
				PG, *PL*	Synthesis of cell wall	Knockdown	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Boase et al. (2018)
				SP, *SP5*, *CLV3*, *WUS*, *GGP1*	Plant growth, photoperiodism, nutritional enrichment of fruits	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Li et al. (2018e)
				SlMBP21	Development of Jointless fruit	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Roldan et al. (2017)
				Solyc08g075770	Fusarium wilt susceptibility	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Prihatna et al. (2018)
				SlDML2	Activation and inhibition of fruit	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Lang et al. (2017)
				ClDMR6	Resistance against downy mildew	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	de Paula et al. (2016)

Table 1 (continued)

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				SlMAPK3	Decrease in drought stress tolerance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Wang et al. (2017)
				PL, *PG2a*, *TBG*	Toughness and texture of fruits	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Wang et al. (2019a)
				BZR1	Decrease in heat stress tolerance	Knockout	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Yin et al. (2018)
				SLALC	Long shelf-life	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Yu et al. (2017)
				L1L4, *NF-YB6*	Leafy cotyledon function in tomato fruit metabolism	Knockout	*Agrobacterium tumefaciens* mediated	ZFN Technology	Gago et al. (2017)
				LEAFY-COTYLEDON1-LIKE4 (*L1L4*)	Highly efficient targeted mutagenesis	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	ZFN Technology	Hilioti et al. (2016)
				LeMADS-RIN	Reduced Ethylene Production	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Jung et al. (2018)
				PROCERA (PRO)	Increased GA response	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Lor et al. (2014)
					Resistance to herbicides	Knockdown	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Van Eck et al. (2019)
Banana	*Musa paradisiaca*	Musaceae	2n = 3x = 33	*MaACO1*	Enhancement of shelf life	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Hu et al. (2020)
Bluewings	*Torenia fournieri*	Linderniaceae	2n = 2x = 18	*F3H*	Variable floral pigmentation	Base editing	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Nishihara et al. (2018)
Capsicum	*Capsicum annum*	Solanaceae	2n = 2x = 24	*CaPAD1*	Seedless pod development	Base editing	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Choi et al. (2024)
				CaERF28	Anthracnose resistance	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Mishra et al. (2021)

Table 1 (continued)

Vegetable crops	Scientific name	Family	Ploidy number	Target gene(s) for editing	Function(s) of the gene/mutant trait	Type of mutation	Mode of transgene delivery	Gene edits tool	References
Pea	*Pisum sativum*	Leguminosae	2n = 2x = 24	*PsPDS*	*Phytoene desaturase*	Site-directed InDel mutagenesis	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Li et al. (2023)
Squash	*Cucurbita pepo* subspecies *pepo*	Cucurbitaceae	2n = 2x = 40	*PDS*	*Phytoene desaturase*	*CpPDS* knockout system	*Agrobacterium tumefaciens* mediated	CRISPR-Cas9	Thakur and Meru (2023)

and ALS-inhibiting herbicides has proven to be effective in producing herbicide-resistant crop varieties. However, there is limited research on the extensive application and successful management of weeds via the use of protoporphyrinogen oxidase and 4-hydroxyphenyl pyruvate dioxygenase, which inhibit herbicides. The NHEJ mechanism can also facilitate precise gene substitution and insertion mediated by introns through the CRISPR-Cas9 system. HDR events are significantly less frequent than NHEJ events and allow for precise modifications of endogenous genes through targeted gene replacements or insertions, unlike NHEJ. In tomato and potato, amino acid mutations are achieved via cytidine base editing (CBE) at key *ALS* sites. Yang et al. (2022) used GE to incorporate herbicide resistance genes such as *ALS* and *5-enolpyruvylshikimate-3-phosphate synthase* (*EPSPS*) in tomato along with the marker gene *phytoene desaturase* (*PDS*). Furthermore, a chimeric Cas9-VirD2 protein was developed to increase HDR efficiency in vegetable plants. This protein merges Cas9 with VirD2, a virus protein that cleaves the lower strands of the Ti plasmid at both the left and right borders. Similarly, in potato, herbicide-inhibiting point mutations in the *StALS1* template were successfully achieved via the CRISPR−Cas system (Butler et al. 2015). A similar investigation was carried out in tomato for the gene encoding *acetohydroxy acid synthase* (*AHAS*) (Danilo et al. 2019). To modify herbicide-related genes such as *ALS* and *EPSPS* in tomatoes, Yang et al. (2022) recently developed and assessed the effectiveness of sgRNA. The results revealed that the target sites could be altered during the transformation process. Furthermore, it has been demonstrated that sgRNAs targeting *ALS2P* or *ALS1W* successfully edited 19 different transgenic tomato plants, with two of these exhibiting three nucleotide changes that could affect herbicide resistance. However, the metabolic degradation of glyphosate is often slow or absent in many other plant species. Rapeseed (*Brassica napus*) and soybean (*Glycine max*) exhibit substantial transformation of the herbicide into its primary metabolite, aminomethylphosphonic acid (AMPA) (Correa et al. 2016). The advancement of the CRISPR-Cpf1 system has broadened the applicability of GE technology reliant on the HDR pathway due to its extended 5′-protruding ends (Zetsche et al. 2015), which may enhance the alignment and incorporation of repair templates; however, the system is hindered by its nonspecific cleavage activity toward single-stranded DNAs.

