CRISPR-Cas9 Applications in Horticultural Crop Improvement: Current Advances and Future Prospects

Suraj Jadhav[1,2 *], Prasad Parekar[2] , Pallavi Pawar[3], Vaibhav Rehpade[4], Trushal Chorage[5], Athoiba Singh[6]

[*1]School of Engineering, Ajinkya DY Patil University, Pune-- 412105, Maharashtra, India. (0000-0003-3758-4815)

[2]Department of Biotechnology, School of Basic and Applied Sciences, JSPM University, Pune- 412207, Maharashtra, India.

[3]Department of Chemistry, Dahiwadi College, Dahiwadi, Satara, Shivaji University, Kolhapur-416004, Maharashtra, India.

[4]Department of Food Technology, School of Mechanical and Manufacturing Sciences, JSPM University, Pune-- 412207, Maharashtra, India.

[5]School Pharmaceutical Sciences, Faculty of Health Sciences, JSPM University, Pune-- 412207, Maharashtra, India.

[6]Department of Plant Biotechnology, Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth (Deemed to be University), Pune-Satara Road, Pune-411046, Maharashtra, India.

Corresponding Author:

Dr. Suraj Jadhav

School of Engineering, Ajinkya DY Patil University, Pune-- 412105, Maharashtra, India

surajjadhav339@gmail.com

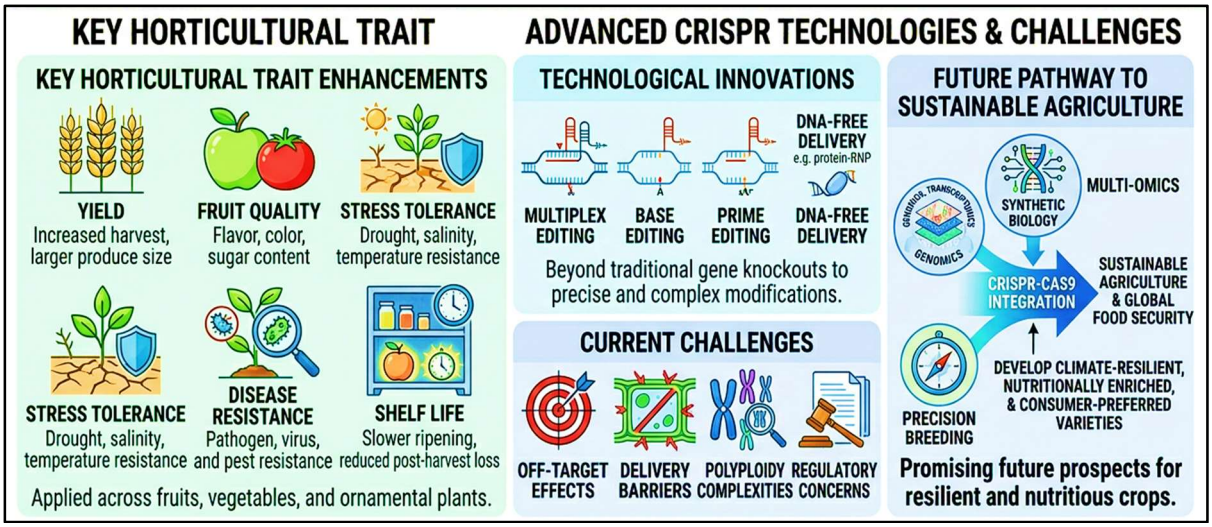
Abstract

CRISPR-Cas9 has revolutionized breeding programs for horticultural crops by facilitating targeted and efficient genetic modifications. These changes enhance key traits such as yield, fruit quality, tolerance to abiotic stresses, resistance to diseases, and post-harvest shelf life in a wide range of fruits, vegetables, and ornamental plants. In addition to conventional gene knockouts, emerging strategies—including multiplexed editing, base editing, prime editing, and DNA-free delivery systems—continue to expand its utility. Nevertheless, several practical limitations remain, notably off-target effects, challenges in delivery methods, complexities associated with polyploid genomes, and regulatory constraints. Integrating CRISPR-Cas9 with multi-omics approaches, synthetic biology, and precision breeding strategies promises to deliver resilient varieties adapted to climate change, enriched with nutrients, and aligned with consumer preferences. This review examines the underlying mechanisms, practical implementations, enduring obstacles, illustrative case studies, and future directions, emphasizing the technology's contributions to sustainable agriculture and global food security.

