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GENETIC ENGINEERING AND CRISPR (CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS) IN HORTICULTURE: A REVIEW

Shubham Jain[1*], Gopa Mishra[2], Deepak Singh[3], Ravi Pratap Singh[4], Rimpika[5], Veersain[6], Ajay Singh[7] and Vivek Kumar[8]

[1]Eklavya University Damoh, Madhya Pradesh 470661, India
[2]Department of Fruit Science FAS, SOADU, Bhubaneswar, Odisha, India
[3]Department of Horticulture, College of Agriculture, JNKVV, Jabalpur (M.P.) 482004
[4]Horticulture Maharishi Markandeshwar (Deemed to be) University Ambala Haryana, India
[5]Department of Fruit Science, College of Horticulture and Forestry Thunag Mandi, Himanchal Pradesh, India
[6]Department of Fruit Science, Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut, U.P., India
[7]Department of Horticulture, College of Agriculture, JNKVV, Jabalpur (M.P.) 482004, India
[8]Uttar Banga Krishi Vishwavidyalaya, Cooch Behar West Bengal, India
*Corresponding author E-mail: shubhu15296@gmail.com
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ABSTRACT

Horticultural crops, such as fruit, vegetable, and aesthetic plants, are an essential part of agricultural production systems and help to maintain human existence. Genome editing technology has the potential to transform the world of horticulture by giving new tools for genetic improvement of fruits and vegetables. Genome editing technology has the potential to revolutionize the horticulture industry by providing new approaches for genetic enhancement of fruits and vegetables. CRISPR-CRISPR-associated protein 9 (Cas9) has emerged as a major genome editing tool, in transforming DNA sequences at specified loci. CRISPR-based approaches like as base editing, prime editing, and RNA editing have expanded the technology's their relevance beyond genome editing.
Keywords **:** CRISPR/Cas9, Horticultural traits, genome editing, mutations, applications, limitations**.**

Introduction

The present global population is 7.7 billion, which is expected to rise to 8.5 billion by 2030, 9.7 billion in 2050, and 10.9 billion by 2100 (UN, 2019). As the human population grows, so does the need for food, and agricultural productivity must keep up (Ort *et al.,* 2015). Recent threats to agricultural output include evolving pests and diseases, droughts, heatwaves, climate change, and other abiotic pressures (Velásquez, 2018). Developing high-yielding crops that are resistant to biotic and abiotic stressors is one strategy to handle agriculture's mounting constraints and meet the rising need for food, feed, and fuel. Traditional horticulture plant breeding depends on natural variation and randomly mixed genes to achieve desired features, which is time-consuming, inefficient, and labor-intensive. Therefore, new breeding methods are urgently needed. Genetic engineering allows for the introduction of desired recombinant DNA into plant genomes, resulting in new genotypes and phenotypes for breeding (Chen *et al.,* 2020; Parmar *et al.,* 2017). To achieve T-DNA insertion-free breeding, gene editing methods are being prioritized. Engineered nucleases, such as zinc finger nucleases (Zinc Finger Nucleases), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9, have been used to enable site-specific gene editing. CRISPR/Cas9 technology is increasingly popular due of its efficiency, accuracy, and simplicity (Xu *et al.,* 2019). CRISPR/Cas9 is one of the most current and commonly used gene editing methods (Jinek *et al.,* 2012). This approach was initially described in the 1980s but just realized its full potential a decade ago. During this short period, there was much interest and

controversy about its usage in human, animal, and plant applications. The approach is used in both forward and reverse genetics (Gurumurthy *et al.,* 2016). CRISPR/Cas9 technology has been widely utilized to enhance agricultural disease resistance by removing susceptibility genes and overexpressing resistance genes. Crops with increased pathogen/disease resistance include powdery mildew resistant wheat (Wang *et al.,* 2014), cucumber vein yellowing virus-resistant cucumber, powdery mildew resistant apples and grapes (Malnoy *et al*., 2016), blast-resistant rice (Wang *et al.,* 2016), and canker-resistant citrus (Peng *et al.,* 2017). Adding and removing spacers inside the CRISPR locus can affect phage resistance and vulnerability (Laanto *et al.,* 2017). CRISPR arrays typically begin with AT-rich leader sequences, followed by arrays containing Cas protein instructions. Microbes acquire CRISPR immunity through three phases: adaptation or spacer acquisition, crRNA synthesis, and target interference.

