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Application of CRISPR/CAS9 for Manipulating Vegetable Crops: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Authors VA and SS designed the study, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

To fulfill the demands of a growing global population, vegetable crop breeding must balance preserving genetic diversity and nutritional value with increasing yields. Traditional breeding procedures are arduous and time-consuming, slowing development. However, recent advances in genome editing tools, notably CRISPR/Cas9, provide exciting opportunities for accelerating vegetable breeding by allowing precise modifications to the plant genome. CRISPR/Cas9 has transformed genetic manipulation by giving a diverse tool for precise gene editing, including knockout, insertion, deletion, and substitution. Its use in improving crop genetics, yield, quality, and resilience to biotic and abiotic stressors has been intensively researched. The practical applications of CRISPR/Cas9 in vegetable crops like tomato, cucumber, and eggplant have shown

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enhancements in drought and salinity tolerance, resistance to diseases, tolerance to herbicides, and fruit ripening regulation, thus affirming the technique's significance across various physiological traits. Despite these advances, challenges remain in successfully delivering CRISPR/Cas9 components into plant cells. While off-target effects are a major concern, cytotoxicity is less commonly emphasized in plant genome editing. It might be clearer to say "ensuring specificity and minimizing unintended genetic modifications. Additionally, the introduction of genome-edited vegetable crops brings forth significant ethical and regulatory issues, especially regarding biosafety evaluations, intellectual property rights, and public acceptance, which differ markedly across various global jurisdictions. Nonetheless, CRISPR/Cas9 has demonstrated tremendous potential in vegetable crop development, permitting researchers to create varieties with increased resilience to environmental stresses, immunity to biological threats, herbicide resistance, and improved ripening time and quality. Especially, certain genome-edited vegetable lines are moving toward commercialization, indicating a change from experimental frameworks to translational research and field-level use. These advances illustrate CRISPR/Cas9 technology's transformational impact on vegetable breeding and its potential for addressing future food security challenges and its rapid advancements offer an urgent and indispensable solution for securing global food supplies amid climate change and population growth.

Keywords: CRISPR; food security; genome editing; vegetable crops.

1. INTRODUCTION

The primary source of nourishment in a human's daily diet is vegetables. Vegetable crop yields are rising, while diversity and nutritious benefits are tumbling down (Khoury et al., 2014). To cope with the world's rapidly increasing population, developing and accelerating vegetable breeding methods is indispensable. Vegetable breeding is carried out primarily via recombination and through the application of genetic markers. fortunately, morphological and genetic analysis of the breeding material is laborious and time-consuming, therefore drastically restricting vegetable breeding. SDNs ("Site-Directed Nucleases") have the probable to be a potent tool for faster breeding by improving the genetics of vegetables (Tran et al., 2021,). Right now, genome editing instruments like ZFNs ("Zinc-Finger Nucleases" (Miller et al., 2007; Porteus and Baltimore., 2003), as well as TALENs ("Transcription Activator-Like Effector Nucleases"), are utilized to modify the DNA sequence at particular sites throughout the genome. The advancement in genome editing techniques reached a significant milestone with the innovation of methods to generate precise DNA fractures. Over the years, genome editing has experienced notable advancements, propelled by the introduction of synthetic sequence-specific nucleases (SSNs). At present, clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) technology, initially pioneered by (Jinek et al., 2012) and subsequently advanced by (Cong et al., 2013), stands as the foremost choice for genome editing. On the other hand, TALEN as well as ZFN technologies were not broadly embraced because of their intricate nature and the technical difficulties they entail (Wood et al., 2011; Zhang et al., 2011). Researchers primarily employ genome editing tools of 3 kinds: ZFN (Kim et al., 1996), TALEN (Boch et al., 2009; Christian et al., 2010), and CRISPR /Cas9 (Jinek et al., 2012; Mali et al., 2013; Cong et al., 2013), to specifically modify particular sites in the plant's genome. However, the genome editing process, which involves accurate alterations, has encountered challenges related to the operation and a relatively elevated failure rate (Nieves-Cordones et al., 2017).

CRISPR/Cas had been effectively utilized for genome editing as well as management across several species since 2013 (Mali et al., 2013; Xie et al., 2015; Ran et al., 2015; Chen et al., 2017). While it has recently gained prominence as the ideal tool for the manipulation of gene in the plants, CRISPR/Cas has proven highly valuable in enhancing crop genetics (Wolt et al., 2016). Recently, CRISPR/Cas has seen extensive utilization in enhancing crop yield (Cai et al.,, 2021; Zhou et al., 2019), improving crop quality (Xu et al.,, 2021; Xing et al., 2020), increasing resistance to abiotic stresses (Bouzroud et al., 2020; Nieves-Cordones et al., 2017), bolstering resistance to the biotic stresses (Oliva et al., 2019; Ji et al., 2018), providing herbicide resistance to crops (Liu et al.,, 2021; Zhang et al.,, 2019b), and even facilitating de novo crop domestication (Zsögön et al., 2018; Li et al., 2018, Standage-Beier et al., 2015).

