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ADVANCING VEGETABLE CROP IMPROVEMENT: STRATEGIES AND PROSPECTS OF CRISPR-CAS9 GENE EDITING

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ABSTRACT

Vegetables are a crucial part of agricultural production systems and play a vital role in sustaining human life. Various biotic and abiotic stresses pose a threat to the growth, yield, and quality of these crops. Addressing these challenges is essential for ensuring food security and agricultural sustainability. While conventional breeding techniques have greatly advanced the development of key varieties, new methods are needed to further enhance horticultural crop production. Recent plant breeding tools such as the CRISPR/Cas9 technique offer rapid, cost-effective, and precise methods for crop improvement. CRISPR-associated protein 9 (Cas9) has proven to be a valuable genome-editing tool, capable of altering DNA sequences at specific loci with precision. With access to whole-genome sequencing data and knowledge about gene functions for important traits, CRISPR-Cas9 editing can precisely mutate key genes. This capability allows for the rapid generation of new germplasm resources to enhance important agronomic traits. This review provides an in-depth overview of CRISPR-Cas9 gene editing technology and explores its potential applications in olericulture as well as the challenges it faces.
Keywords **:** CRISPR, crop breeding, genome editing, new breeding techniques, trait improvement

Introduction

Global population dynamics are experiencing unprecedented shifts. Despite a recent decline in the growth rate, the total population continues to rise (Mason *et al.,* 2022). The global population is anticipated to reach 10 billion by the year 2050. At the same time, water and arable land resources are dwindling each year (El-Mounadi *et al.,* 2020) creating major challenges for the economy and the sustainable use of agricultural resources, including food production and safety. Interest in the benefits of vegetable consumption has been increasing due to their wide array of nutritional compounds such as vitamins, minerals, antioxidants, dietary fiber and numerous phytochemical's (Miller *et al.,* 2017). Vitamins and minerals play essential roles as important nutrients for human health, while the antioxidant compounds found in fruits and vegetables are recognized for their ability to reduce cellular oxidative stress. This reduction is important because it lowers the risk of developing chronic diseases such as cancer, diabetes, and cardiovascular conditions. (Septembre-Malaterre *et al.,* 2018; Aune *et al.,* 2017).

Plant breeding an intricate process aimed at creating novel crop varieties with favourable characteristics has grown significantly in importance (Singh *et al.,* 2024). This method integrates a range of strategies to generate superior varieties (Glenn *et al.,* 2017). Plant breeding an intricate process aimed at creating novel crop varieties with favorable characteristics has grown significantly in importance. This method integrates a range of strategies to generate superior varieties (Bigliardi *et al.,* 2013). Embracing emerging crop biotechnology methods has the potential to improve the efficiency and precision of varietal breeding (Parmar *et al.,* 2009). Genetic engineering has been utilized specifically to bolster resistance against both biotic and abiotic stresses, as well as to enhance the quality of fruits and vegetables. A significant milestone in this field occurred in 1994 when the FDA granted approval for a genetically modified tomato that exhibited enhanced storage capabilities (Kramer *et al.,* 1994).

Researchers have discovered and extensively studied numerous molecular and genetic mechanisms, enabling the reproduction of experiments in vitro. CRISPR-Cas9 stands out as one of the most recent and extensively embraced gene editing techniques Jinek *et al.,* 2012). Although it was first identified in the 1980s, it's full potential has been harnessed primarily over the past decade, generating considerable interest and debate about its applications in humans, animals and plants. CRISPR-Cas9 is utilized in both forward and reverse genetics (Gurumurthy *et al.,* 2016).

This review provides a comprehensive look at the various methods related to intrinsic CRISPR-Cas technology, highlighting its recent uses in vegetable crops and the progress made in CRISPR-Cas systems. Furthermore, we delve into the regulatory frameworks linked with CRISPR-Cas which aid in commercialization of gene edited crops across diverse countries.