Adaptation (Spacer acquisition): In the first step, when a bacterium comes into contact with a foreign genetic element (for example, viral DNA), particular CRISPR system proteins, notably Cas1 and Cas2 in most organisms, detect it. These proteins cut a short segment of foreign DNA, known as a protospacer, and integrate it into the host genome's CRISPR array. The CRISPR array is made up of repetitive DNA sequences separated by spacers, with each representing a portion of a previously encountered intruder. Protospacers are often chosen from areas surrounding a certain sequence known as the PAM (Protospacer Adjacent Motif), which is critical for determining the target for future interference. The adaption mechanism guarantees that each new spacer functions as a memory record of previous infections. The new spacer is placed into the leader end of the CRISPR locus, guaranteeing that the most recent viral infection is recorded first, allowing the bacteria to respond to new threats faster (Barrangou *et al.,* 2007).

crRNA Synthesis (Expression): Once a new spacer is added to the CRISPR locus, the CRISPR array is transcribed into a lengthy precursor RNA known as pre-crRNA. The pre-crRNA, which contains all of the repeat and spacer sequences, must be processed before it can function. During this step, specialized endonucleases, such as Cas proteins or RNase III in certain systems, cleave the pre-crRNA into smaller components known as CRISPR RNAs. Each crRNA consists of a single spacer (which is complementary to the invading DNA or RNA) and portions of the neighboring repeat sequence. The crRNA is subsequently associated with Cas proteins to form a surveillance complex, such as Cas9 in type II CRISPR systems or multi-Cas complexes in other kinds (Jinek *et al.,* 2012).

Target Interference: In the penultimate phase, the crRNA directs Cas proteins to locate and break foreign genetic material. When the same invader infects the microbe again, the crRNA in the monitoring complex identifies the corresponding sequence in the invader's DNA or RNA. In the well-studied Type II system, the Cas9 protein, directed by the crRNA and frequently a trans-activating crRNA (tracrRNA), recognizes foreign DNA based on the matching sequence of the spacer and protospacer, as well as the presence of a PAM sequence. (Makarova *et al.,* 2012) Cas9 causes a double-strand break at a specific spot in the invader's DNA, thereby eliminating the threat. Other CRISPR systems, like as Type I or Type III, employ various processes and multi-subunit protein complexes to interfere, but the final outcome is destruction of the invader's genome (Doudna *et al.,* 2014).

In recent years, various research on genome editing of horticulture plants have been published, including plants with resilience to biotic and abiotic challenges, changed blooming timings, increased fruit quality, altered flowers, and changing fruit colour (Shao *et al.,* 2020). CRISPR/Cas9 genome editing has the benefit of being able to alter several target genes at the same time. Plants with specific features can be generated faster than through standard breeding or transgenic approaches (Zhu *et al.,* 2019). However, horticultural plant genome editing has drawbacks, including protracted juvenile periods for fruit trees, polyploidy, and difficulty creating homozygous lines (Charrier *et al.,* 2019).