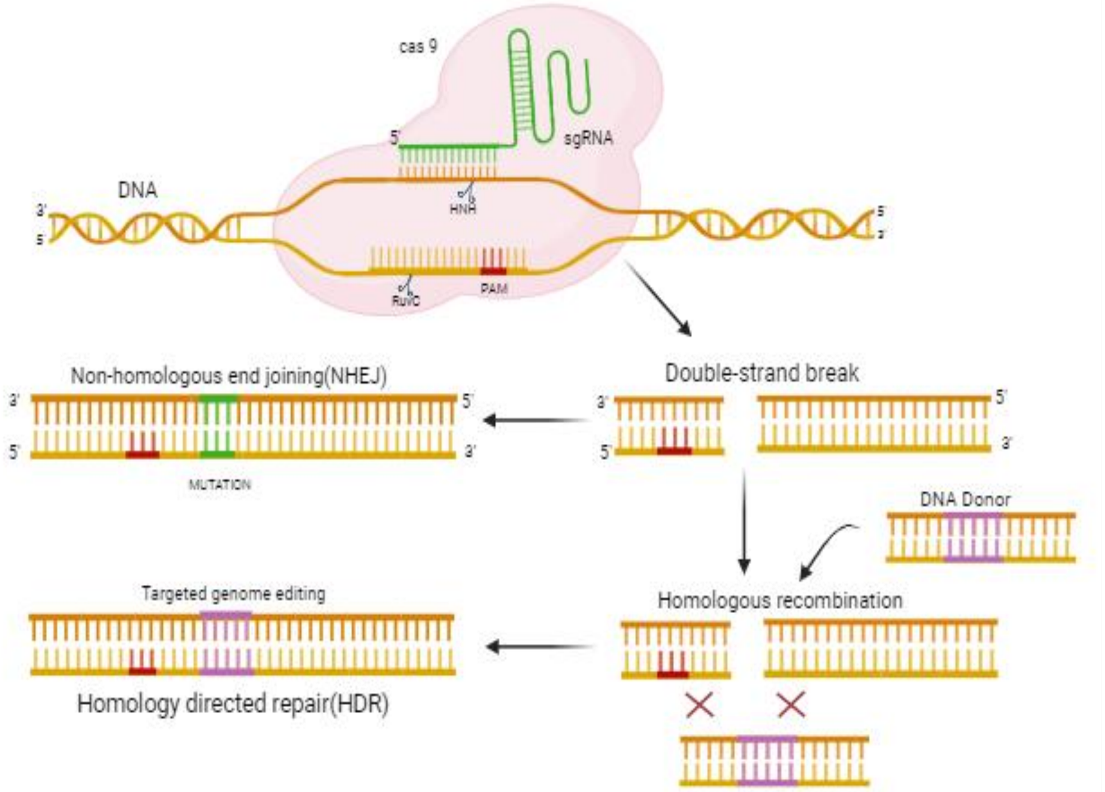


Fig. 1. An illustrated representation of "CRISPR/Cas9-mediated genomic modification

Fig. 1 reveals Cas9, guided by a sgRNA, cleaving double-stranded DNA to generate a DSB ("Double-Strand Break"). Following this, DNA repair transpires through one of 2 pathways: HDR or NHEJ. CRISPR/Cas9 and PAM stand for protospacer adjacent motif.

CRISPR/Cas9 represents the most recent gene editing method employed for altering favorable characteristics (Biswas et al., 2022; Bhattacharyya et al., 2022; Mitra et al., 2022; Sirohi et al., 2022; Nidhi et al., 2021; Pickar-Oliver & Gersbach,2019; Cong et al., 2013). CRISPR/Cas9 systems are found in only a limited number of bacteria and archaea, where they function as components of the immune system aimed at removing foreign intruders like viruses as well as plasmids (Koonin & Makarova. 2009, 2013). The 2 primary components of CRISPR/Cas9 are Cas9 and sgRNA (single-stranded guide RNA) (Negi et al., 2022, Martín-Pizarro, & Posé, 2018). The sgRNA directs the RNA-driven Cas endonuclease and is a truncated form of crRNA as well as tracrRNA (Hu et al., 2018,). By cleaving the DNA target, the nucleases HNH, as well as RuvC of the Cas9-sgRNA complex, produce complementary along with non-complementary breaks. This process triggers various forms of DNA repair, like MMEJ ("Microhomology-Mediated End Joining"), NHEJ ("Non-Homologous End Joining"), and HDR ("Homology-Directed Repair"), allowing for specific knockout of the gene, deletion, insertion, and replacement (Hua et al., 2018, Ma, et al., 2019). SgRNAs have been crafted to target exact sequences of DNA positioned just 3 bp before the PAM ("Protospacer Adjacent Motif") (Liu et al., 2016; Barrangou et al., 2015).

2. THE EMERGENCE AND EVOLUTION OF THE CRISPR/CAS9 SYSTEM

Yoshizumi Ishino first documented the system of CRISPR in 1987, although its biological significance remained undiscovered then (Mirza. Z et al., 2019). This system had been classified into 2 primary classes with 6 subtypes distinguished by effector proteins (Manghwar H et al., 2019; Wright A. et al., 2016). The kind 2 CRISPR-Cas9 system, broadly utilized in genome editing, comprises three key components: CRISPR RNA (crRNA), the enzyme Cas9, and tracrRNA (Martinez-Lage M et al., 2018). This system is comprised of 2 essential parts: the protein of Cas9, responsible for DNA cleavage, and the "guide RNA, designed to target specific DNA sequences containing A's, T's, G's, and C's. The guide RNA pairs with the DNA-cutting enzyme Cas9" to form a complex introduced into target cells. Cas9 then accurately identifies the target sequence and initiates DNA cleavage at that location, allowing for modifications or additions to the existing genome. Thus, CRISPR-Cas9 operates as a DNA editing tool comparable to 'cutting and pasting' (Doudna et al., 2014; Barrangou et al., 2007, Li et al., 2016, 2018, Ito et al., 2015).

This technology enables precise alterations to any identified genomic sequence using a short guide RNA (Jiang et al., 2017). In the year 2013, it has been 1st applied "to target the human genome (Cong et al., 2013). Since then, CRISPR-Cas9 has been" broadly utilized to edit genes in samples of animals, plants, and humans. Its applications span several scientific fields, involving therapeutics, medical science, as well as animal & plant sciences (Zhang Y et al., 2020; Sun et al., 2020; Li et al., 2019, Ahmad S et al., 2020; Tahir et al., 2020, Hu et al.,2018).