The Emergence and Advancement of CRISPR Technology

In 1987, scientists initially discovered CRISPR-Cas associated genes in the genome of *Escherichia coli*. A Dutch scientist later coined the term "CRISPR" after identifying these genes (Rouillon *et al.,* 2013). In 2005, scientists uncovered that many CRISPR spacers contain short sequences that closely match fragments of extra chromosomal DNA. The Cas proteins have the ability to bind with both the CRISPR derived RNA products and the corresponding foreign DNA sequences. This process leads to the creation of a protein-RNA complex that can effectively cut foreign DNA. In both bacteria and archaea, the main role of the CRISPR complex is to incorporate specific fragments of foreign DNA, such as those from viral attacks, into their own genetic material. CRISPR-Cas technology has been successfully applied across various fields, transforming the landscape of genetic engineering, advancing research in disease and potential therapeutic interventions and editing the genomes of humans, animals and plants. Additionally, CRISPR-Cas technology has been adapted for diverse applications including drug screening, animal domestication and research in food science, showcasing it's versatility and impact across various fields. This versatile tool continues to revolutionize various fields by enabling precise genetic modifications (Cong *et al.,* 2013; Kaboli *et al.,* 2018; Shan *et al.,* 2013). Three distinct categories of CRISPR-Cas systems are recognized, each with unique characteristics. Notably, Types I and III demonstrate a complex interplay among multiple Cas proteins that is essential for their interference functions, effectively distinguishing them within the broader context of the CRISPR framework (Rouillon *et al.,* 2013). Type II uses a simple framework to enable interference. It takes advantage of its two specific nuclease domains, RuvC and HNH. This simplicity makes Type II CRISPR-Cas systems particularly advantageous for various genome editing applications. Gasiunas *et al.,* 2012). Among the various CRISPR nucleases, the type II Cas9 from Streptococcus pyogenes (SpCas9) is distinguished as the most widely used in CRISPR-Cas technology due to its broad applications and adaptability in genetic manipulation (Doudna *et al.,* 2014). The sgRNA-Cas complex identifies the protospacer adjacent motif (PAM), which activates Cas9 to cleave the target DNA. This process creates a double-strand break (DSB) in the DNA, then initiating the cell's DNA repair pathways, either through non-homologous end joining (NHEJ) or homology-directed repair (HDR).

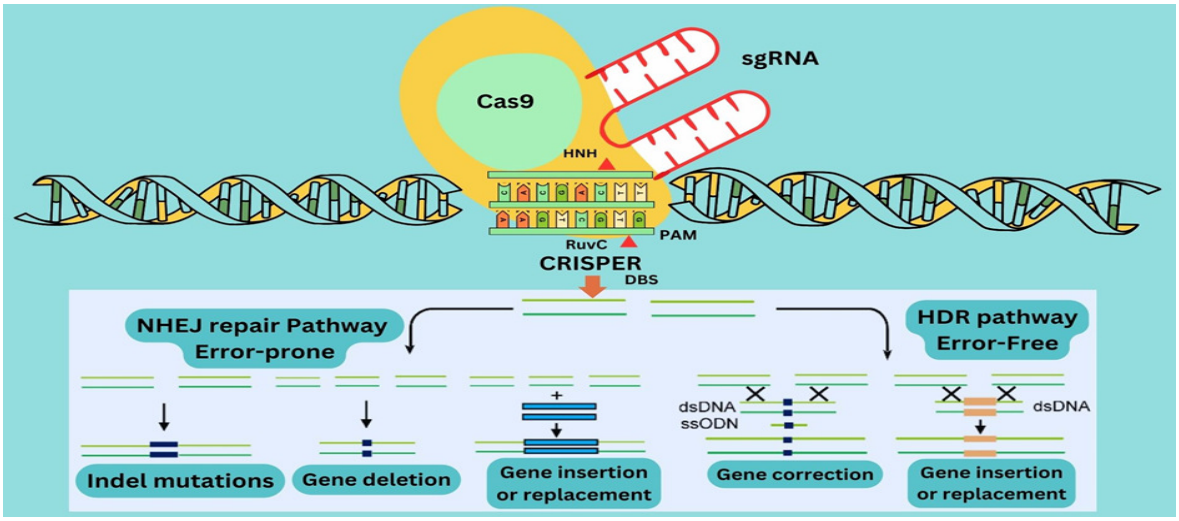

Fig 1 : The potential applications of CRISPR-Cas systems in genome editing.