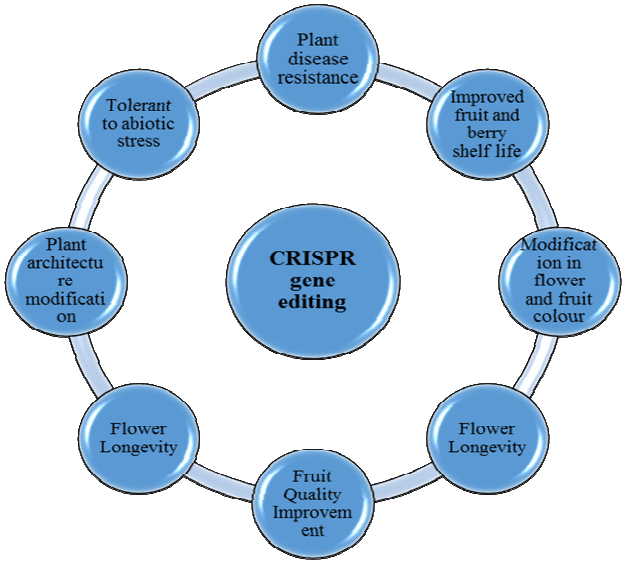

Fig. 1 : Horticultural traits that can be improved by genome editing

Cas9 nuclease and the guide RNA: The CRISPR-Cas9 system requires two components: Cas9 nuclease and guide RNA (gRNA). They enable precise DNA modification for horticulture plants. Cas9 nuclease has a recognition domain (Pattanayak *et al.,* 2011). This domain is endonuclease-active, which means it targets and cleaves DNA at specific locations. The guide RNA (gRNA) directs the Cas9 nuclease to the target DNA sequence, activating pathways for cellular DNA repair (Osakabe *et al.,* 2015). The CRISPR-Cas9 technology can enhance disease resistance in crops like tomatoes, providing greater protection against diseases like powdery mildew and bacterial spot. Tomato cultivars were engineered with CRISPR-Cas9 technology to improve resistance to the Tomato Mosaic Virus (ToMV). This was done by targeting and altering the eIF4E gene, which is a critical component of the ToMV infection process. Citrus canker is a deadly bacterial disease that infects citrus crops (Khanzadi *et al.,* 2020). CRISPR-Cas9 technology has been used to produce resistance against citrus canker. Researchers successfully developed citrus plants resistant to citrus canker infection by targeting and modifying the susceptibility gene CsLOB1 (Noman *et al.,* 2016). Research used CRISPR-Cas9 to knock out the gene CHS (chalcone synthase) in petunia plants. CHS is an important enzyme involved in flavonoid production (Rasheed *et al.,* 2022). Disrupting CHS caused significant alterations in pigment synthesis, revealing the role of flavonoids in determining the color of petunia flowers. This study suggests that CRISPR-Cas9 can effectively analyze gene activity related to horticultural crop traits (Urnov *et al.,* 2010).

Gene-editing technologies (Types and its mechanism)

Gene-editing tools use designed endonucleases that recognize and cleave particular DNA sequences using DNA-binding domain (DBD) proteins or guide RNAs, resulting in double-strand breaks (DSBs) at the target location. The DSBs are subsequently repaired using one of two types of cellular DNA repair mechanisms: homology-directed repair (HDR) or error-prone non-homologous end-joining breaks (NHEJ), which cause changes to the target sites (Wyman and Kanaar, 2006). Zinc Finger Nuclease was the first designed endonucleas with specialized DBDs for gene editing (Bibikova *et al.,* 2002, 2003). Zinc Finger Nuclease's DBD consists of several Cys2-His2 zinc fingers (ZF). Each ZF has around 30 amino acids that identify three nucleotides. A chimeric Zinc Finger Nuclease is created by fusing two pairs of tailored ZF arrays that recognize the upstream and downstream sequences of target genes' cleavage sites with the non-specific catalytic domain of endonuclease FokI. Zinc Finger Nucleases can create DSBs and induce mutations in certain gene sequences (Lloyd *et al.,* 2005).

TALENs (Transcription Activator-Like Effector Nucleases) are potent genome-editing tools that combine TALE proteins' DNA-binding selectivity with nucleases' ability to cleave DNA (Christian *et al.,* 2010). These proteins are produced from the Xanthomonas bacterium and may be designed to target specific DNA sequences during genome editing (Joung and Sander, 2013). TALE Domains: TAL effectors have repetitions that identify specific nucleotides. Each repetition has 33-35 amino acids, and the RVD at positions 12 and 13 determines DNA recognition specificity. FokI Nuclease: TAL effectors unite with the FokI nuclease, which causes double-strand breaks in DNA. Two TALENs must attach to contiguous sequences on opposing DNA strands before FokI can dimerize and cut. TALENs have been employed in genetic research, plant and animal model creation, and are being investigated for therapeutic genome editing. They have great specificity and are adaptable to target diverse sections of the genome (Boch *et al.,* 2009).

CRISPR/Cas9, a novel approach for genome editing, is increasingly replacing ZFNs and TALENs. Over the past decade, CRISPR/Cas9 technology has become the most popular gene-editing method due to its efficacy, simplicity, and convenience of use. In 1987, (Ishino *et al.,* 1987) discovered the first CRISPR sequence in Escherichia coli, which encodes an alkaline phosphatase isozyme conversion gene. The CRISPR/ Cas system consists of CRISPR RNA (crRNA), trans-activating crRNA (tracrRNA), and Cas proteins. CrRNA and tracr-RNA generate a guide RNA (sgRNA) that leads the Cas protein to the target gene location, resulting in DSBs (Jinek *et al.,* 2012). Streptococcus pyogenes is responsible for the most extensively utilized CRISPR/Cas9 system (type II in class 2). Unlike previous types of CRISPR systems, the CRISPR/Cas9 system is designed to function with two key components: a Cas9 endonuclease and a streamlined sgRNA (Sorek *et al.,* 2013). The sgRNA is built with a 20-nt DNA sequence upstream of the Cas9 recognition sequence, a short, trinucleotide (5-NGG-3 or 5-NAG-3) protospacer adjacent motif (PAM) in the target gene DNA. Cas9 protein goes to the target region under the guidance of sgRNA, identifies PAM sequences, and then cleaves the 3 nt location upstream of the PAM sequence to cause a DSB, which starts the HDR pathway or NHEJ pathway to insert, delete, or replace DNA at the target site. Streptococcus pyogenes is responsible for the most extensively utilized CRISPR/Cas9 system (type II in class 2). Unlike