3. CRISPR/CAS 9 APPLICATIONS

The technology of CRISPR/Cas9 rapid advancement is unparalleled. Much of the research conducted so far has concentrated on mechanisms like gene silencing or gene knockout through NHEJ, that is imprecise but widely utilized. However, strategies involving "gene knock-in or gene replacement after targeted mutagenesis by HDR" have shown significant promise in both mammalian along plant cells. Previously, achieving HDR in plants posed challenges because of lower effectiveness and ineffective homologous donor sequence delivery into the transfected cells of plants (Steinert et al., 2016; Puchta and Fauser, 2014). Various methods have been utilized to enhance HDR effectiveness, leading to favorable results (Humanes et al., 2017; Collonnier et al., 2017). Analyzing the genomic characteristics of woody plants proves difficult due to their extended periods of vegetative growth, low efficiency in genetic transformation, and the scarcity of mutant variants available for study. The targeted disruption of the native PtoPDS (Phytoene Desaturase Gene) in the Populus tomentosa Carr led to the generation of both homozygous and heterozygous pds mutants in initial generation (Fan et al., 2015).

The technology of CRISPR/Cas9 utilization has offered to various Plantae kingdom lower members, including bryophytes, algae, and pteridophytes. Liverworts, in particular, have gained prominence as model organisms for examining the evolutionary aspects of land plants. Researchers have employed CRISPR/Cas9 for precise mutagenesis in *Marchantia polymorpha* L (Sugano et al., 2014, González et al., 2020). Apart from genome editing, the technology of CRISPR/Cas9 is advancing quickly and finding applications in diverse areas of functional genomics and molecular biology, expanding its utility beyond targeted genetic modification. Presently, there is a concentration on examining gene functionalities utilizing both loss-of-function along gain-of-function methodologies, alongside the identification of gene modules and comprehension of genetic expression patterns. CRISPR has supplanted RNA interference (RNAi) as a gene silencing technology, providing a more effective as well as accurate method for gene knockdown. It has successfully addressed several shortcomings of RNAi, including "incomplete loss-of-function evaluation and notable off-target effects. The concurrent expression advancement of numerous guide RNAs (sgRNAs) has empowered the CRISPR/Cas9 system to facilitate multiplex genome editing. It is an effective tool for diminishing genetic redundancy in the paralogous sequences by generating multiple gene knockouts. Furthermore, it had been utilized to induce chromosomal deletions spanning numerous base pairs of DNA in plant species like *Nicotiana benthamiana* and Arabidopsis, among others.

4. DELIVERY OF CRISPR/CAS AGENTS IN PLANTS

Due to the swift advancement of CRISPR-Cas" technologies, which encompass diverse functionalities, capacities, and specialized uses, CRISPR/Cas utilization for the editing of genome in plants has markedly increased in efficiency and efficacy, particularly in the domains of enhancing crops and advancing translational research (Vats et al., 2019). To begin, the procedure entails identifying a distinct segment within the gene of interest, positioned before a 5'-NGG-3' PAM sequence (Jiang et al., 2013). The sgRNA is crafted to exhibit similarity to the target sequence, typically spanning around 20 base pairs, while also including a "scaffold" sequence crucial for interacting "with the Cas9 protein (Doudna et al., 2014)". Once expressed, the Cas9 protein as well as sgRNA unite to create a complex capable of attaching to any genomic sequence featuring a PAM site. However, cleavage of the target site occurs solely if it closely aligns with the sgRNA (Kleinstiver et al., 2016). The sgRNAs main function is to direct Cas9 to particular genomic sites, and also with their accurate design is pivotal for the effectiveness of gene editing. Additionally, it is essential to mitigate the risk of off-target impacts, that may arise when sgRNAs match other sites resembling the target sequence (Doench et al., 2016). When aiming to activate the mechanism of HDR, it is important to include a template of DNA repair consisting of the required mutation along with the Cas9 nuclease and the sgRNA(s). To streamline this procedure, it's important to have homologous sequences situated directly preceding and following the target site, referred to as the left and right homology arms. The repair template could be administered as a “single or double-stranded oligonucleotide, or as a double-stranded DNA plasmid (Li, J. Meng et al., 2016; Sun et al., 2016, Globus, & Qimron, 2018).

The Cas9” along with sgRNA expression elements are subsequently introduced into the plant’s genome, which had been commonly achieved via AMT (Agrobacterium-Mediated Transformation) techniques “(Fan et al., 2015; Zhou et al., 2014). The presence of Cas9” and sgRNA leads to the generation of DSBs at the designated site of the target (Yin et al., 2017, Gil-Humanes et al., 2017, Danilo et al., 2019, Alomari et al., 2018). Following repair by either NHEJ or HDR mechanisms, the intended mutations in the regenerated transgenic plants are detected by utilizing methods such as restriction enzyme assays or sequencing analysis (Soda et al., 2018). Transgenic populations frequently display diverse mutations or edits within the target gene, yielding 4 genotypic outcomes: homozygotes (identical mutations in both alleles), heterozygotes (mutation in 1 allele), biallelic (distinct mutations in both alleles), and chimeras (multiple differing mutations) (Yang et al., 2017). Certain plants may remain unaltered due to the lack of Cas9/sgRNA expression or inadequate target cleavage. Despite the provision of a DNA repair template to encourage HDR repair, some DSBs may still undergo repair via NHEJ, leading to unintended mutations (Xingliang et al., 2016). While the Cas9/sgRNA transgene as well as the region of the target are frequently situated at separate sites within the genome, the the Cas9/sgRNA transgene elimination via segregation in successive generations is feasible.