Disease resistance

Owing to the diverse range of diseases caused by fungi, bacteria, viruses, and nematodes, numerous attempts have been made to improve vegetable disease resistance. Broad-spectrum resistance to both fungal (*Phytophthora*) and bacterial pathogens (such as *Pseudomonas* and *Xanthomonas*

spp.) is produced in tomato by a mutation generated in *SlDMR6-1* via CRISPR-Cas9-driven knockout (Thomazella et al. 2016). Similarly, GE for disease-resistant cucumber production against Potyvirus and Ipomovirus was carried out (Chandrasekaran et al. 2016). Additionally, CRISPR-mediated mutation of the *SlMlo1*gene resulted in tolerance to powdery mildew in tomato (Nekrasov et al. 2017). Gomez et al. (2019) demonstrated resistance to Cassava brown streak disease (CBSD) via the use of CRISPR-Cas9 to knockout the *eIF4E* gene associated with the disease. Tashkandi et al. (2018) presented tomato yellow leaf curl virus (Begomovirus) resistance through CRISPR-Cas9. Similarly, Sun et al. (2018b) reported that introducing a mutation in *BnWRKY70* enhanced rapeseed resistance to *Sclerotinia* spp. The viral genes P3, CI, Nib, and CP have been effectively targeted via CRISPR-Cas13a to confer resistance to three PVY strains (Zhan et al. 2019). A potato virus Y (PVYO-FL, PVYN-Jg, PVYN: OMb112)-resistant potato cultivar was created by knocking out the *Colin* gene, which is responsible for CRISPR-Cas9 (Makhotenko et al. 2019). Ortigosa et al. (2019) reported resistance to bacterial speck disease by targeting Jasmonate ZIM-domain 2 via CRISPR. Ghorbani Faal et al. (2020) reported tomato yellow leaf curl disease resistance through GE. Resistance to powdery mildew was investigated in tomato against the responsible gene *Powdery mildew Resistance 4* (*PMR4*). Atarashi et al. (2020) reported resistance to mild mosaic disease in tomato via CRISPR via the targeting of eukaryotic translation initiation factor *4E1*. *A Botrytis cinerea*-resistant tomato cultivar was produced by targeting the *MYC2* gene via CRISPR-mediated editing (Shu et al. 2020). Furthermore, CRISPR uses *Pectate Lyase* and *Histone H3 Lysine methyltransferase* genes for knockdown to develop resistant tomato lines to gray mold and *Botrytis cinerea*, respectively (Silva et al. 2021; Bvindi et al. 2022). Mishra et al. (2021) revealed anthracnose resistance in chili by knocking out *ethylene response factor 28* via CRISPR editing.