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Table 1: Comparison of gene-editing technologies

Feature	TALENs	ZFNs	CRISPR/Cas9
Mechanism of action	Introduction of DSBs in target DNA	Introduction of DSBs in target DNA	Introduction of DSBs in target DNA
DNA cleavage domain	FokI	FokI	Cas9
Operation difficulty level	Moderate	Difficult	Easy
DNA recognizing domain	TALE	Zinc finger domain	sgRNA
Target DNA interaction pattern	Protein-DNA	Protein-DNA	RNA-DNA

Mutation of CRISPR/Cas Components to Plant Cells

There are various methods for delivering CRISPR/Cas components to plant cells. Vector constructions containing these components are commonly used to genetically change plants using the microorganisms Agrobacterium tumefaciens or A. rhizogenes. Several binary vectors with sgRNA and Cas nuclease genes have been produced. These genes are often inserted under the control of cauliflower mosaic virus 35S RNA, ubiquitin, or RNA polymerase III U6 and U3 promoters (Ren *et al.,* 2019; 2021). The downside of this editing strategy is the constitutive production of sgRNA and Cas nucleases, which may result in improper editing (Yin *et al.,* 2015). Some laboratories have developed virus-based vectors for plant genome editing (Ali *et al.,* 2015; 2018). The vectors are supplied to plants by agroinfiltration or biolistic transformation procedures. Protoplast transformation by PEG or electroporation can deliver vector constructs, including ribonucleoproteins (RNPs) carrying purified Cas nuclease and sgRNA. Grapevine and apple plants' genomes were altered using protoplast transformation with Cas9 and sgRNA-based RNPs (Malnoy *et al.,* 2016). The foreign DNA was not incorporated into the plant genome, so the gene editing was more targeted. Similar studies were conducted to improve the editing of the phytoene desaturase (PDS) gene in banana protoplasts (Wu *et al.,* 2020). Researchers have refined genome editing strategies for fruit and berry crops utilizing the PDS gene. In *Vitis vinifera* L. plants, high GC content in the sgRNA sequence was found to be the most important factor in boosting the frequency of mutations, rather than Cas9 nuclease expression (Ren *et al.,* 2019). Selecting and utilizing appropriate host plant promoters for sgRNA and Cas9 expression is crucial (Kaur *et al.,* 2018; Wilson *et al.,* 2019).

CRISPR/Cas9 genome editing in Fruit Crops

Although fruit crops are crucial for nutrition, they have received less attention due to their time-consuming breeding process and perennial nature. Therefore, transgenics or genome engineering are the most appropriate options for them. Citrus mutated the Phytoene desaturase (CsPDS) gene in the carotenoid biosynthesis pathway (Jia and Wang, 2014). (Jia *et al.,* 2017) used CRISPR/Cas9 to generate citrus canker-resistant plants by knocking out the susceptibility gene CsLOB1. Citrus alleles of the CsLOB1 gene include an effector binding element (EBEPthA4) that Xcc recognizes and activates CsLOB1 production. (Peng *et al.,* 2017) used a different method to produce citrus canker resistance by targeting an effector binding site in the CsLOB1 promoter. CRISPR/Cas9 genome editing has been demonstrated in watermelon (Tian *et al.,* 2017) and grape (Nakajima *et al.,* 2017), targeting the Phytoene desaturase gene in both crops.