In instances where a homologous repair template is absent, the non-homologous end joining (NHEJ) pathway is triggered at the site of the double-strand break (DSB), ultimately resulting in the disruption of gene function. Numerous strategies are devised to augment the frequency of homologous recombination between a genomic target and an external homologous template donor, each aimed at optimizing the efficiency of gene editing processes. Many of these strategies focus on increasing the number of donor repair templates through the utilization of virus replicons (Baltes *et al.,* 2014), in plant cells, tactics for enhancing homologous recombination frequency include the suppression of the non-homologous end joining (NHEJ) pathway (Endo *et al.,* 2006), and synchronizing the induction of double-strand breaks (DSBs) at target sites with the delivery of donor repair templates (Gil-Humanes *et al.,* 2017).

Enhancing Vegetable Cultivation through CRISPR-Cas9 Technology

Through disruption of the native phytoene desaturase (PDS) gene the first needle-leaf mutant in tomatoes was produced using CRISPR-Cas9. This disruption led to a distinctive albino phenotype providing a visual indicator of successful gene editing. Numerous studies have explored it's potential applications in enhancing plant resilience against biotic and abiotic challenges, along with enhancing the quality of fruit, modifying plant architecture and prolonging shelf life (Brooks *et al.,* 2014). CRISPR-Cas9 technology is currently under research for a range of fruits and vegetables including tomato, cabbage, mustard and watermelon showing promising potential for crop enhancement.

Many gene-editing investigations have assessed mutation efficiency based on the yield of albino plants resulting from the alteration of the native phytoene desaturase gene. Disrupting PDS hinders chlorophyll and carotenoid production, leading to a distinct albinism phenotype in plants. The results obtained from gene editing utilizing this approach do not possess substantial economic relevance (Ma *et al.,* 2019; Sun *et al.,* 2018). Due to it's significant economic importance and the accessibility of Agrobacterium-mediated transformation, tomato has emerged as a prime crop for evaluating the applications of CRISPR-Cas9 technology (Figure 2).