Keywords: CRISPR-Cas9, Horticultural crops, Genome editing, Multiplex editing, Base editing, Sustainable agriculture.

How to cite this article: Jadhav S, Parekar P, Pawar P, Rehpade V, Chorage T, Singh A. CRISPR-Cas9 applications in horticultural crop improvement: current advances and future prospects. Int J Drug Deliv Technol. 2026;16(79s): 337-357. DOI: 10.25258/ijddt.16.79s.37

Graphical Abstract:



Introduction

Horticultural crops—including fruits, vegetables, and ornamentals—are integral to global agriculture, contributing significantly to food security, nutrition, and economic development. These crops serve as important providers of essential nutrients such as vitamins, minerals, and dietary fiber, while also holding significant cultural and ornamental importance across the globe (Klee & Tieman, 2018). Nevertheless, the yield and quality of horticultural crops are consistently threatened by a range of biotic and abiotic stress factors, such as pest infestations, plant diseases, water scarcity, soil salinity, and extreme temperature conditions (Zhang et al., 2020). Traditional breeding methods have been employed for centuries to improve horticultural crops. Although these approaches have contributed to notable progress, they are frequently constrained by the available genetic diversity within the species and tend to be time-intensive and labor-demanding (Varshney et al., 2014). Moreover, the complex genetic architecture of many horticultural traits and the long generation times of perennial crops further complicate conventional breeding efforts (Hickey et al., 2019). The advent of modern biotechnology has revolutionized plant breeding, offering tools that allow for precise manipulation of plant genomes. Among the various approaches, genome editing technologies—especially the CRISPR-Cas9 system—have gained prominence as highly effective tools for enhancing crop traits (Jaganathan et al., 2018).

Genome editing encompasses a range of advanced techniques that allow for accurate and targeted alterations in an organism's DNA. Initial genome editing methods, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), utilized custom-designed proteins to identify and bind to specific DNA sequences (Gaj et al., 2013). Although effective, earlier genome editing systems like ZFNs and TALENs are often complex and time-intensive to design and implement. The advent of the CRISPR-Cas9 system has revolutionized genome editing by offering a simpler and more efficient approach. Originating from the adaptive immune system of bacteria, CRISPR-Cas9 employs a guide RNA (gRNA) to accurately direct the Cas9 enzyme to a specific DNA sequence, where it introduces a double-strand break (DSB). This break is subsequently repaired by the cell's innate mechanisms—either non-homologous end joining (NHEJ) or homology-directed repair (HDR)—resulting in precise mutations or insertions at the targeted site (Jinek et al., 2012). CRISPR-Cas9 offers several advantages over previous genome editing tools, including ease of design, high efficiency, and the ability to target multiple genes simultaneously (multiplexing) (Cong et al., 2013).

In recent years, the use of CRISPR-Cas9 in horticultural crops has rapidly expanded, with a growing body of research highlighting its potential to improve key traits such as resistance to diseases, tolerance to abiotic stresses, as well as enhancements in yield and overall crop quality (Zhang et al., 2019). For example, CRISPR-Cas9 has been successfully employed to develop resistance to powdery mildew in tomato by targeting the SlMlo1 gene (Nekrasov et al., 2017). Likewise, editing the MaPDS gene in banana led to the emergence of albino phenotypes, thereby validating the effectiveness of CRISPR-mediated genome editing pathways in this crop (Kaur et al., 2023). For example, editing the SlAGL6 gene in tomato led to parthenocarpic fruit development,

which is desirable in certain market segments (Ueta et al., 2017). In strawberries, targeting the FaMYB10 gene resulted in changes to fruit color, demonstrating the potential for modifying aesthetic traits (Zhou et al., 2018). The CRISPR-Cas9 system has further been explored for boosting abiotic stress resilience in horticultural crops. For example, in lettuce, targeted disruption of the LsNCED4 gene led to improved drought tolerance by altering the production of abscisic acid, a critical hormone in plant stress signaling pathways (Park et al., 2019). In a similar approach, modifying the CsWRKY50 gene in cucumber resulted in enhanced resistance to cold stress (Wang et al., 2020).