CRISPR/Cas9 genome editing in Vegetable Crops

CRISPR/Cas9 investigations mostly focus on tomato, targeting 18 distinct genes. Table 1 provides details on how this technology is used. (Brooks *et al.,* 2014) announced the first CRISPR/Cas9-mediated genome editing in tomato, targeting the ARGONAUTE7 (SlAGO7) gene, which is important in leaf formation. Tomato biosynthetic pathway genes, including Anthocyanin 1 (ANT1), are involved in anthocyanin biosynthesis. (Cermak *et al.,* 2015) identified phytoene desaturase (SlPDS), phytochrome interacting factor (SlPIF4) (Pan *et al.,* 2016), and phytoene. CRISPR/Cas9 modified PSY1 (Hayut *et al.,* 2017), a carotenoid biosynthetic enzyme. The use of CRISPR/Cas9 to boost vegetable crops was first

reported in 2017. Two groups successfully developed parthenocarpic tomato fruits, which are highly demanded in the processing business. (Klap *et al.,* 2017) knocked out the Slagamous-like 6 (SlAGL6) gene, allowing mutant plants to produce parthenocarpic fruits under heat stress, which would ordinarily hinder fertilization-dependent fruit set. Alternatively, the other group used a different technique by altering the SlIAA9 gene, which is implicated in the auxin signaling system and inhibits the onset of fruit development without fertilization (Ueta *et al.,* 2017). This method of generating parthenocarpy is suitable for horticulture crops such as watermelon, pointed gourd, and bitter gourd that require seedless or less seeded fruits. (Andersson *et al.,* 2017) generated a "waxy genotype" in hexaploid potato by utilizing CRSIPR/Cas9 to mutate the granule bound starch synthase (GBSS) gene, which produces solely amylopectin-containing starch, similar to the one seen in maize (Klosgen *et al.,* 1986). The starch analysis revealed that one of the genome-edited lines generated exclusively amylopectin. Knocking down all four GBSS gene results in a total absence of amylose. This experiment established the usage of CRISPR/Cas9 may effectively mutate several alleles in polyploid crops. It has been used in potato to identify StMYB44 gene function, making it a useful tool for reverse genetics (Zhou *et al.,* 2017). Metabolic engineering in vegetables can enhance nutritional value. Targeting several genes in a pathway for metabolic changes can be tricky.

CRISPR/Cas9 genome editing in other horticultural Crops

CRISPR/Cas9 genome editing has expanded beyond fruits and vegetables to include medicinal and decorative plants. Lotus japonicus, a model legume, was the first ornamental plant to undergo genome editing using CRISPR/Cas9. (Wang L. *et al.,* 2016) found that targeting symbiotic nitrogen fixation-related genes, including symbiosis receptor-like kinase and leg hemoglobin loci (LjLb1, LjLb2, and LjLb3), resulted in both single and multiple gene mutant plants. *Chrysanthemum morifolium,* a significant hexaploid decorative plant, serves as another example. (Shishi-Kaboshi *et al.,* 2017) used a transgenic chrysanthemum with a stably producing yellowish-green fluorescent protein (CpYGFP) gene for genome editing. Two sgRNAs were targeted at distinct sites to alter the gene. Researchers believe that the PcUbi promoter for Cas9 and the AtU6 promoter for sgRNA are appropriate for genome editing in Chrysanthemum. Editing the genome of medicinal plants can aid in understanding the biosynthesis routes of secondary metabolites, leading to improved productivity and quality.

Table 2: CRISPR/Cas9 gene editing

Fruit plants				
Plant	**Botanical Name**	**Trait**	**Targeted Gene**	**References**
Apple	*Malus domestica*	Fire blight disease resistance	DIPM-1, 2, 4 MdDIPM4	(Malnoy *et al.,* 2016; Pompili *et al.,* 2020)
Banana	*Musa acuminata*	Photo bleaching, albinism	PDS	(Ntui *et al.,* 2020)
Blueberry	*Vaccinum corymbosum*	Dwarfism, lack of precocious flowering	CEN	(Omori *et al.,* 2021)
Grapes	*Vitis vinifera L*	Resistance to powdery mildew	MLO-7	(Malnoy *et al.,* 2016)
Kiwifruit	*Actinidia chinensis*	Compact growth, terminal flowering	AcCen4, AcCen	(Varkonyi-Gasic *et al.,* 2019)
Orange	*Citrus sinensis*	Delayed citrus canker symptoms	CsWRKY22	(Wang *et al.,* 2019)
Papaya	*Carica papaya L*	Photo bleaching, albinism	CpPDS	(Brewer *et al.,* 2022)
Pear	*Pyrus communis L*	Herbicide resistance	Md/PcALS	(Malabarba *et al.,* 2020)
Strawberry	*Fragaria vesca*	Dwarfism	FveARF8	(Zhou *et al.,* 2018)
Sweet orange	*Citrus sinensis*	Citrus canker resistance	CsLOB1	(Peng *et al.,* 2017)
Watermelon	*Citrullus lanatus*	Resistance to *Fusarium oxysporum* f. sp. niveum	Clpsk1	(Zhang *et al.,* 2020)
Vegetable plants				
Tomato	*Solanum lycopersicum*	Leaf development	ARGONAUTE7 (SlAGO7)	(Brooks *et al.,* 2014)