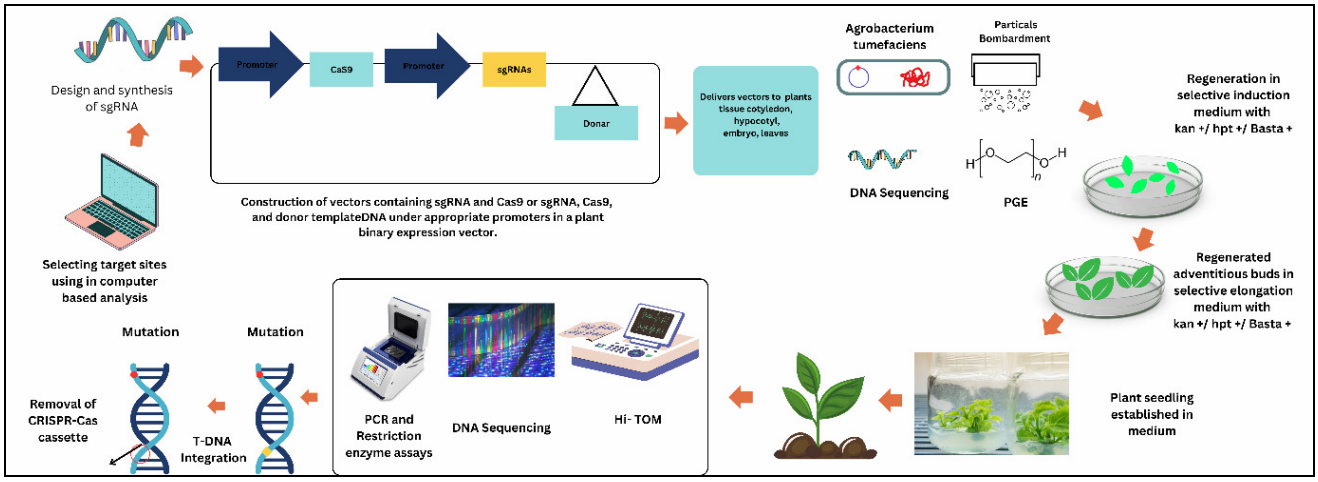

Fig. 2 : CRISPR-Cas9 mediated genome editing

Revolutionary Application of CRISPR/Cas9 Technology

The application of the CRISPR-Cas9 technique for crop modification has shown remarkable efficacy, with efficiency rates reaching up to 91.6% in rice and 79% in maize. The versatility and effectiveness of CRISPR-Cas9 make it a valuable tool for advancing plant genetic research and crop improvement (Tian *et al.,* 2017; Strygina *et al.,* 2020). CRISPR-Cas9 potential as a transformative plant breeding tool has not gone unnoticed. The plant breeding community has taken a keen interest in this technology, recognizing it's immense value in accelerating crop improvement and addressing global challenges in food security and sustainability. A significant portion of it's popularity stems from its simplicity, allowing for easy design and it's ability to multiplex, enabling the simultaneous editing of multiple loci by introducing multiple double-stranded breaks (Mao *et al.,* 2013). With the use of Cas-9 technology numerous vegetable crops have been successfully modified to meet a variety of scientific objectives. These goals include elucidating the functions of specific genes and achieving various applied breeding objectives as highlighted in Table 1.

Table 1 : List of Targeted gene and trait modified via CRISPR in vegetable crops

Vegetable	Targeted gene	Targeted trait	Method of trait modification	References
Brassica campestris	Pectin-methylesterase genes Bra003491, Bra007665, and Bra014410	methylation of pectin	Agrobacterium-mediated transformation	(Ma *et al.,* 2018).
Cabbage	PDS and FRI	Albino phenotype and flowering	PEG-mediated protoplast transfection	(Sun *et al,* 2018).
Carrot	Flavanone-3-hydroxylase	Anthocyanin biosynthesis blockage	Agrobacterium-mediated callus transformation	(Ueta *et al,* 2017).
Carrot	DcMYB113-like	Anthocyanin biosynthesis	Agrobacterium-mediated transformation	(Klap *et al,* 2017).
Chinese kale	BaPDS1 BaPDS2	Albino phenotype	Agrobacterium-mediated transformation	(Sun *et al*, 2019).
Chicory	Chicory	Albino phenotype	Agrobacterium-mediated leaf sections and protoplast transfection	(Bernard *et al,* 2019).
Cucumber	eIF4E	Virus resistance	Agrobacterium-mediated cotyledon transformation	(Bastet, *et al,* 2019).
Eggplant	*SmelPPO*	Agrobacterium-mediated transformation	KO/lowered enzymatic browning in eggplant berries	Maioli *et al.,* 2020
Lettuce	*LsBIN2*	PEG-mediated protoplast transfection	KO/targeted gene disruption in whole plants regenerated from protoplasts	Woo *et al.,* 2015
Potato	16 alfa hydroxylation (St16DOX)	Steroidal glycoalkaloids (SGAs) biosynthesis	Agrobacterium- mediated shoots transformation	(Nakayasu *et al.,* 2018)
Tomato	Carotenoid cleavage dioxygenase 8	Agrobacterium- mediated transformation	Resistance against *Phelipanche aegyptiaca*	(Bari *et al.,* 2019)
Tomato	NPR1	Agrobacterium-mediated cotyledon segments transformation	Drought tolerance	(Wang *et al.,* 2015).
Tomato	Alcobaca (SLALC)	Long shelf-life	Agrobacterium-mediated hypocotyls transformation	(Wang *et al.,* 2019).
Watermelon	PDS	Albino phenotype	Agrobacterium-mediated callus transformation	Tian *et al.,* 2017).

The CRISPR-Cas9 technology has been widely utilized to demonstrate its capabilities by introducing mutations in the phytoene desaturase gene across different vegetable crops. This includes successful applications in cabbage, Chinese kale, tomato, and watermelon showcasing the system versatility and effectiveness in gene editing. This gene plays a crucial role in carotenoid biosynthesis, and its disruption results in an albino phenotype, serving as a visual marker for successful gene editing. The CRISPR-Cas9-mediated disruption of the phytoene desaturase (PDS) gene has been successful in generating albino plants, which can be utilized to investigate the impact of PDS disruption on plant development and stress tolerance. Furthermore, CRISPR-Cas9 system has been leveraged to introduce desirable traits into these crops including enhanced disease resistance and improved nutritional profiles, thereby expanding its potential applications in crops improvement and breeding (Li, Wang *et al.,* 2018; Bertier *et al.,* 2018; Bari *et al.,* 2019; Ito *et al.,* 2015). Among vegetables, tomatoes have received the most extensive research attention utilizing the CRISPR-Cas9 system driven by the crops substantial economic value and the ease of genetic transformation via Agrobacterium. This has resulted in an emphasis on favorable characteristics like parthenocarpy which is greatly esteemed by consumers and holds considerable significance in processing applications (Ledford, H., 2017). Tomatoes represent a model crop for artificial domestication through the application of CRISPR-Cas9 technology. Over years of selective breeding based on harvesting practices, cultivars with jointless fruit stems have been developed ensuring that the fruit remains attached to the plant even after maturation. This trait