Improving agronomic traits and yields

Vegetable domestication affects a number of agronomic traits as well as yield. In tomato, artificial domestication was achieved via CRISPR−Cas-mediated GE (Soyk et al. 2017). To facilitate the *de novo* domestication of wild *Solanum pimpinellifolium*, Zsogon et al. (2018) applied CRISPR-Cas9 to target six key loci essential for yield and productivity in contemporary tomato varieties. Compared with the wild parent, the engineered lines presented a tenfold increase in fruit quantity and a threefold increase in fruit size. Zheng et al. (2020) knocked out the *BnaMAX1* gene via CRISPR to increase yield in rapeseed. In cucumber, *Cswip1* mutants were developed via the CRISPR−Cas9 technique and were further used in heterosis breeding to generate high-yielding hybrids (Hu et al. (Hu et al. 2017)). Additionally, shorter flowering and earlier harvesting were achieved in tomatoes by generating CRISPR-induced *SP5G* mutations (Soyk et al. 2017). Both tomato and *Arabidopsis* have *blade-on-petiole (BOP)* genes related to leaf complexity and silique dehiscence. The absence of the *BOP* gene, which may be achieved by CRISPR-based knockout, leads to morphological differences in the inflorescence. Compared with wild-type plants, CRISPR-Bop1/2/3 triple mutants blossom more quickly because of accelerated gene expression (Xu et al. 2016). Indeed, a range of *cis*-regulatory alleles were generated through multiplexed CRISPR−Cas9 mutagenesis in the promoter region of *SlCLV3* (Rodríguez-Leal et al. 2017). These genes caused a number of changes, such as changes in the expression levels of *SlCLV3* and changes in the number of locules, the size of the fruit, and the shape of the flower. Parthenocarpy is a crucial agronomic characteristic of vegetables. To produce seedless tomatoes, *auxin response factor 7* (*SlARF7*) and *indole-3-acetic acid inducible 9* (*SlIAA9*) have been effectively knocked out in tomato (Ueta et al. 2017). Klap et al. (2017) successfully achieved parthenocarpy under high-temperature stress by using CRISPR-Cas9 to knock out the tomato gene *SlAGAMOUS-LIKE6* (*SlAGL6*) without compromising fruit weight, shape, or pollen viability. This technique can also induce parthenocarpy in various vegetable crops.

Improvements in quality and shelf-life

Consumer choice is significantly influenced by the quality of vegetables, including their flavour, color, and presence of nutrient-rich, health-promoting components. CRISPR-Cas9, an innovative GE technology, has transformed numerous areas of biological research and has profound implications for agriculture, especially concerning the enhancement of vegetable quality and longevity by modifying genes related to amino acids, carbohydrates, vitamins, fatty acids, and carotenoids in vegetables. Yellow, pink, and purple tomatoes were created via the use of CRISPR−Cas to modify the genes encoding *phytoene synthase 1* (*PSY1*), *MYB transcription factor 12* (*MYB12*), and *anthocyanin 2* (*ANT2*) (Deng et al. 2018). Solanine and chaconine, two bitter and poisonous glycoalkaloids, were unable to accumulate in potato tubers after CRISPR-mediated silencing of the *St16DOX* gene (Nakayasu et al. 2018). Similarly, in eggplant, three polyphenol oxidase (PPO) genes, *SmelPPO4*, *SmelPPO5*, and *SmelPPO6*, were eliminated via CRISPR-Cas9-mediated mutagenesis to limit the browning of fruit flesh (Maioli et al. 2020). Using granule-bound starch synthase I (*Ib-GBSS I*) and starch branching enzyme II (*Ib-SBE II*) as target genes, along with seed fatty acid reducer 4 (*BnSFAR4*) and seed fatty acid reducer 5 (*BnSFAR5*) as additional targets, CRISPR technology successfully increased the total