Potato	*Solanum tuberosum*	Herbicide resistance	ACETOLACTATE SYNTHASE1 (StALS1)	(Butler *et al.,* 2016)
Cabbage	*Brassica oleracea*	Plant development, fruit dehiscence	Gibberellin3-beta-dioxygenase 1 (BolC.GA4.a)	(Lawrenson *et al.,* 2015)
Lettuce	*Lactuca sativa*	Plant development	BRASSINOSTEROID INSENSITIVE 2 (BIN2)	(Woo *et al.,* 2015)
Ornamental Crops				
Chrysanthemum	*Dendranthema grandiflora*	Fluorescence	CpYGFP	(Kishi-Kaboshi *et al.,* 2017)
birdsfoot trefoil	*Lotus japonicus*	Nitrogen fixation	SYMRK, LjLb1, LjLb2, LjLb3	(Wang L. *et al.,* 2016)

Applications Of Crispr/Cas9 in Horticultural Crops

Fruit quality improvement

Fruit quality is crucial for horticulture production and sale, categorized as intrinsic quality (nutrition, flavor) and extrinsic quality (size, color, shelf life) (Hermanns *et al.,* 2020; Zhang & Hao, 2020). CRISPR/Cas9 technology has been utilized extensively to research tomato fruit quality (Wang *et al.,* 2019). Tomato fruits contain gamma-aminobutyric acid (GABA), which has been shown to relieve nervous tension and reduce blood pressure (Takahashi *et al.,* 1961; Bachtiar *et al.,* 2015). (Nonaka *et al.,* 2017) used CRISPR/Cas9 technology to boost tomato GABA content by deleting the autoinhibitory domains of GABA biosynthesis enzymes Glutamic acid decarboxylase 2 and GAD3. Fruit colors are determined by endogenous pigments like carotenoids and anthocyanins. Knocking down genes in the carotenoid metabolic pathway using CRISPR/Cas9 leads to increased lycopene accumulation in tomatoes (Li *et al.,* 2018c). Fruit quality is closely linked to its shelf life during post-harvest storage. During tomato ripening, pectate lyases (PLs) play an important role in softening the fruit through cell wall remodeling. CRISPR/Cas9 gene modification in tomatoes improved shelf life without affecting flavor or nutrition (Uluisik *et al.,* 2016; Wang *et al.,* 2019). (Yu *et al.*, 2017) found that deleting the alcobaca (ALC) gene using CRISPR/Cas9 methods increased the shelf life of alc mutant apples.

Modification of cultivation traits

Semi-dwarfism is a desirable horticultural characteristic for resilience to wind and rain, as well as ease of management. (Chen *et al.,* 2014) identified several QTLs and genes associated with dwarf phenotypes in plants. (Blazquez *et al.,* 2020) found that gibberellin-insensitive and GA-deficient mutants had a dwarfish phenotype. Mutating the MaGA20ox2 gene in banana using the CRISPR/Cas9 system results in decreased active GA content and a semi-dwarf phenotype, highlighting the importance of GA 20-oxidases (GA20ox) in GA biosynthesis. CRISPR/Cas9 mutation of the GA response repressor DELLA leads to a dominant dwarf mutant. Shortening the juvenility phase speeds up the breeding of woody horticulture crops. Knocking down the Terminal Flower 1 (TFL1) gene in apples and pears using CRISPR/Cas9 technology resulted in early blooming (Charrier *et al.,* 2019). TFL1 is a kind of flowering repressor.

Enhancing the resilience of horticultural plants to both biotic and abiotic stresses.