not only reduces post-harvest losses but also enhances the efficiency of mechanical harvesting, making tomato cultivation more sustainable and cost-effective (Soyk *et al.,* 2015; Ledford, 2017). The CRISPR-Cas9 system utilized as a breeding tool to develop parthenocarpic tomato varieties. By precisely editing specific genes researchers can create tomato varieties that produce fruit without fertilization, leading to seedless tomatoes that can improve both yield and quality. By precisely targeting and modifying genes involved in fruit set and development researchers can create tomato varieties that set fruit without fertilization leading to improved yields and reduced dependence on pollination. Additionally, CRISP-Cas9 mediated parthenocarpy can enhance the consistency and predictability of fruit production, making it a valuable tool for tomato breeding programs (Ueta *et al.,* 2017). Seedless tomatoes are produced from the T0 generation of bi-allelic and homozygous SlIAA9 mutants derived from the Micro-Tom and Ailsa Craig cultivars (Xu *et al.,* 2017). The number of flowers which is determined by the structure of the inflorescence, has a significant impact on plant productivity. In both tomatoes and Arabidopsis, the BLADE-ON-PETIOLE genes are crucial for regulating leaf complexity and silique dehiscence. Using CRISPR-Cas9 to eliminate BOP function has been shown to alter inflorescence morphology in tomatoes potentially impacting flower production and overall yield. The CRISPR-Bop1/2/3 triple mutant exhibited accelerated flowering compared to wild-type plants, yet it displayed remarkably simplified inflorescences. This mutation highlights the crucial role of BOP1/2/3 genes in regulating inflorescence complexity and flowering time in tomatoes. The simplified inflorescences suggest that the BOP genes are essential for the proper development and architecture of tomato flowers, indicating their potential as targets for genetic manipulation to enhance flowering traits and overall plant productivity (Parkhi *et al.,* 2018). To expedite the ripening process and reduce the time it takes for tomato fruits to ripen, researchers employed CRISPR-Cas9 gene editing technology to modify key genes involved in fruit development. Specifically, they targeted the APETALA2a (AP2a), NON-RIPENING (NOR), and FRUITFULL (FUL1/TDR4 and FUL2/MBP7) genes, which play crucial roles in regulating fruit ripening. This approach involves knocking out the encoding genes for these transcription factors, which are known to regulate tomato fruit ripening in collaboration with plant hormones like ethylene and their downstream effector genes (Soyk *et al.,* 2017). Crop cultivation is often limited by the sensitivity of plants to photoperiod. However, manipulating genes associated with photoperiod can expedite the domestication process. By disrupting the self-pruning 5G (SP5G) gene, a swift flowering response is initiated, resulting in an earlier fruit harvest Boscaiu *et al.,* 2020).

Advanced Breeding Strategies for Improved Vegetable Traits

Agriculture has advanced dramatically due to genome editing's ability to precisely modify plant genomes. This breakthrough has achieved a long-standing objective of plant breeders worldwide. Vegetables are acknowledged as vital crops for human nutrition because of their high vitamin content and phytochemicals which help to prevent disease and preserve health. Vegetable crops are susceptible to various abiotic stresses, including drought, salinity, flooding, and nutrient deficiencies, in addition to a range of pests and diseases caused by bacteria, fungi, and viruses. (Erpen-Dalla Corte *et al.,* 2017). Genome editing, particularly with CRISPR-Cas9 systems has been widely used in a variety of crops. Generally, it has been employed for total knockout through certain indel mutations to determine the function of the genes to be edited or generate crops with desired characteristics Table 1 (Ito *et al.,* 2015).