starch and seed oil contents in sweet potato and rapeseed, respectively (Wang et al. 2019b). A similar study involving the complete knockout of the GBSS, SS6, SBE1 and SBE2 genes was performed on potatoes to improve the quality of their starch (Sevestre et al. 2020; Zhao et al. 2021). Malate and gamma-aminobutyric acid (GABA) in vegetables offer various health benefits for humans. Tomatoes engineered with CRISPR-Cas9 to alter *Al-activated malate transporter 9* (*SlALMT9*) were found to accumulate higher levels of malate (Ye et al. 2017). Tomato leaves and fruits contain elevated levels of the nonproteinogenic amino acid GABA (Nonaka et al. 2017). Various efforts have been undertaken to modify the genes associated with fatty acid metabolism in rapeseed and camelina to increase oil quality (Okuzaki et al. 2018). Another problem in the vegetable industry is browning in cuts, which decreases market value. The primary cause of browning is the enzyme polyphenol oxidase (PPO), which accelerates the oxidation that changes polyphenols into quinones. In eggplant, CRISPR-mediated editing of three target PPO genes, *SmelPPO4*, *SmelPPO5*, and *SmelPPO6*, results in nonbrowning of the cut parts (Maioli et al. 2020). Similar outcomes were observed when the RNP complex was composed of two sgRNAs and the Cas9 nuclease was used to target *StPPO2* in potatoes (González et al. 2021). Lycopene, a pigment that enhances the visual appeal of tomatoes, also offers nutritional benefits by serving mainly as a source of antioxidants. Thus, increasing lycopene accumulation is beneficial for both crop yield and consumer attractiveness. The carotenoid metabolic pathway converts lycopene into α- and β-carotene. Consequently, the goal is to increase lycopene levels by promoting lycopene biosynthesis and inhibiting lycopene conversion, with a focus on the key enzymes involved in the pathway. When multiplex genome editing via CRISPR was employed, the*stay green-1* (*SGR1*) genes were mutated, and as a consequence of this silencing, the mutants presented a notable increase in lycopene content, which improved the activity of enzymes such as *phytoene synthase* (*PSY1*), *lycopene β-cyclase 1* and *2* (*LCY-B1*, *LCY-B2*), and *lycopene ε-cyclase* (*LCY-E*) (Ku and Ha 2020). By utilizing targeted site-specific CRISPR, a bidirectional approach can promote lycopene accumulation in carrot plants while inhibiting lycopene conversion to β- and α-carotene (Li et al. 2018d). In a different approach, CRISPR targets *ANT1*, a transcription factor that regulates anthocyanin biosynthesis, resulting in increased accumulation of anthocyanins and creating purple tomatoes (He et al. 2023). Klimek-Chodacka et al. (2018) reported that a mutation in the anthocyanin biosynthesis gene *F3H* resulted in reduced accumulation of anthocyanin and a discoloured callus in carrots. Compared with their wild relatives, commercial cultivars present a variety of fruit colours, textures, and sizes. The pink phenotype in tomatoes arises partly from a deficiency of yellow flavonoids, such as naringenin chalcone. In a previous study, when the carotenoid isomerase gene (*BoaCRTISO*) in Chinese kale was inactivated via CRISPR, the plants changed from green to yellow, resulting in an accumulation of lycopene (Sun et al. 2020). On the other hand, yellow tomatoes were generated via the use of CRISPR to knock out two genes related to carotenoid biosynthesis, namely, *Psy1* and *CrtR1-b2* (Li et al. 2018e). Additionally, tomatoes with a tangerine hue were developed because of a gain-of-function mutation in *carotenoid isomerase* (*CRTISO*) (Ben Shlush et al. 2020). Similar methods were employed to downregulate *DcMYB7*, an *R2R3-MYB* regulator linked to structural genes that facilitate anthocyanin production in carrots, resulting in yellow-rooted carrots (Wang et al. 2020). The ripening inhibitory gene (*RIN*) in tomato is responsible for controlling ripening; CRISPR-mediated knockout mutation of *RIN*-producing mutants results in longer shelf lives and a delay in ripening (Xu et al. 2020). One such mutation in *alcobaca* (*alc*) occurs when adenine is substituted for thymine at position 137 in the coding region of the *nonripening* (*NOR*) gene. Modification of the *ALC* gene via CRISPR led to the development of tomato *alc* mutants that exhibit longer shelf lives (Yu et al. 2017). Compared with the rin mutants, the *alc* mutants presented a significantly enhanced flavour. Additionally, research on the *LeMADS-RIN* gene in the tomato varieties ‘Mamirio’ and ‘Golden bell’ demonstrated that the mutants presented lower ethylene levels and prolonged shelf lives compared with their wild-type counterparts (Ku and Ha 2020). The remodelling of vegetable cell walls also influences shelf-life, and pectin-degrading enzymes are integral to this process. This extended shelf-life is due to the presence of three specific genes, *pectate lyase* (*PL*), *polygalacturonase* (*PG2a*), and *β-galactanase* (*TBG4*), which are associated with pectin degradation and were selected for mutagenesis through CRISPR (Wang et al. 2019a).

Vegetable breeding strategies through genome editing

The global population is growing, demanding increased food production while climate change intensifies, posing significant challenges to agriculture. Vegetables are cultivated and consumed worldwide, as they are rich sources of vitamins, minerals, dietary fibre proteins, and other important phytochemicals (Ahmad 2023). To date, techniques of conventional plant breeding have played important roles in the qualitative and quantitative improvement of major vegetable crops. Conventional breeding techniques utilize available genetic variation in the population and generate new improved vegetables by combining the desired gene pools. Backcrossing or introgression breeding, inbreeding, hybrid breeding, and mutation breeding are some conventional