Although CRISPR-Cas9 holds great promise, its application in horticultural crops is hindered by several challenges. One significant obstacle is the efficient delivery of CRISPR-Cas9 components into plant cells, particularly in species that are resistant to transformation and regeneration (Altpeter et al., 2016). Additionally, off-target effects, where unintended genomic sites are edited, pose concerns regarding the specificity and safety of the technology (Zhang et al., 2015). Regulations for genome-edited crops differ around the world; some nations classify them on par with genetically modified organisms (GMOs), whereas others adopt more relaxed regulatory approaches (Wolt et al., 2016). Societal views and approval of genome-edited crops also impact their market introduction and widespread use.

Advancements in CRISPR-based technologies, such as base editing and prime editing, offer new opportunities for precise and efficient genome editing, which do not require the introduction of double-strand breaks (DSBs) (Anzalone et al., 2019). These new technologies can potentially extend the application of genome editing in horticultural crops. Integration of CRISPR-Cas9 with other omics technologies, including genomics, transcriptomics and metabolomics, can provide comprehensive information regarding the functions and regulations of genes and help precisely pinpoint the candidate genes for editing (Chen et al., 2019). Furthermore, the development of non-transgenic genome editing approaches - such as transient expression systems and DNA-free editing - can help address regulatory issues and improve public acceptance of genome-edited crops (Zhang et al., 2016). The use of CRISPR-Cas9 to improve traits in horticultural crops is discussed here, focusing on molecular mechanism, applications, challenges and prospects.

Mechanism of CRISPR-Cas9

Origin and Discovery

CRISPR-Cas9 is a naturally-occurring system found in bacteria and archaea, which targets viruses that infect them (bacteriophages) (Barrangou et al., 2007). It uses sequences known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and proteins (Cas) to recognise and eliminate foreign genetic elements. Jinek and colleagues (2012) demonstrated that by engineering synthetic single guide RNAs (sgRNAs), Cas9 could be targeted to cut DNA sequences of choice and enable genome engineering.

Molecular Components of CRISPR-Cas9

This gene-editing tool consists of two main elements: the Cas9 nuclease, an RNA-guided DNA endonuclease from Streptococcus pyogenes (SpCas9), which creates double-stranded breaks (DSBs) in the DNA (Doudna & Charpentier, 2014); and the guide RNA (gRNA), a synthetic chimeric RNA made up of a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA), which guides the nuclease to cut the DNA through base-pairing (Cong et al., 2013). The guide RNA usually consists of a 20-nucleotide sequence (complementary to the target DNA) and a scaffold region that is recognised by the Cas9 protein (Jinek et al., 2012). The DNA-cutting enzyme also requires a short sequence of DNA referred to as a Protospacer Adjacent Motif (PAM) to target and cut the DNA. For the enzyme from Streptococcus pyogenes, this sequence is generally 5'-NGG-3' (Anders et al., 2014). This motif allows the system to accurately differentiate between foreign and self-sequences and avoids self-targeting (Fig.1).

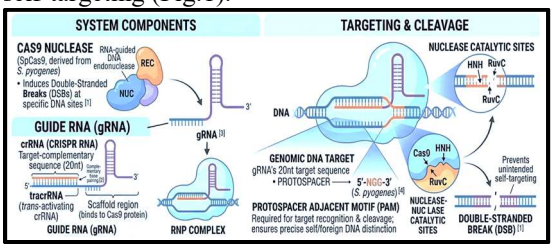


Fig 1: Detailed mechanism of Crisper Cas9 genome editing

Mechanism of DNA Cleavage

The nuclease is activated by conformational changes upon binding to the PAM. The guide RNA then anneals to the complementary target strand forming an R-loop (Soyk et al., 2017). This protein contains a nuclease domain that cleaves the non-target strand (RuvC) and another domain that cleaves the target strand (HNH) (Nishimasu et al., 2014). These cleave the DNA to generate a

blunt double-strand break (DSB) three base pairs upstream of the PAM.

The repair of double-strand breaks (DSBs) introduced by Cas9 occurs through two main processes: Non-Homologous End Joining (NHEJ) is a blunt-end rejoining process that can cause small insertions or deletions (indels) and cause the gene to be disrupted (Puchta, 2005). In contrast, Homology-Directed Repair (HDR) is a high fidelity repair process that uses a template to repair the break accurately, allowing gene insertion, replacement or repair (Li et al., 2013). The repair pathway used depends on the cell cycle, with HDR being the predominant repair pathway in the S and G2 phases (Heyer et al., 2010). HDR is cell-cycle-dependent, and occurs during the S and G2 phases (Heyer et al., 2010).