Tomato

The tomato (*Solanum lycopersicum* L.) serves as the model vegetable crop for testing CRISPR-Cas9 techniques aimed at crop improvement, due to its significant economic importance and the availability of extensive genomic resources. Targeted mutations have also been introduced into an exon and an untranslated region (UTR) of the RIPENING INHIBITOR (RIN) gene using the CRISPR-Cas9 technology. The MADS-domain transcription factor that controls the ripening of tomato fruit is encoded by this gene Alonge, (M., *et al.,* 2020). CRISPR-Cas9-mediated disruption of six distinct genes in the wild tomato (*Solanum pimpinellifolium*) led to a tenfold increase in both fruit number and size. In functional genomics CRISPR-Cas9 has established itself as a standard technique, especially for confirming potential genes found in genome-wide association studies (Maioli *et al.,* 2020).

Brinjal

Enzymatic browning in Brinjal (*Solanum melongena* L.), caused by polyphenol oxidase activity, leads to undesirable discoloration of the fruit flesh upon exposure to air. The three PPO genes, SmelPPO4, SmelPPO5 and SmelPPO6, identified with high transcript levels post cutting play crucial roles in this process. By employing CRISPR-Cas9 based mutagenesis, researchers have targeted these PPO genes for simultaneous knockout aiming to mitigate

enzymatic browning and extend the shelf life of eggplant fruits. This approach not only addresses a longstanding issue in eggplant post-harvest quality but also showcases the potential of genome editing technologies in crop improvement strategies (Chandrasekaran *et al.,* 2016).

Cucumber

When CRISPR-Cas9 was first utilised in cucumbers (*Cucumis sativus* L.), it's primary goal was to eliminate the eukaryotic translation initiation factor 4E (eIF4E) gene in order to confer broad virus resistance. Since this gene is essential for viral replication, CRISPR-Cas9 was used to damage it in an attempt to increase cucumber resistance to viral infections. This could increase crop yields and decrease losses from viral diseases (Hu B. *et al.,* 2017). In cucumber farming gynoecious inbred lines are prized for their higher production potential and lower labour expenses related to hand pollination. By focusing on the WPP trp/pro/pro domain Interacting Protein1 (CsWIP1) gene, CRISPR-Cas9 techniques were utilised to produce mutants of Cswip1. This gene encodes a zinc finger transcription factor that may have an impact on gynoecy-related features and is known to alter cucumber plant development. In order to improve yield and agricultural efficiency targeted mutations like these present a possible way to advance cucumber breeding efforts. Cucumber carpel formation is likely inhibited by the Cswip1 T_0 mutation, as seen by the gynoecious phenotype of plants yielding solely female flowers (Maoto *et al.,* 2019).

Watermelon

Watermelon (*Citrullus lanatus*), eulogized as a "Mood Food" crop belonging to the Cucurbitaceae family, is renowned for it's rich content of citrulline, vitamins and lycopene Tian *et al.,* 2017). Mutations induced by CRISPR-Cas9 in the phytoene desaturase (ClPDS) gene which plays a pivotal role in carotenoid synthesis resulted in the predictable albino phenotype in watermelon plants (Zhang, *et al.,* 2020b). The phytosulfokine1 (ClPSK1) gene which is linked to watermelon sensitivity to *Fusarium oxysporum* f. sp. *niveum* (FON) infection was subjected to a knockout mutation using the CRISPR-Casp-9 technology. The watermelon seedlings' resistance to FON infection was enhanced by the loss-of-function mutant of ClPSK1 (Zhang *et al.,* 2020a). A gynoecious gene called ClWIP1 expresses itself specifically in carpel primordia seen in male flower buds. Additionally, it is linked to the termination or abortion of carpel primordia in the early stages of floral development (Woo *et al.,* 2015). It has been possible to successfully create gynoecious watermelon lines by targeting the ClWIP1 gene with the CRISPR-Cas9 technology (Lee *et al.,* 2020).