Plants are susceptible to ailments produced by bacteria, fungus, and viruses. Infections can negatively impact plant development and productivity, leading to considerable agricultural losses and higher production costs (Mushtaq *et al.,* 2018). CRISPR/Cas technology improves plant resilience to biotic stressors. To generate virus-resistant plants, two techniques are used: viral genome editing and altering virus-sensitive plant genes. Viruses often employ host-plant transcription and translation machinery. To defend plants from viruses, CRISPR/Cas technology can be used to impair the expression of sensitivity genes (S genes), such as knocking off translation initiation components. CRISPR/Cas9 technology produced bananas resistant to the endogenous banana streak virus (Tripathi *et al.,* 2019). Citrus canker, caused by Xanthomonas bacteria, has been successfully treated using genome editing. Citrus cultivars including *Citrus sinensis* (orange) and *C. paradisi* (grapefruit) were produced using genome editing, resulting in high resistance to diseases. Citrus plants' susceptibility to *Xanthomonas citr*i subsp. citri disease is determined by the CsLOB1 gene. The promoter region of this gene allows for the binding of the bacterium's pathogenicity factor PthA4, which causes illness symptoms. CRISPR/Cas9 was used to change the binding sites of

the PthA4 factor, limiting the bacteria's capacity to infect Citrus sinensis. Researchers used vector constructs to modify the promoter region of the CsLOB1 gene in the Wanjincheng orange cultivar (Jia *et al.,* 2019). Fungal infections are a major hazard to plants, producing a variety of illnesses. CRISPR/Cas9 technology has enabled the development of plants that are resistant to harmful fungus. This resistance is frequently developed by altering the genes responsible for plant sensitivity to certain diseases, which play a role in pathogen penetration and infection. However, subsequent research improved the editing procedure utilizing RNPs, resulting in alterations in three MLO genes that reduced grapevine plant sensitivity to powdery mildew by up to 77%. Grapevine plants evolved resistance to gray mold Botrytis cinerea by knocking out the gene encoding the transcription factor WRKY52. In addition to powdery mildew resistance, grapevine plants with improved resistance to the gray mold Botrytis cinerea were created by deleting the gene that encodes the transcription factor WRKY52, which functions as a negative regulator of the jasmonic acid pathway. Using CRISPR/Cas9 technology to alter pathogen sensitivity genes in plant cells provides a fast and reliable way to generate plants resistant to infections from viruses, bacteria, and fungi (Pattanayak *et al.,* 2011).

Modifying the agronomic characteristics of fruit plants through genome editing.

CRISPR/Cas9 editing of the MaGA20ox2 genes, which regulate gibberellin biosynthesis, resulted in the semi-dwarf phenotype of the banana plant *Musa acuminate* "Gros Michel" (Shao *et al.,* 2020). Mutants exhibited lesser growth but thicker, darker green leaves compared to the normal plants. The modified plants have different cell structures compared to the wild-type plants. These investigations are crucial for selecting dwarf banana types, as tall plants are vulnerable to severe winds, causing significant crop losses. CRISPR/Cas9 technology can change the color of strawberries from red to white. The scientists employed a deletion of the RAP gene, which encodes the glutathione S-transferase enzyme that transports anthocyanins from the cytosol to the vacuole (Gao *et al.,* 2020). Editing the RAP gene has the potential to create popular white strawberry cultivars. A mutation in the GlBG1 β-glucosidase gene in watermelon (*Citrullus lanatus*) reduced seed size and increased germination by lowering abscisic acid levels (Wang *et al.,* 2021). This gene regulates seed size and germination, an essential feature for watermelon breeding. Using CRISPR/Cas9 gene deletion, researchers were able to examine and increase the shelf life of fruit ripening genes in important plants for human consumption. Bananas were produced by altering the 1-aminocyclopropane-1-carboxylate oxidase 1 (MaACO1) gene, which is involved in ethylene production (Hu *et al.,* 2021).