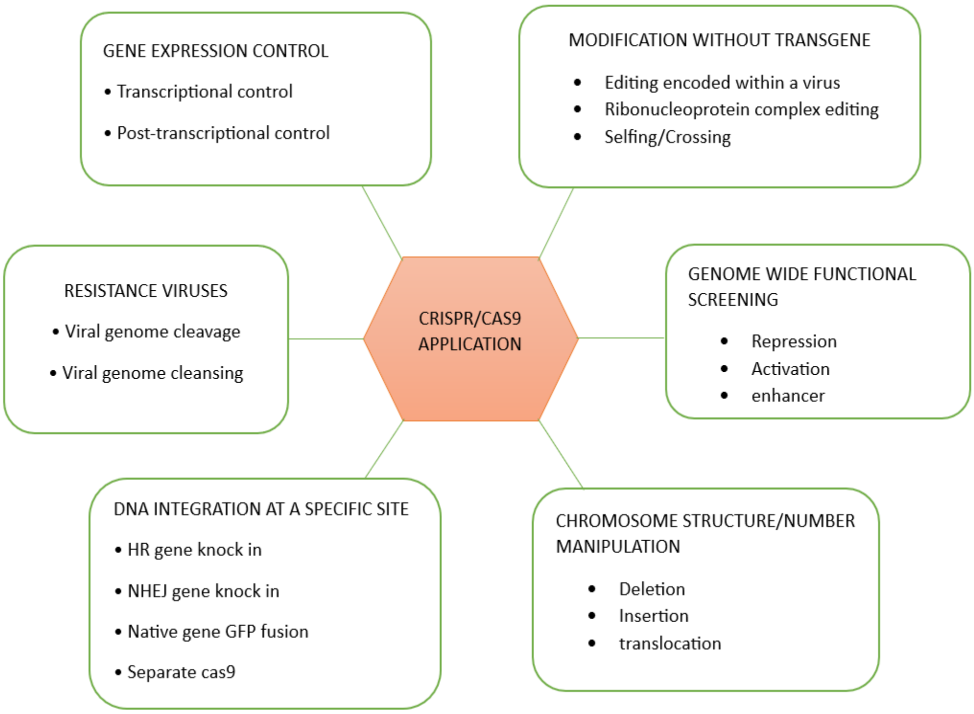
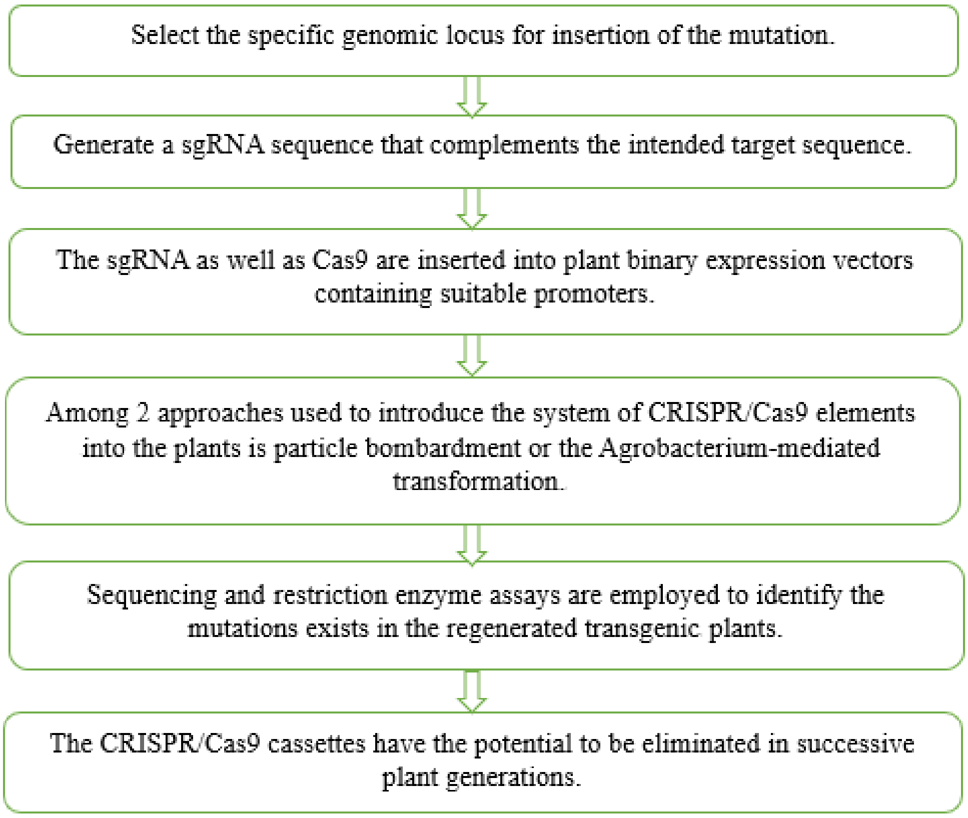


Fig. 2. CRISPR/CAS9 application



Select the specific genomic locus for insertion of the mutation.

Generate a sgRNA sequence that complements the intended target sequence.

The sgRNA as well as Cas9 are inserted into plant binary expression vectors containing suitable promoters.

Among 2 approaches used to introduce the system of CRISPR/Cas9 elements into the plants is particle bombardment or the Agrobacterium-mediated transformation.

Sequencing and restriction enzyme assays are employed to identify the mutations exists in the regenerated transgenic plants.

The CRISPR/Cas9 cassettes have the potential to be eliminated in successive plant generations.