Lettuce

In lettuce, Cas-9 and Cpf-1 ribonucleoproteins (RNPs) have been introduced into protoplasts using a DNA-free genome-editing method. PEG-mediated transfection was used to introduce CRISPR-Cas9 RNPs into lettuce protoplasts and the result was the regeneration of plants bearing the desired alterations. Based on the results of phytoene desaturase 1 (PDS1) sgRNA delivery, electroporation is more effective than PEG-mediated transfiguration for RNP delivery to protoplasts in cabbage. It's possible that less chemical toxicity is the cause of this improved efficiency (Woo *et al.,* 2015)

Enhancing Disease Resistance with CRISPR-Cas9 Genome Editing

CRISPR-mediated plant engineering for disease resistance has been documented in significant vegetable crops like tomato, cassava, and cucumber (Zaidi *et al.,* 2020). The ability of these genetic loci to confer resistance against a broad range of pathogen species or strains makes broad-spectrum resistance an effective strategy for managing crop diseases (Zhou *et al.,* 2018). This approach not only enhances crop resilience but also reduces the need for chemical pesticides promoting sustainable agriculture. Recent advancements in genome editing have enabled precise modifications, allowing for the fine tuning of resistance traits without affecting the plant's overall growth and productivity. The powdery mildew pathogen *Oidium neolycopersici* was more resistant to the tomato's Powdery Mildew Resistance 4 (PMR4) knock-out lines. This suggests that PMR4 plays a crucial role in the tomatoes defence mechanism against this pathogen (Martinez *et al.,* 2020).

Enhancing Abiotic Stress Resistance in Vegetable Crops

Vegetable crops encounter various abiotic stresses such as extreme temperatures, drought, salinity and heat all of which can significantly reduce crop productivity. Although traditional breeding techniques can mitigate stresses to a certain extent, innovative technologies like CRISPR-Cas9 offer the potential to create more resilient germplasm, enhancing the ability to cope with these stresses (Haque *et al.,* 2028). CRISPR-Cas9 gene editing has accelerated the process of developing new varieties. The advent of CRISPR-Cas9 gene editing has revolutionized the speed and precision of developing new plant varieties significantly reducing the traditional breeding timeline.

One such pivotal gene, Brassinazole-resistant 1 (BZR1), is integral to diverse developmental pathways regulated by brassinosteroids (BR), highlighting it's critical role in plant growth and adaptation. Disrupting BRASSINAZOLE RESISTANT 1 (BZR1) function hindered the activation of RESPIRATORY BURST OXIDASE HOMOLOG1 (RBOH1), leading to increased hydrogen peroxide (H_2O_2) production and enhanced heat tolerance in tomatoes. The application of exogenous H_2O_2 restored heat tolerance in tomato BZR1 mutants, indicating the potential for H_2O_2 to mitigate the effects of BZR1 disruption on heat stress response (Yin *et al.,* 2018). In tomato, the SlDMR6-1 orthologue Solyc03g080190.2 exhibits up-regulation in response to infection by both *Pseudomonas syringae* pv. tomato and *Phytophthora capsici.* CRISPR-Cas9 technology was employed to knock out tomato homologous genes, inducing mutations in DMR6. This genetic modification conferred broad-spectrum resistance against pathogens such as *Pseudomonas, Phytophthora* and various *Xanthomonas spp*.

Enhancing biotic Stress Resistance in Vegetable Crops

Globally, diseases represent a major threat to vegetable crop production causing substantial economic losses and food insecurity. A sustainable approach to meeting the needs of the world's growing population with food involves the development of cultivars resistant to diseases (Thomazella *et al.,* 2016). For centuries, traditional plant breeding has been instrumental in developing new varieties. However, modern technologies such as genome editing offer the potential to rapidly enhance varieties by precisely integrating beneficial alleles into locally adapted cultivars (Nekrasov *et al.,* 2017). In tomatoes, using CRISPR-Cas9 resulted in increased susceptibility to Fusarium wilt disease (Thomazella *et al.,* 2016).