Changing flower color and shape, flowering time, and extending flower longevity

CRISPR/Cas9 technology has been used to modify genes in both wild and domesticated strawberry plants, clarifying their roles in flower and fruit development. The first genes altered were FveARF8 and FveTAA1, both involved in auxin production, which is crucial for strawberry growth (Zhou *et al.,* 2018). Strawberry FveARF8 gene mutants exhibited larger and quicker growth than control plants. Mutations in strawberry genes (FaTM6 and FveSEP3) cause aberrant development of petals, anthers, and pollen grains, as well as parthenocarpy and an erroneous fruit phenotype (Pi *et al.,* 2021). These genes have a role in the growth of strawberries, including blossoms and berries. Genome editing allows for modifications to fruit plant blooming mechanisms. Knocking down the TFl1 flowering repressor gene has resulted in apple and pear plants (Charrier *et al.,* 2019). The Centroradialis (CEN) gene in blueberry plants (*Vaccinium corymbosum* L.) was modified (Omori *et al.,* 2021). The scientists predicted that knocking down this gene would result in early blooming, similar to previous studies on TFl1/CEN-like genes in apple, pear, and kiwi. Attempts to modify blueberry flowering by altering the CEN gene were unsuccessful. Furthermore, mutant plants exhibited significantly slower development compared to controls. A study on altered blueberry plants suggests that mutations in the CEN gene may contribute to the dwarf phenotype (Omori *et al.,* 2021). Researchers employed genome editing to explore genes regulating aging and changing the color of corollas in ornamental flowers such petunia, lily, chrysanthemum, ipomoea, gentian, torenia, and orchid. The PhACO1 gene, which regulates ethylene production, was altered in petunia cultivar "Mirage Rose" plants (Xu *et al.,* 2020).

Limitations and future prospects for genetic engineering's in horticulture crops

CRISPR/Cas9 genome editing technology has sped the production of superior horticultural plant types, although it still has limitations and challenges to use. Fruit and berry crops reproduce vegetatively, making it difficult to acquire modified offspring. Fruit trees have a protracted juvenile phase, polyploidy, and might be challenging to acquire homozygous lines. Few garden plants have been obtained from the T1

generation, such as kumquat CCDb4 mutants (Zhu *et al.,* 2019) and strawberry FveARF8 mutants (Zhou *et al.,* 2018). Optimal strategies for transforming and regenerating altered plants are necessary for certain fruit and berry harvests. To avoid chimerism in apple and pear trees, specific procedures with an additional regeneration step were established (Charrier *et al.,* 2019). New marker genes for selecting plants with mutations should be identified, as mutations in the commonly used PDS gene can result in aberrant and non-viable phenotypes. CRISPR/Cas9 genome editing does not necessarily result in homozygous plants. Instead, chimeric and biallelic lines are more common since the nuclease in transformed plants works throughout development. To address these shortcomings, promoters should be both constitutive and inducible. Recently, many very effective CRISPR/Cas9-based approaches for editing a variety of genes in pear microcalli were established (Ming *et al.,* 2022). The Cas12a and Cas12b nucleases used in the same investigations produced poor editing efficiency.The authors propose that gene editing with distinct nucleases is dependent on the kind of plant. Researchers have also developed additional Cas12 nuclease variants. Using the temperature-tolerant ttLbCas12a nuclease to modify the LOB1 gene was more efficient than Cas9 nuclease, resulting in canker-resistant citrus maximum plants (Jia *et al.,* 2022).

Summary and Conclusion

CRISPR/Cas9 systems are widely used in horticultural crops for breeding and trait improvement. Optimizing the system can speed up its application in more crops. Selecting the most active interspecies U6 or U3 promoters for sgRNA expression and tissue-specific and strong promoters for Cas9 expression can improve editing efficiency. SpCas9 variants and orthologs that recognize different PAMs broaden the genome-wide r. The CRISPR/Cas technology has constraints that preclude its widespread deployment. Most horticultural woody crops have challenges in obtaining homologous T-DNA free mutants by segregation because to lengthy juvenility and incompatibility with certain species. To achieve transgene-free plants, a gene edit system with heat-shock-inducible FLP/FRT and CRISPR/Cas9 expression cassettes was designed. The heat-shock inducible FLP/FRT recombination mechanism removes foreign DNA following gene editing (Pompili *et al.,* 2020). This approach shows potential for transgene-free breeding. STU-Cas9 systems are simple and small, making them ideal for gene editing activities. Effective sgRNA design is crucial for achieving improved editing outcomes. CRISPR/Cas9 vector delivery strategies differ per horticultural species, however nanoparticle-based alterations provide a viable approach. The CRISPR/Cas system is efficient, accurate, simple, and user-friendly due to its collaborative methods. These methods may be designated as non-transgenic crops, making them more acceptable in countries where the public opposes transgenic plants. The team is hopeful that genome-editing technologies, like as CRISPR/Cas, will be integrated into horticultural plant breeding, despite initial challenges. Establishing a policy for developing technologies and distinguishing between genetically modified and genome-edited organisms is crucial. Genome editing technology can enhance the appearance and nutritional value of fruits, vegetables, and flowers when combined with other breeding methods. This will improve our quality of life by making it more enjoyable and healthier.

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