Fig. 3. The CRISPR/Cas9 system fundamental process in editing plant genomes involves several key steps

5. DIFFICULTIES IN IMPLEMENTING CRISPR/CAS9 IN VEGETABLE CROPS

5.1 System of Efficient Delivery

Introducing CRISPR/Cas9 components into the plant's somatic cells presents difficulties. The genomes of plants can be altered by a range of methods, including particle bombardment, PEG-mediated protoplast transformation, floral-dip transfer, and AMT. However, AMT may encounter challenges due to the requirement for binary vectors, while the transfer of floral dip is viable mainly in plants with prolific flowers and seeds. Particle bombardment, though effective, tends to be less efficient and more expensive (Baltes et al., 2017). These difficulties could be mitigated by employing techniques such as pollen magnetofection along with nanoparticle-mediated delivery (Sandhya et al., 2020).

Agrobacterium-mediated transformation stands as the predominant method for delivering components of CRISPR/Cas9. This process entails inserting the target DNA fragment into CRISPR vectors like pRGEB31 and pRGEB32, then transferring these vectors into Agrobacterium. Following this, plant Calli or entire plants have been transformed using these bacteria loaded with the CRISPR components. Floral dip, unlike explant transformation via tissue culture, is a more straightforward and user-friendly method. It is commonly employed with Arabidopsis thaliana, where the target plant inflorescences are submerged in a solution consisting of Agrobacterium cells carrying the desired genetic modifications.

Seeds harvested from these types of plants which have been cultivated on selection media to detect transformed individuals. Additional validation is conducted through PCR (“Polymerase Chain Reaction”), “and the transgenic lines have been propagated across many generations until homozygous lines” have been attained.Additional transformation methods, including particle bombardment as well as PEG-mediated protoplast transformation, have been commonly utilized. Though, their efficacy changes depending on the plant species, as different plants exhibit diverse responses to these various transformation techniques.

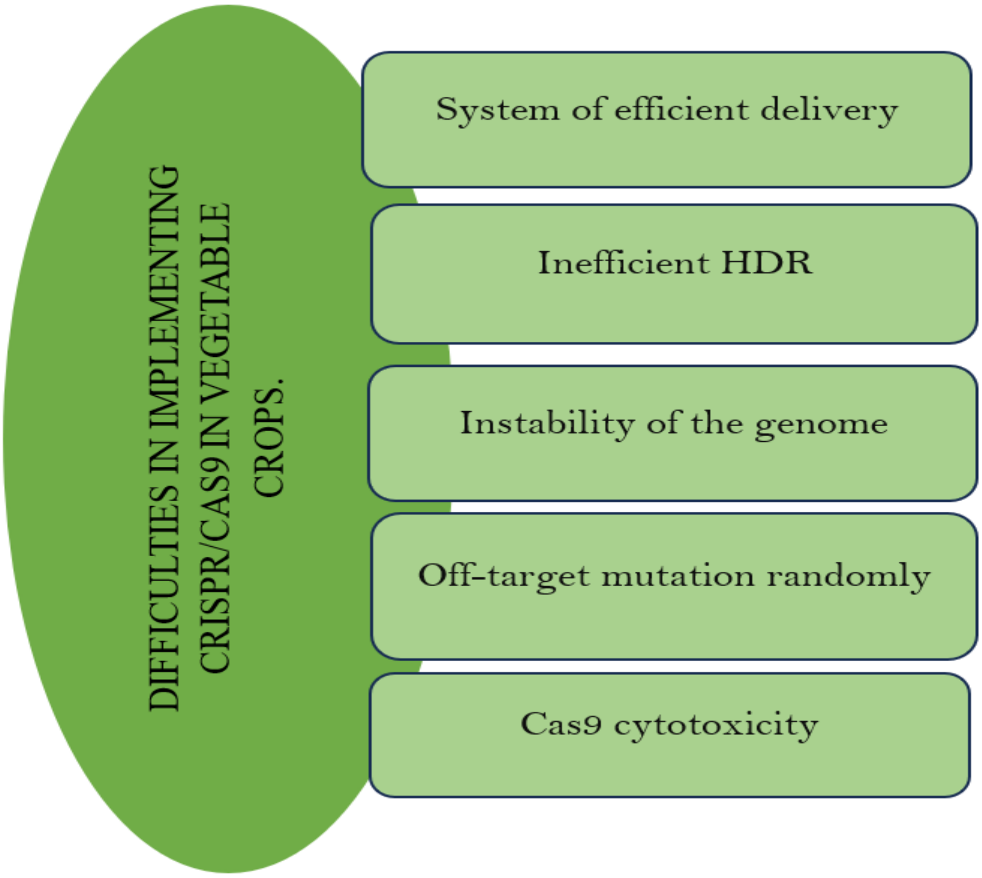


Fig. 4. Difficulties in implementing CRISPR/CAS9 in vegetable crops

5.2 Inefficient HDR

"In the majority of plant genome editing applications employing CRISPR/Cas9", NHEJ is the main mechanism involved in introducing indel mutations during the repair of DSBs. Indel mutations frequently result in gene knockouts. Nonetheless, NHEJ has its constraints. Conversely, HDR involves the recombination of a single DNA strand with a repair template, offering a more accurate method of DNA repair compared to NHEJ. HDR also facilitates the integration of overall DNA sequences. Efforts had been directed towards enhancing the initiation of HDR repair more efficiently. One strategy involves incorporating NHEJ inhibitors (such as resveratrol, Scr7, and L755507) or enhancers into HDR to accelerate the procedure (Chu et al*.,* 2015, Aird et al*.,* 2018).

5.3 Instability of the Genome

Unrepaired DSBs have the potential to induce cell death, which leads to instability of the genome, including "chromosomal rearrangements or aneuploidy, thereby limiting the utility of CRISPR/Cas9. Alternatively, highly effective DSB-free base editors such as the CRISPR/Base Editing System (CRISPR-BEST) and CRISPR" are utilized to alter the coding site through mutations (Eid et al*.,* 2018).