breeding strategies that traditional plant breeders have successfully employed for vegetable improvement (Rajasree and Pugalendhi 2021; Wang and Zhang 2022). However, conventional breeding methods are more time-consuming and laborious, and vegetable breeders are continuously looking for new and advanced breeding strategies (Mirza and Ghuge 2021). Moreover, owing to genetic erosion, hybridization bottlenecks, genetic drag, and complex selection processes, traditional vegetable breeding programs have achieved limited success in the postgreen revolution era. Through targeted genetic improvement, breeders can develop vegetable varieties with increased nutritional content, disease and pest resistance, and adaptability to changing climates (Ndudzo et al. 2024). In such a way, improved cultivars contribute to food security by increasing crop yield and reducing postharvest losses. Additionally, vegetable breeding plays a vital role in sustainable agriculture, enabling the development of resource-efficient varieties that require less water, fertilizer, and pesticides. The genetic diversity of vegetables serves as a valuable resource for breeders, allowing them to select desirable traits and address specific challenges faced by farmers and consumers (Salgotra et al. 2024).

Advanced breeding methods

Modern plant breeding approaches have developed due to the rise of plant genome engineering technologies and tremendous progress in plant biotechnological approaches such as molecular marker systems and transgenics methods. Unlike in the pregenomic era, where important agroeconomic traits are blindly incorporated into crops, modern scientists have identified and incorporated the desired genomic regions for the qualitative and quantitative improvement of vegetables (Zhang et al. 2018). The next-generation breeding strategies include sophisticated technologies such as genomic-assisted breeding (GAB), genome editing, artificial intelligence (AI) and machine learning (ML) as alternatives to speed up complex and lengthy breeding programs (Devi et al. 2022). A number of cutting-edge technologies, such as genotyping by sequencing (GBS) coupled with next-generation sequencing (NGS), genome-wide association studies (GWAS), and marker-assisted selection (MAS), have facilitated multitrait gene identification and alteration. Thus, comprehensive knowledge of key genes and their regulatory pathways has assisted multidisciplinary breeding programs for the development of next-generation crop varieties (Razzaq et al. 2021). The available technologies can be broadly classified into three categories: novel breeding techniques, which have been introduced; established genetic modification techniques; and conventional breeding procedures. The methods involving site-directed nucleases (SDNs) and oligonucleotide-directed mutagenesis (ODM) allow a range of techniques for genome editing, such as precision-directed mutagenesis and gene transfer, along with control over gene expression (Liu et al. 2017). These transgenic materials, as in the case of nuclease genes, can be expressed, delivered into plant cells, and produced, either transientlyly or after stable genome integration, via a variety of transformation techniques. The direct methods used were PEG-mediated protoplast transformation and biolistic-based techniques; among the indirect methods, *Agrobacterium* and viral vector-based methods using TRV and Geminiviruses were applied. Recently, the direct delivery of pure, preassembled Cas9-sgRNA RNP complexes into a variety of species by protoplast transfection or biolistics has been demonstrated to be successful (Altpeter et al. 2016). Marker-assisted selection (MAS) is a cornerstone of modern breeding, allowing for the identification and selection of desired traits on the basis of specific genetic markers. This accelerates the breeding process by enabling breeders to focus on individuals with the desired genes, reducing the time and resources required (Roychowdhury 2014; Roychowdhury et al. 2014). Genome-wide selection (GWS) takes this concept further, analysing an organism's entire genome to predict its performance for multiple traits simultaneously, leading to even more efficient breeding programmes.

Incorporation of genome editing in vegetable breeding

Genome editing is revolutionizing vegetable breeding by offering unprecedented precision and efficiency. This approach significantly reduces the time required for traditional breeding methods, accelerating the development of new and improved vegetable cultivars. As research progresses, genome editing holds immense potential to address global food security challenges and provide sustainable solutions for agriculture. Owing to the availability of large amounts of genomic data on vegetables, genome editing has emerged as the most potent option for the development of new crop varieties with targeted gene modification(s). Genome editing technologies enable plant breeders to develop new and improved plant varieties with high speed and precision. Genome editing tools enable the engineering of plant genomes via the insertion, deletion, or replacement of desired DNA sequences at specific locations in the target genome (Anzalone et al. 2020). Both spontaneous and induced mutations have been extensively utilized to develop genetic resources with a range of characteristics for breeding purposes. The infrequency and unpredictability of these mutations have led scientists to seek methods for introducing precise mutations at specific target sites. Currently, plant breeders are increasingly utilizing genome editing technologies for two reasons: first, tools for genome editing enable the identification of

candidate genes associated with any desired trait, increasing genetic diversity; second, genome editing helps in understanding gene function, which is necessary for direct conventional breeding techniques.