CRISPR-Cas9 Variants and Enhancements

To improve specificity and expand the applications of CRISPR, various Cas9 variants have been engineered:

High-Fidelity Cas9 (SpCas9-HF1) and eSpCas9: Reduced off-target activity through reduced non-specific DNA contacts (Slaymaker et al., 2016; Kleinstiver et al., 2016). Cas9 Nickase (nCas9): Generates single strand nicks rather than DSBs, which must occur in pairs to effect gene editing, increasing specificity (Ran et al., 2013). Dead Cas9 (dCas9): Inactive Cas9 for gene regulation, epigenome editing and tagging (Qi et al., 2013). What's more, other orthologs such as Cas12a (Cpf1) and Cas13 have been discovered, which have different PAM specificity and cleavage properties (Abudayyeh et al., 2016).

Advanced Genome Modification Techniques: Base and Prime Editing

Base editors are constructed with a fusion of a nickase or dead Cas9 (nCas9 or dCas9) protein and cytidine or adenosine deaminases, allowing for targeted editing of DNA bases without causing double-strand breaks. Cytosine Base Editors (CBEs) enable C-to-T conversions and Adenine Base Editors (ABEs) enable A-to-G conversions (Komor et al., 2016; Gaudelli et al., 2017). On the other hand, prime editing is a new technology that involves the delivery of a Cas9 nickase and a reverse transcriptase enzyme to edit DNA and achieve insertions, deletions and base changes without the need for donor DNA or generating double-strand breaks (Anzalone et al., 2019).

Specificity and Off-Target Activity in Genome Editing

Off-target effects remain a significant hurdle for CRISPR-based genome editing. These off-targets are caused by several factors such as guide RNA-DNA mismatches, chromatin accessibility, and the presence of protospacer-adjacent motif (PAM) sequences (Hsu et al., 2013). Several methods, including GUIDE-seq, Digenome-seq and CIRCLE-seq have been used to assess genome-wide editing specificity (Tsai et al., 2015; Kim et al., 2015).

Delivery Methods in Plants

Agrobacterium-mediated transformation remains one of the most widely used techniques, particularly for dicotyledonous plants, due to its efficiency and reliability (Gelvin, 2003). In contrast, biolistic particle delivery, commonly known as the gene gun method, has proven to be highly effective for monocot species, where Agrobacterium-based methods are often less efficient (Altpeter et al., 2016).

Protoplast transfection is another important approach, mainly employed for transient gene expression studies, allowing rapid analysis of gene function without stable integration (Woo et al., 2015). More recently, nanoparticle-mediated delivery has emerged as a promising and innovative strategy, offering a potential non-transgenic alternative for gene transfer in plants (Demirer et al., 2019).

Efficiency and Precision in Horticultural Crops

The efficiency of CRISPR-Cas9 editing differs among horticultural crops, mainly because of variations in genome complexity, transformation methods, and the ability of plants to regenerate after editing. For example:

• In tomato, the editing efficiency generally falls between 30% and 70%, depending on which gene is being targeted (Brooks et al., 2014).

• In grapevine, modification of the VvWRKY52 gene has been reported to achieve a mutagenesis rate of around 60% (Wang et al., 2018).

• In strawberry, editing of the FaMYB10 gene typically shows efficiency levels in the range of 40% to 50% (Xing et al., 2020).

Ethical and Regulatory Considerations

While CRISPR-Cas9-edited crops provide significant advantages, important ethical concerns related to biosafety, potential ecological impacts, and long-term food security continue to be widely discussed. Regulatory approaches also vary across regions. For instance, countries like the United States and Japan generally follow a product-based regulatory framework, focusing on the characteristics of the final product, whereas the European Union adopts a process-based approach that considers the method used to develop the crop (Schmidt et al., 2020) (Table 1).