5.4 Off-target Mutation Randomly

In genome editing, CRISPR can occasionally result in uncontrolled off-target effects, posing challenges to achieving the desired outcomes of the technique. Although some studies have indicated minimal off-target effects, eliminating all off-target mutations remains challenging. However, when employing CRISPR to improve desirable traits, it is essential to recognize the existence of off-target impacts, that might lead to unintended gene mutations like deletions, insertions, inversions, and translocations. The primary strategy to decrease the risk of off-target impacts entails enhancing the sgRNA target specificity to decrease the likelihood of mismatches "(Doench et al*.,* 2016) and regulating levels of Cas9 within cells to mitigate off-target binding (Shen et al., 2019)".

5.5 Cas9 Cytotoxicity

The endonuclease enzyme SpCas9 is commonly utilized for genome editing in bacteria, and the Cas9 enzyme expression may sometimes exhibit toxicity. The system of CRISPR/Cas9 is perceived as toxic by numerous organisms, potentially leading to chromosome breaks as well as eventual failure of the editing of genes (Zhao et al., 2020). Cas9 toxicity could be mitigated by substituting stronger promoters with weaker ones or reducing endogenous Cas9 levels by utilizing alternative nucleases (eSpCas9, HypaCas9, and SpCas9-HF1) "(Standage-Beier et al., 2015). Additionally, employing editors of base to decrease DNA DSBs and then thus cytotoxicity could also alleviate Cas9 toxicity (Kleinstiver et al., 2016, Chen et al., 2018)". "To date, there had been no documented cases of Cas9 toxicity in the plants.

6. UTILIZATION OF CRISPR/CAS9

Table 1. Utilizing CRISPR/Cas9 gene editing in vegetable crops

Crop	**Target gene**	**trait targeted**	**References**
Tomato (*Solanum lycopersicu m L.)*	SlHyPRP1	Stress to salinity	Tran *et al.,* (2021)
	SlARF4	Stress to salinity	Bouzroud *et al.,* (2020)
	NPR1	Drought tolerance	Li *et al.,* (2019)
	MAPK3	Stress to drought	Wang *et al.,* (2017)
	SlGID1	Stress to drought	Illouz-Eliaz *et al.,* (2020)
	SlLBD40	Stress to drought	Liu *et al.,* (2020)
	UVR8	UV-B stress tolerance	Liu *et al.,* (2020)
	DMR6	Disease resistance against *Phytophthora capsica L, Xanthomonas spp., Pseudomonas syringae L*	Paula de Toledo Thomazella *et al.,* (2016)
	Mlo1	Disease resistance against *Oidium neolycopersici*	Nekrasov *et al.,* (2017)
	MAPK3	Disease resistance to Botrytis cinerea	Zhang *et al.,* (2018)
	MAX1	Disease resistance against *Phelipanche aegyptiaca*	Bari *et al.,* (2021)
	SlMAPK3	High temperature	Yu *et al.,* (2019)
	RIN	Ripening of fruit	Ito *et al.,* (2015)
	lncRNA1459	Ripening of fruit	R. Li, Fu, *et al., (*2018)
	SlDML2	Ripening of fruit	Lang *et al.,* (2017)
	GAD	Quality improvement	Nonaka *et al.,* (2017)
	Sl-ALMT9	Quality improvement	Ye *et al.,* (2017)
	CP sequence of TYLCV	Virus resistance	Tashkandi *et al.,* (2018)
	CCD8	Resistance to herbicide	Bari *et al.,* (2019)
	ALS1	Resistance to herbicide	Danilo *et al.,* (2019)
	IAA9	Parthenocarpy	Ueta *et al.,* (2017)
Potato *(Solanum tuberosum L.)*	StALS1	Resistance to herbicide	Butler *et al.,* (2015)
	GBSS	Starch quality	Andersson *et al.,* (2017)
	PPO2	Reduce enzymatic browning	Gonzalez *et al.,* (2019)
	GBSS1	Starch quality	Kusano *et al.,* (2018)

Crop	**Target gene**	**trait targeted**	**References**
	SBE1, SBE2	Starch quality	Tuncel *et al.,* (2019)
	PPO2	Reduce enzymatic browning	Gonzalez *et al.,* (2019)
	PDS	Albino phenotype	Tian *et al.,* (2017)
	GBSSI, SBEII	Quality improvement	H. Wang *et al.,* (2019)
Sweet potato (*Ipomoea batatas L.) Lam*.)	PDS	Phenotype	Ma *et al.,* (2019)
	FRI, PDS	Phenotype	Murovec *et al.,* (2018)
Cabbage (*Brassica oleracea var. capitata* L.)	MAX1	Improves plant architecture and increases yield	Zheng *et al.,* (2020)
Rapeseed (*Brassica napus L.)*	SFAR	Oil degradation	Karunarathna *et al.,* (2020)
	EOD3	Seed development	Khan *et al.,* (2021)
	FAD2	Fatty acid metabolism	Okuzaki *et al.,* (2018)
Camelina sativa	FAD2	Quality improvement	Jiang *et al.,* (2017)
	GIGANTEA	Flowering time	Park *et al.,* (2019)
Eggplant (*Solanum melongena L.)*	SmelPPO1-10	Enzymatic browning	Maioli *et al.,* (2020)
	eIF4E	Virus resistance	Chandrasekaran *et al.,* (2016)
Cucumber (*Cucumis sativus L.)*	RBOHD	Salt tolerance	Huang *et al.,* (2019)
	ALS	Herbicide resistance	Tian *et al.,* (2018)
Citrullus lanatus	PSK1	Biotic resistance	Zhang *et al.,* (2020b)
	F3H	Anthocyanin biosynthesis	Klimek-Chodacka *et al.,* (2018)
Daucus carota	PDS, FRI	Albino phenotype, flowering	Murovec *et al.,* (2018)
Brassica rapa	AP2a, AP2b	Sepal to carpal modification	Zhang *et al.,* (2018)
Pumpkin (*Cucurbita moschata* L.)	RBOHD	Salt tolerance	Huang *et al.,* (2019)