Evolution of plant breeding in vegetable crops

The evolution of plant breeding in vegetable crops has been a transformative journey that has significantly shaped global agriculture, enhancing food security, nutrition, and crop resilience. Vegetable crop improvement programs can be broadly categorized into three groups, i.e., traditional breeding methods, biotechnological innovations, and new breeding techniques, each involving the use of several other alternative methods (Cardi et al. 2017). The traditional plant breeders select natural populations and intraspecific cross hybrids and use mutagenesis and wide hybridization methods involving interspecific hybrids for the breeding and improvement of vegetable crops. On the other hand, biotechnological innovations rely on tissue culture technologies such as somatic hybridization of both sexually compatible and noncompatible species and transgenic approaches for achieving similar objectives. Interestingly, recent advancements in molecular biology and the emergence of different omics approaches offer a wide range of precise techniques to plant breeds. Some of the new methods used in the breeding of vegetable crops include oligonucleotide-directed mutagenesis (ODM), nuclease-based genome editing, cisgenesis and intragenesis, grafting, RNA-dependent DNA methylation (RdDM), reverse breeding, agroinfiltration, and synthetic genomics.

Domestication of vegetable crops

Domestication is the process by which wild crop plants are adapted and made suitable for human use. Plant domestication involves the accumulation and alteration of numerous agro-economic traits, such as the time of flowering and maturation, seed setting, fruit size and nutritional value, and photoperiodic activity. Such vegetable domestication involves millennia of selective breeding, where cultivated plants with desirable traits, such as increased yield, larger size, and improved taste with nutritive benefits, are cultivated. For example, cabbage, broccoli, cauliflower, and kale all share a common ancestor—wild mustard. Over time, farmers have carefully selected plants with specific characteristics, leading to the development of these distinct vegetables. The process often includes genetic modifications, either through natural selection or, more recently, through advanced biotechnological techniques. This domestication not only provided a reliable food source but also shaped agricultural practices, economies, and cultures, making it a cornerstone of human civilization. For a very long time, traditional plant breeders augmented the domestication process by introducing favourable genes and alleles from wild relatives into cultivated species. Owing to its rapid and precise genome editing ability, CRISPR-Cas9 has substantially accelerated the *de novo* domestication process (Ledford 2017; Soyk et al. 2017). Research indicates that through precise genetic manipulation of domesticated genes, significant phenotypic changes can be achieved in wild tomatoes, which exhibit strong potential for natural stress tolerance (Chen et al. 2019).

Efficiency of genetic transformation

An efficient genetic transformation and plant regeneration system is a prerequisite for the accurate modification of plant genomes by GE tools. However, the accuracy and efficiency of genetic transformation and regeneration vary among genotypes. Therefore, the success of genome editing is often limited due to the availability of compatible genotypes. Thus, effective genome editing requires the development of methods that overcome the genotype bottleneck. Presently, two different methods are being used to overcome this genotype limitation: one involves the overexpression of key genes associated with plant development, and the other involves the coexpression of developmental regulatory genes. To date, a number of genes, such as *LEAFY COTYLEDON1*, *LEAFYCOTYLEDON2*, *WUSCHEL*, *GRF-GIF* chimeric protein, and *BABY BOOM*, have been successfully overexpressed and shown to improve plant regeneration efficiency in tissue culture media (Debernardi et al. 2020). On the other hand, numerous attempts have been made for the direct production of genome-edited plants through bypassing the tissue culture stage via the coexpression of developmental regulatory genes (Maher et al. 2020).

Production of nongenetically modified (transgene-free) vegetables

To date, selfing or hybridization after genetic transformation is the routine procedure for eliminating foreign genes and obtaining transgene-free modified vegetables via GE. However, this is quite hard in a few notably heterozygous and clonally propagated vegetable species. The advancement of genetic engineering accelerated crop improvement programs by generating different transgenic lines, which were developed by inserting foreign DNA fragments into endogenous genes that affect adjacent gene expression. Cardi et al. (2017), on the other hand, show how easy it is to obtain multiple homozygous mutations in a single generation that can be passed down to offspring via stable or temporary transformation methods that do not require the addition of foreign DNA. Moreover, CRISPR-Cas9 expression systems can be removed by subsequent selfing or crossing due to the different locations of the CRISPR-Cas9