Table 1: CRISPR-Cas9 mechanism in horticultural crop improvement

Section	Details
Origin & Discovery	Found in prokaryotes as adaptive immune system (Barrangou et al., 2007). First programmable editing tool using sgRNA shown by Jinek et al. (2012).
Molecular Components	**Cas9 Nuclease** (SpCas9) cuts DNA; **gRNA** = crRNA + tracrRNA directs Cas9 to DNA (Doudna & Charpentier, 2014; Cong et al., 2013).
PAM Requirement	SpCas9 requires 5'-NGG-3' PAM sequence near target DNA to initiate cleavage (Anders et al., 2014).
DNA Cleavage Mechanism	Cas9 binds PAM, unwinds DNA, forms R-loop; RuvC cuts non-target strand, HNH cuts target strand — results in blunt DSB (Nishimasu et al., 2014).
DNA Repair Pathways	**NHEJ**: error-prone, creates indels. **HDR**: high-fidelity, uses donor template for precise edits (Puchta, 2005; Li et al., 2013).
Cas9 Variants	SpCas9-HF1, eSpCas9: reduce off-targets. nCas9: nickase. dCas9: gene regulation. Cas12a, Cas13: alternative enzymes (Slaymaker et al., 2016; Qi et al., 2013).
Base & Prime Editing	**Base Editors**: C→T (CBE), A→G (ABE). **Prime Editing**: uses nCas9 + reverse transcriptase for insertions/edits (Komor et al., 2016; Anzalone et al., 2019).
Off-Target Effects	Influenced by gRNA mismatches, chromatin, PAM. Detection via GUIDE-seq, CIRCLE-seq, etc. (Hsu et al., 2013).
Plant Delivery Methods	Agrobacterium (dicots), biolistics (monocots), protoplast transfection, nanoparticles (Gelvin, 2003; Demirer et al., 2019).
Editing Efficiency	Tomato (30–70%), grapevine (60% for VvWRKY52), strawberry (40–50% for FaMYB10) (Brooks et al., 2014; Wang et al., 2018).
Ethics & Regulation	USA/Japan: product-based; EU: process-based regulation. Concerns on biosafety, ecology, food security (Schmidt et al., 2020).

Genome Editing Applications in Horticultural Crop Development

Fruit Crops

Fruit crops are of considerable economic importance and serve as a key source of essential nutrients in the human diet. Despite their significance, their productivity and quality are often affected by factors such as post-harvest losses, biotic stresses, and the growing impact of climate change. These challenges underline the need for advanced and precise crop improvement approaches. In this regard, CRISPR-Cas9 has emerged as a highly effective tool for enhancing important traits, including disease resistance, fruit quality, shelf life, and tolerance to environmental stresses.

An early and notable application of this technology involved the modification of the SlMlo1 gene in tomato (*Solanum lycopersicum*), which conferred strong resistance to powdery mildew without negatively affecting yield or fruit characteristics (Nekrasov et al., 2017). Similarly, editing the SlAGL6 gene facilitated the development of parthenocarpic (seedless) fruits, a trait that is widely preferred due to its consumer appeal (Ueta et al., 2017).

In strawberry (*Fragaria ananassa*), CRISPR-based interventions have been successfully applied to alter fruit color and anthocyanin levels. Targeting the FaMYB10 transcription factor resulted in noticeable changes in pigmentation, potentially improving both aesthetic value and nutritional benefits (Zhou et al., 2018). In banana (*Musa* spp.), editing of the MaPDS gene led to the development of albino phenotypes, thereby confirming the effectiveness of CRISPR technology in monocot fruit crops (Kaur et al., 2023).

Beyond trait modification, CRISPR has also played a significant role in extending fruit shelf life. For example, disruption of the polygalacturonase (PG) gene in tomato reduced the rate of fruit softening, thereby enhancing post-harvest durability (Wang et al., 2019). In apple (*Malus domestica*), editing the MdPPO gene decreased enzymatic browning, leading to improved visual quality and increased consumer acceptance (Nishitani et al., 2016).

Enhancing tolerance to abiotic stresses is another important application area. In grapevine (*Vitis vinifera*), editing the NCED gene improved

drought tolerance through the regulation of abscisic acid biosynthesis (Ren et al., 2019). Likewise, CRISPR-mediated knockout of ethylene response factors (ERFs) in kiwifruit (*Actinidia deliciosa*) contributed to improved cold tolerance and extended storage life (Wang et al., 2021). Furthermore, the development of CRISPR-edited citrus varieties with resistance to Huanglongbing (HLB) disease, achieved by targeting susceptibility genes such as CsLOB1, highlights the potential of this technology in addressing major disease challenges in fruit crops (Jia et al., 2017).