6.1 Enhancing Resilience to Environmental Stressors

“CRISPR/Cas9 genome editing gives a potential avenue for studying the functions of genes related to” stress-responsive proteins in vegetable crops, especially in the face of environmental difficulties like temperature extremes, UV radiation, salinity, and drought. By targeting specific genes, the goal of the researchers is to enhance the adaptability of higher-yield vegetable crops to adverse conditions. For example, in tomato plants, targeted deletion of specific domains within the SlHyPRP1 gene had been shown to notably improve tolerance of salinity at the time of both the germination as well as vegetative stages (Tran et al., 2021). Likewise, altering the SlARF44 gene has demonstrated efficacy in boosting salt stress resilience in crops (Bouzroud et al., 2020). Through the utilization of “CRISPR/Cas9 to modify GID1, researchers have successfully produced tomato plants exhibiting augmented leaf water content, consequently improving resistance to drought “(Illouz-Eliaz et al., 2020). Moreover, the CRISPR/Cas9-induced mutation of the SlLBD40 gene has markedly enhanced drought resistance in the” tomatoes (Liu et al., 2020). Increased temperatures, especially during critical growth stages such as establishment, gametophytogenesis, and flowering, can affect the entire growth cycle of crops (Jagadish et al., 2021). “CRISPR/Cas9-induced mutations, such as those in the slmapk3 gene, have” assisted tomato plants in maintaining a balance of ROS, regulating the antioxidant enzymes expression, and HSPs/HSF genes, ultimately improving heat tolerance (Yu et al.,, 2019). Additionally, altering the photoreceptor SlUVR8 through CRISPR-CAS9 knock-out mutants has demonstrated potential in regulating acclimation to lower-dosage “UV-B and enhancing tolerance to increase the stress of UV-B, potentially improving tomato plant performance in UV-B-exposed environments (Liu et al., 2020). Nevertheless, it is important to acknowledge that CRISPR/Cas9”-induced mutations, such as slnpr1 and slmapk3 in tomatoes, may lead to heightened vulnerability to drought stress in comparison with “wild-type plants (Li et al.*,* 2019, Wang et al.*,* 2017). Likewise, disrupting the RBOHD gene” in pumpkins utilizing CRISPR/Cas9 editing has caused the salt-sensitive characteristics attributed to modifications in root apex H2O2 and K+ content, and alterations in gene expression patterns (Huang et al.*,* 2019).

In a recent research, researchers improved the CRISPR/Cpf1-mediated HDR pathway to introduce a precise K+ transporter HKT1 amino acid point mutation; 2 into “the genome of tomato through the insertion of a gene” sequence. This development resulted in a stable salt-tolerant tomato variety with heritable characteristics (Vu et al.*,* 2020)

6.2 Boosting Immunity against Biological Stressors

CRISPR/Cas9 technology has developed as a valuable tool for bolstering plant resilience against diverse biotic stressors which are bacteria, viruses, nematodes, and fungi, that pose considerable threats to crop health. To address fungal diseases such as downy mildew and powdery mildew, which pose significant risks to tomato crops, CRISPR/Cas9 technology has facilitated the formation of a fungal disease-resistant tomato cultivar known as "Tomelo" through the deletion of the SlMlo1 gene (Nekrasov et al.*,* 2017). Likewise, manipulation of the DMR6 gene in tomatoes has been conducted to confer resistance against pathogens like *L*, *Phytophthora capsica*, *Pseudomonas syringae. “*and Xanthomonas spp. (Paula de Toledo Thomazella et al.*,* 2016). For example, the removal of the Jas domain from SlJAZ2 by utilizing CRISPR/Cas9 conferred resistance to P. syringae. L Tomato (Ortigosa et al.*,* 2019)”. Additionally, SAMPK3 CRISPR/Cas9-mediated mutation effectively combated the fungal pathogen Botrytis cinerea, which is the reason for Gray Mold disease in the crops, notably impacting vegetables as well as fruits. Therefore, there has been an increase in ROS levels and a decrease in defense enzyme activities, leading to modifications in the SA as well as JA defense signaling pathways as well as providing resistance to the B. cinerea “(Zhang et al.*,* 2018).

CRISPR/Cas9 had been utilized to edit the biosynthetic gene” MAX1 (MORE AXILLARY GROWTH 1) responsible for strigolactones (SLs) production to combat *Phelipanche aegyptiaca*. L, a root parasitic weed. The genetic alterations were passed on to subsequent plant generations, leading to decreased height, enhanced growth of axillary buds, and diminished formation of adventitious roots (Bari et al.*,* 2021). Cucumber plants were able to achieve heightened virus resistance through the eIF4E (“Eukaryotic Translation Initiation Factor 4e”) gene editing using “CRISPR/Cas9 (Chandrasekaran et al.*,* 2016). Similarly, tomato plants have been made resistant to the tomato yellow leaf curl virus by utilizing the system of CRISPR/Cas9 to target the genomic coat protein and well sites of replicase (Tashkandi et al.*,* 2018)”.