expression cassettes and their target sites in the genome. Therefore, the CRISPR system can produce transgene-free genome-edited plants due to the degradation of foreign DNA molecules by endogenous proteases and the nonintegration of the CRISPR-Cas9 expression cassette into the genome (Malnoy et al. 2016). To date, two CRISPR-Cas9-mediated genome editing methods have been successfully used to produce transgene-free plants. Transient delivery techniques have potential advantages in the editing of transgene-free and DNA-free genes. Preassembled RNPs with Cas9 and sgRNA have been used for the development of an efficient plasmid-free system for genome editing in plants. In the case of lettuce, a rate as high as 46% was reached for regenerated mutants. This expanded technique is now ready to be applied to some *Brassica* vegetable crops and potatoes. The plant breeding of vegetable crops involves the same important task of genome editing in noncoding regions. GWAS provide data that noncoding plant areas instrumentally promote domestication, breeding, and evolution. Compared with coding sequence changes, mutations in the noncoding regions of genes tend not to abolish gene functions. In general, these noncoding region mutations influence gene expression levels, patterns, or timing and lead to only slight phenotypic changes. The use of the CRISPR-Cas9 system in conjunction with sgRNA libraries to screen noncoding genomic regions can help pinpoint functional areas associated with phenotypes and genes. The first is the Flp/FRT system, which involves the recognition of 34 bp-long flippase recognition target site (FRT) sequences by a site-specific recombinase flippase (Flp) and the removal of specific foreign DNA sequences (Pompili et al. 2020). The second method is based on Cas9 enzyme-mediated T-DNA removal at cleavage target sites (Dalla Costa et al. 2020).

Nonspecific (off-target) gene editing

The CRISPR system often results in nonspecific editing at nontarget sites, which are also known as off-target effects. Off-target effects result in undesirable genome mutations, which are in many cases uncontrollable and greatly affect the editing specificity of the CRISPR-Cas system. Although much emphasis has been given to improving its specificity, CRISPR technologies are still not able to eliminate the impact of off-target effects. Therefore, genome editing technologies such as CRISPR systems need optimization to minimize or completely avoid off-target effects (Nerkar et al. 2022).

Gene knock-in by CRISPR for gain of function

Gene insertion via CRISPR−Cas has made significant progress, particularly in vegetable crops, primarily through site-directed mutations that result in loss-of-function alterations to improve certain traits. CRISPR-mediated knock-in experiments rely on HDR, where double-strand breaks (DSBs) in DNA are precisely repaired by using homologous templates. In-frame gene knock-ins by CRISPR−Cas resulted in a gain of function" and often produced new alleles in breeding practices (Zhang et al. 2021). Furthermore, knock-ins that aggregate multiple genes within a single variety can be leveraged to modulate several elite traits in crops, offering substantial benefits for crop trait enhancement (Chen et al. 2019). As a result, the potential for the use of a GE tool for precise substitution or insertion at specific sites has increased. However, the efficiency of plant GE technology in terms of fragment site-directed insertion, substitution, and single-base gene substitution remains relatively low. This poses significant challenges in effectively and accurately editing most positive regulatory genes that govern key agronomic traits. Consequently, this limitation could significantly hinder the practical and large-scale use of gene-editing technologies in the genetic engineering of vegetables.

Government regulation and safety guidelines for gene-edited vegetables

The regulation of gene-edited plants varies widely across countries, reflecting diverse perspectives on agricultural innovation and food safety. Countries such as the United States and Canada have adopted relatively lenient regulations, often exempting gene-edited crops without foreign DNA from rigorous approval processes. This approach is based on the argument that these plants are indistinguishable from those produced through traditional breeding methods. On the other hand, the European Union has taken a more cautious stance, classifying gene-edited plants (GEPs) as GMOs and subjecting them to strict regulations. This decision stems from concerns about potential environmental and health risks, despite the lack of scientific consensus on these issues. Australia and Argentina represent intermediate positions, with regulatory frameworks that consider the specific nature of the GE technique and the potential risks associated with the modified plant. This global patchwork of regulations highlights the challenges of balancing agricultural advancement with public safety and environmental protection. The development of innovative plant breeding technologies such as GE enables quick and accurate changes in plant genomes without the need to insert any foreign DNA sequences. While CRISPR−Cas can mediate the loss of gene function editing, Cas cuts the target sequence and produces double-strand breaks, whereas gene knock-ins by CRISPR involve homologous sequences derived from the same species or related interbreeding species. Therefore, GE technologies result in small targeted genetic changes in the genome that can also be induced by natural methods. Thus, there is great controversy over