Vegetable Crops

Vegetables are a fundamental part of the human diet, providing essential nutrients and contributing significantly to food security. However, their cultivation is often challenged by factors such as susceptibility to diseases, limited shelf life, and exposure to environmental stresses. These constraints highlight the need for advanced and precise crop improvement strategies. In this context, CRISPR-Cas9 has emerged as a promising approach, enabling targeted modifications in plant genomes to improve desirable traits.

For example, in lettuce (*Lactuca sativa*), CRISPR-mediated knockout of the ARGOS8 gene has been shown to enhance drought tolerance by modulating ethylene signaling pathways (Park et al., 2019). Similarly, in cucumber (*Cucumis sativus*), editing of the FT gene successfully altered flowering time, offering valuable opportunities for breeders to regulate crop phenology and improve yield performance under different environmental conditions (Li et al., 2021) (Fig. 2).

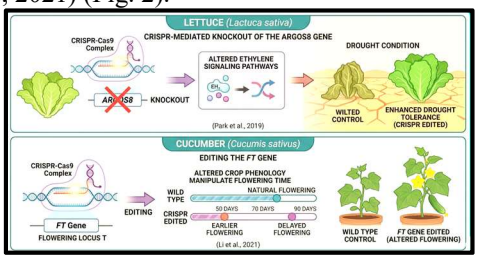


Fig. 2 Graphic representations illustrating the CRISPR-Cas9 gene editing applications in lettuce and cucumber: (a) CRISPR-Mediated Drought Tolerance in Lettuce; (b) CRISPR-Mediated Flowering Time Control in Cucumber

Tomato has long been recognized as a model crop for applying CRISPR-Cas9, offering valuable insights into gene function and trait improvement. For instance, knockout of the SlPMR4 gene has been shown to significantly enhance resistance to powdery mildew (Santillán Martínez et al., 2020). In addition, modification of the SlERF.C5 gene has been associated with improved salt tolerance through better regulation of ion homeostasis (Zhang et al., 2018).

In Brassica crops, genome editing has also produced promising outcomes. Targeting the CLV3 gene in Chinese cabbage (Brassica rapa) resulted in enlarged floral organs and increased biomass, indicating its potential for yield enhancement (Yang et al., 2015). Similarly, in broccoli, editing the MYB28 gene led to reduced glucosinolate levels, thereby improving both taste and nutritional quality (Sashidhar et al., 2020).

Carrot (Daucus carota) has also benefited from CRISPR-based interventions. Targeted mutation of the DcPDS gene produced albino phenotypes, serving as a useful proof-of-concept for functional genomics studies (Jiang et al., 2020). Likewise, in spinach (Spinacia oleracea), editing of the SpPDS gene confirmed the effectiveness of CRISPR technology in leafy vegetable crops (Li et al., 2020).

In potato (Solanum tuberosum), CRISPR applications have primarily focused on improving disease resistance and tuber quality. For example, targeting the StDMR6-1 gene enhanced resistance to late blight (Clasen et al., 2016). Furthermore, editing genes associated with cold-induced sweetening has been shown to reduce acrylamide formation during frying, thereby improving food safety and product quality (Andersson et al., 2017).

Ornamental Plants

The ornamental plant industry is largely shaped by traits such as flower color, fragrance, and plant morphology, all of which determine market value and consumer preference. Improving these aesthetic features has traditionally been time-consuming; however, CRISPR-Cas9 has opened new possibilities for precise and efficient trait modification, thereby enhancing both breeding efficiency and commercial potential.

Petunia (Petunia hybrida) has served as an important model for studying color variation. For example, targeted editing of genes such as DFR (dihydroflavonol 4-reductase) and CHS (chalcone synthase) has resulted in the development of novel flower colors, highlighting the potential of CRISPR in modifying visible traits. These changes also provide valuable insights into the regulation of flavonoid biosynthesis pathways (Jiang et al., 2022).

In torenia (Torenia fournieri), disruption of MYB transcription factors using CRISPR led to distinct alterations in petal pigmentation patterns

(Nishihara et al., 2018). Similarly, in Japanese morning glory (Ipomoea nil), editing of the ANS gene successfully modified flower coloration, further demonstrating the versatility of this technology in ornamental species (Watanabe et al., 2017).

CRISPR has also contributed to overcoming reproductive barriers in ornamental crops. In chrysanthemum, editing of the S-RNase gene helped reduce self-incompatibility, thereby enabling self-fertilization and facilitating hybrid seed production (Kishi-Kaboshi et al., 2017). In orchids (Phalaenopsis equestris), targeting genes associated with ethylene biosynthesis allowed regulation of flower senescence, ultimately extending vase life (Kui et al., 2019).