Enhancing quality of Vegetable Crops

Fruits and vegetables are highly perishable and require advanced post-harvest technologies to ensure their storage stability and extend shelf life (Yu *et al.,* 2017). CRISPR/Cas9 technology successfully employed to delete the SlAGL6 gene in tomatoes, enabling the plants to produce fruit through parthenocarpy even when subjected to high-temperature stress. This genetic modification ensured that the fruit maintained it's desired weight, shape, and pollen viability (Klap *et al.,* 2017). Lycopene, an essential plant nutrient prized for its powerful antioxidant benefits, shields cells from oxidative stress. Researchers have amplified lycopene accumulation in tomato fruit by suppressing select genes in the carotenoid pathway using CRISPR/Cas9 technology. This approach led to a remarkable 5.1-fold increase in lycopene levels, underscoring CRISPR/Cas9's efficacy in enhancing nutritional content with minimal genetic disruption and reliable inheritance (Zhang *et al.,* 2018a). Enhancing potato starch quality for various food applications has been successfully achieved through CRISPR-mediated genome editing. This approach involved fully knocking out genes such as granule-bound starch synthase (GBSS), starch synthase (SS6), and starch-branching enzymes (SBEs) like SBE1 and SBE2. These genetic modifications have resulted in improved starch characteristics, showcasing CRISPR technology's potential to customize potato starch properties to suit diverse industrial and culinary requirements Andersson *et al.,* 2017 and Zhao *et al.,* 2021). CRISPR-Cas9 technology has been used in brinjal to remove the three-polyphenol oxidase (PPO) genes SmelPPO4, SmelPPO5, and SmelPPO6, which cause enzymatic browning in the fruit flesh Maioli *et al.,* 2020).

Enhancing herbicide resistance of Vegetable Crops

Vegetable farming faces a significant obstacle from weeds which can reduce both crop yield and quality. Selective herbicides are commonly utilized to effectively manage weed growth during cultivation. The application of CRISPR-Cas9 technology has been used to alter the acetolactate synthase (ALS) gene in crops such as tomato, watermelon, soybean and potato improving herbicide resistance and ensuring plant health and productivity Danilo *et al.,* 2019). The CRISPR-Cas9 system was employed to edit the Carotenoid dioxygenase 8 (CCD8) gene, a critical enzyme involved in the carotenoid biosynthesis pathway responsible for strigolactone production in tomatoes. Additionally, the More Axillary Growth1 (MAX1) gene, which is also implicated in strigolactone synthesis, was targeted for modification. These genetic alterations led to a significant decrease in the strigolactone (SL) content in tomatoes, resulting in the development of plants that are resistant to *Phelipanche aegyptiaca,* a parasitic plant (Bari *et al.,* 2019). Recently, the effectiveness of sgRNA in editing the herbicide-related genes pds, ALS, and EPSPS in tomatoes was evaluated utilising the CRISPR/Cas system. ALS2 P and ALS1 W sgRNAs successfully edited 19 transgenic tomatoes confirming the successful targeting and transformation. Of them, two tomato showed three-base alterations that might confer resistance to herbicides this underscores the accuracy and potency of CRISPR/Cas technology in agricultural genetic manipulation Yang *et al.,* 2022).

Challenges of Application of CRISPR-Cas Genome editing

The application of CRISPR-Cas9 technology for genome editing has profoundly revolutionized the development of diverse germplasm resources by leveraging insights gained from whole genome sequencing and functional genomics studies in fruit and vegetable crops. Gene-editing technology significantly influences the modification of gene expression in plants, albeit potentially restricting their ability to adapt. Hence, it is essential to have efficient and specific control over gene functions in order to achieve accurate genome editing. Cis-regulatory elements (CREs) play a vital role in controlling gene transcription and are comprised of noncoding DNA sequences. Changes such as mutations, insertions, deletions, inversions, and epigenetic modifications within CREs are strongly associated with crop domestication (Sapkota *et al.,* 2020).

The CRISPR-Cas system has proven to be an efficient tool for causing mutations in promoter regulatory regions, leading to the creation of different alleles with different phenotypes. These alleles are important genetic assets in breeding initiatives, making it easier to produce crops with specific characteristics. After identifying a target gene, the next important hurdle is to effectively transport CRISPR-Cas gene-editing agents into plant cells and then regenerate the potentially edited plants (Chuang *et al.,* 2021). The efficiency of genetic transformation in vegetable crops depends on multiple factors, such as the quantity and GC content of sgRNA the expression levels of sgRNA and Cas9 and the secondary structure of paired sgRNA and target sequences. These factors are essential in influencing the precision and effectiveness of CRISPR-Cas gene editing in agricultural settings (Xian *et al.,* 2019). The current challenges in genome editing for vegetable crops are expected to be effectively addressed with the emergence of strategies due to the vast potential of this technology.

Competing Interest

The authors affirm that they have no known conflicting financial interests or personal relationships that could have appeared to influence the work described in this research paper.

Author Contributions

ES conceived the idea and drafted the manuscript. All listed authors have made substantial intellectual contributions to the work and have approved the final version of the manuscript for publication.

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