6.3 Stress Induced by Herbicides

Weeds present a significant threat to the productivity of vegetable crops as they compete with them for crucial resources such as space, light, water, and nutrients, leading to stress and yield reduction. To address this challenge, researchers utilize CRISPR/Cas9 gene editing, a technique employed in developing fruits and vegetables resistant to herbicides. An integral part of this genetic editing procedure involves the meticulous selection of target genes. CRISPR/Cas9 had been utilized to target P. aegyptiaca, a plant parasite that relies on strigolactones (SL) from its host's roots to stimulate seed germination, by mutating the MAX1 genes responsible for SL biosynthesis. This resulted in the formation of tomatoes resistant to P. aegyptiaca. Furthermore, to confer herbicide resistance in tomatoes and potatoes, Cytidine base editing (CBEs) techniques were utilized to convert a C-to-T base (Veillet et al.*,* 2019). In addition, scientists achieved the development “of herbicide-resistant watermelon plants by incorporating a mutation of a single point using CRISPR/Cas9-based base editing at a conserved position in the ALS (Acetolactate Synthase) gene (Tian et al.*,* 2018)”.

6.4 Improving the Ripening Time

Regulating the ripening process is a fundamental aspect of studying fleshy fruit and vegetable species. Tomatoes are particularly suitable for such investigations owing to their shorter cycle of life, ease of genetic modification, and efficient propagation. Utilizing CRISPR/Cas9 technology enables the genetic alteration of genes influencing ripening, consequently slowing down the ripening of tomatoes (Martín-Pizarro & Posé, 2018). An instance entails the disruption of the tomato DNA demethylase gene SlDML2 by utilizing technology of CRISPR/Cas9, leading to heightened DNA methylation. This modification impacts genes involved in both promoting and suppressing ripening, underscoring the substantial influence of DNA demethylation on the tomato ripening process (Lang et al., 2017). The RIN protein ("RIPENING INHIBITOR") plays a pivotal role in regulating ethylene biosynthesis in tomatoes. Utilizing the CRISPR/Cas9 system, scientists have introduced minor insertions or deletions in the RIN gene, leading to fruits that are not as fully matured and have less red "pigmentation than fruits of the wild type (Ito et al., 2015).

Additionally, another investigation revealed that CRISPR/Cas9-mediated editing of the lncRNA1459 gene notably inhibited ethylene production as well as lycopene accumulation (R. Li, Fu, et al., 2018). These results emphasize the efficiency of CRISPR/Cas9" in altering genes to delay the maturation process, indicating a promising avenue for further research.

6.5 Quality Enhancement

Some vegetable crops are essentially consumed as fruits, and the quality of these produce encompasses diverse aspects, including external attributes like color, size, and shelf life, along with internal characteristics like flavor, taste, and nutritional content (Patel et al., 2015). Improving vegetable quality and prolonging shelf life can be accomplished by integrating different strategies (Toivonen, 2009). Vegetables are crucial for supplying essential nutrients to human diets. Through "the utilization of CRISPR/Cas9 genome editing" methods, genes involved in synthesizing fatty acids, amino acids, vitamins, carbohydrates, and carotenoids could be efficiently modified. For example, editing the SlALMT9 gene (Aluminium-activated malate transporter 9) using CRISPR/Cas9 lead to an improved accumulation of malate in the "tomatoes (Ye et al., 2017). For tomatoes, the nonproteinogenic amino acid GABA" was observed to be increased in both fruit and leaves (Nonaka et al., 2017).

The technology of CRISPR/Cas9 had been effectively utilized to enhance the overall starch as well as seed oil content in sweet potatoes and rapeseeds. This was accomplished by targeting specific genes like "IbGBSSI (granule-bound starch synthase I) or IbSBEII (starch-branching enzyme II) for starch enhancement, and BnSFAR4 (seed fatty acid reducer 4) and BnSFAR5 (seed fatty acid reducer 5) for increased oil production (H. Wang et al., 2019, Karunarathna et al., 2020). Mutations introduced in the StPPO2 gene lead to a significant decline in tuber PPO (Polyphenol Oxidases) activity and enzymatic browning in potatoes (Gonzalez et al., 2019). Furthermore, Cas9-mediated mutagenesis of SBE along with GBSS genes holds promise in creating novel and potentially beneficial starch characteristics in potatoes (Tuncel et al., 2019; Kusano et al., 2018; Andersson et al., 2018, Andersson et al., 2017). Numerous endeavors have been made to enhance oil quality in Brassica napus L. along with Camelina sativa (L.) Crantz by targeting genes included in fatty acid metabolism (Okuzaki et al., 2018; Ozseyhan et al., 2018; Morineau et al., 2017, Jiang et al., 2017). In recent advancements, CRISPR/Cas9" modifications have been successfully established in carrot cells, showing promise as a valuable application. Klimek-Chodacka demonstrated that mutations in the anthocyanin biosynthesis gene F3H caused decreased callus discoloration as well as anthocyanin accumulation in the carrots (Klimek-Chodacka et al., 2018).

6.6 Parthenocarpy

Fruit crops exhibiting parthenocarpy, a process leading to seedless fruit development irrespective of fertilization, are considered to possess excellent agronomic characteristics. One possible tool for parthenocarpy tomato plant breeding is "the CRISPR/Cas9 system. A feature of tomato parthenocarpy, seedless fruit production was demonstrated by mutants created via CRISPR/Cas9 knockout of the SLIAA9 (indole-3-acetic acid inducible 9) (Ueta et al., 2017)".

7. CONCLUSION

The CRISPR/Cas genome editing utilization within vegetable crops presents a hopeful path for agricultural progress. It holds the promise of transforming the cultivation and consumption of vegetables by generating crops possessing sought-after characteristics. Nonetheless, ethical, regulatory, and environmental concerns necessitate meticulous attention to guarantee the conscientious application of technology. In essence, while CRISPR/Cas holds immense potential for enhancing vegetable crop yield, its effectiveness hinges on a harmonious strategy that underscores both innovation and safety.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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