whether genome-edited plants are transgenic. Nevertheless, this lack of clear and consistent conclusions regarding genome-edited plants makes their consideration critical owing to the management and evaluation of traditional and GMOs (Globus and Qimron 2018). According to the United States Department of Agriculture (USDA), US Food & Drug Administration (FDA), and Environmental Protection Agency (EPA), plant GE via the CRISPR−Cas system is equivalent to crop improvement via conventional breeding programs; therefore, genome-edited plants are not considered GMOs for regulatory purposes (Wan et al. 2021). This regulatory approval from United States regulatory agencies not only accelerated the genetic improvement of crops but also promoted the introduction of an increasing number of genome-edited crops to the market (Callaway 2018; Metje-Sprink et al. 2020). In Europe, the rules for CRISPR-Cas9 are quite strict, and the European Court of Justice has already ruled that gene-edited crops are subject to GM crop regulation. It is a decision that has previously seen Australia take a more measured approach, allowing GE but not including any foreign genetic material. There are reports that gene-edited crops have already been planted in the field; additionally, gene-edited crops have gained fewer rigid attitudes in countries such as China and Japan. Moreover, some countries apply their frameworks on a case-to-case basis, considering the methodology of breeding, newly added traits or characteristics, and indications of genetic modification in the product.

Future challenges of CRISPR−cas genome editing in vegetables

After several positive results from whole-genome sequencing and functional genomics studies in vegetable crops were obtained, it became clear that the CRISPR-Cas9 GE method could be used to obtain many genetically diverse crop resources. Two major challenges prevent its future application: first, how precisely the target changes are identified and the types of changes associated with key genes. Modification of a single gene does not affect the phenotype, but key agronomic traits are often complex quantitative traits. Therefore, pyramiding mutant alleles via efficient target site-specific insertion mediated by CRISPR−Cas and chromosomal recombination methods is theoretically possible. GE techniques, which suppress the expression of some genes, have produced less adaptable plants. Thus, efficient and specific regulation of gene activities is essential for precision GE. Mutations in gene exons alter protein function, but mutations in the exon−intron splice site produce alternative splicing variants. The targeted induction of DSBs via CRISPR−Cas can lead to significant genome rearrangements, including large deletions, chromosomal translocations, and inversions, in addition to smaller mutations such as base substitutions and InDels. Such editing provides an effective means of eliminating unwanted allergenic genes in vegetable crops. However, the incidence of these chromosomal rearrangements is lower than what is typically achieved through conventional targeted mutagenesis. The second challenge in the use of gene-editing technology lies in the intricate process of introducing the CRISPR−Cas system into plant cells and subsequently producing regenerated plants. The precise GEs using cytosine base editors (CBEs) and adenine base editors (ABEs), along with prime editing and genetic mutation vectors mediated by traditional Cas9 or its variants through various methods, including Agrobacterium-mediated transformation, gene gun techniques, polyethylene glycol treatments, and electroporation, have been successfully applied. These studies have thus established a groundwork for potential applications in other vegetables. However, creating an efficient and universally applicable system for genetic transformation and regeneration in vegetable crops presents significant challenges. Specifically, the ability of CRISPR−Cas cassettes to facilitate genetic transformation and the capacity of modified tissues to produce new plants have emerged as limiting factors. By overexpressing genes that regulate plant morphogenesis during the transformation of the CRISPR−Cas expression cassette, it is possible to improve the regeneration potential of explants following transformation, as well as enhance meristem induction. This describes the genetic transformation and regeneration process using CRISPR−Cas, where plant RNA and DNA viruses serve as vectors for the transformation of plant cells. Consequently, this approach is appropriate for in planta transformation and simplifies the process of developing gene-edited plants that lack transgenic elements.

Conclusion

In conclusion, GE holds immense potential for enhancing the nutritional quality and food security of vegetable crops in the face of a changing climate. Techniques such as CRISPR-Cas9 allow for targeted modifications that can improve crop resilience to environmental stresses, increase yield, and enhance nutrient profiles. These innovations are crucial, as global agriculture faces challenges such as rising temperatures, unpredictable weather patterns, and declining arable land. By leveraging GE, researchers can develop vegetable varieties that are more adaptable to these shifts, ensuring a stable food supply and addressing malnutrition. However, the successful integration of GEs into agricultural systems will require careful consideration of regulatory policies,

ethical concerns, and equitable access to these technologies. As we move forward, GE stands as a vital tool for building sustainable and resilient food systems in a rapidly evolving climate.

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