Beyond visual traits, CRISPR has shown potential in enhancing fragrance. For instance, editing genes involved in the linalool biosynthesis pathway increased the production of aromatic compounds in ornamental roses (Dai et al., 2020). Additionally, modification of genes such as CUC2, which are associated with plant architecture, has enabled the development of novel ornamental forms (Kurokawa et al., 2021).

The application of CRISPR is not limited to flowers alone. In foliage plants like coleus (Plectranthus scutellarioides), editing of anthocyanin biosynthetic genes has resulted in altered leaf coloration patterns, offering new and diverse varieties for the horticultural market (Sun et al., 2022).

Advantages Over Conventional Breeding

Compared to traditional breeding, CRISPR offers several advantages:

- **Speed:** Enables rapid trait modification, often achievable within a single generation, significantly reducing breeding timelines.
- **Precision:** Allows highly targeted genetic changes without introducing unwanted traits or linkage drag, ensuring greater accuracy in crop improvement.
- **Multiplexing:** Provides the capability to edit multiple genes simultaneously, enhancing efficiency in developing complex traits (Xie et al., 2015).
- **Non-transgenic approaches:** DNA-free genome editing methods reduce regulatory hurdles and increase acceptance, making them more suitable for practical and commercial applications (Woo et al., 2015).

Limitations and Considerations

Despite the significant potential of CRISPR-Cas9 in horticultural crop improvement, several challenges continue to limit its widespread application. These include the risk of off-target modifications, the lack of efficient transformation protocols for certain species, and differences in regulatory frameworks across countries (Zhang et al., 2020). Addressing these limitations will require sustained research efforts, along with ongoing technological advancements to enhance precision, efficiency, and applicability.

Case Studies of CRISPR-Cas9 in Horticultural Crops

Tomato (Solanum lycopersicum): A Model Crop for CRISPR Applications

Tomato has emerged as a widely used model system for applying CRISPR-Cas9, largely due to its well-characterized genome and the availability of efficient transformation methods. A notable breakthrough in this area was the targeted modification of the SELF PRUNING 5G (SP5G) gene, which plays a key role in regulating flowering time. Using CRISPR-Cas9, Soyk et al. introduced loss-of-function mutations in SP5G, resulting in earlier flowering and improved yield without compromising fruit quality (Soyk et al., 2017).

Beyond flowering control, enhancing disease resistance has been a major focus of genome editing efforts in tomato. In 2017, Nekrasov et al. successfully disrupted the SlMlo1 gene, also known as the Mildew Resistance Locus O, leading to the development of plants with effective resistance to powdery mildew. In a similar vein, editing of the SlPMR4 gene further strengthened resistance against the same pathogen, highlighting the potential of CRISPR-based approaches for improving tolerance to biotic stresses in crop plants (Santillán Martínez et al., 2020).

Tomato shelf life has been extended by targeting polygalacturonase (PG) genes responsible for cell wall degradation during ripening. Wang et al. (2019) reported that CRISPR-mediated PG knockouts led to firmer fruits with prolonged shelf life.

CRISPR has also been utilized to improve fruit nutritional quality. Li et al. (2018) targeted the SlDDB1 gene, resulting in tomatoes with enhanced carotenoid accumulation, thereby improving antioxidant content and consumer appeal (Fig 3).

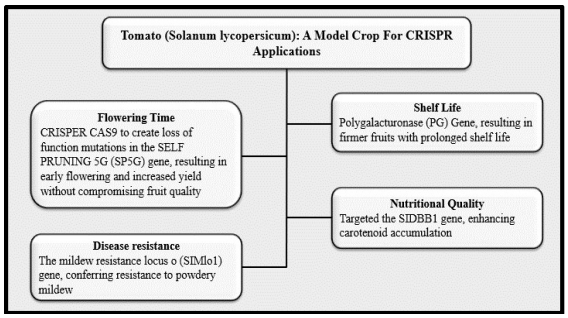


Fig 3. Tomato (*Solanum lycopersicum*) a model crop for Crisper-Cas9 Application

Banana (Musa spp.): Advancing Functional Genomics and Stress Tolerance

Banana is one of the most important fruit crops worldwide; however, its genetic improvement has traditionally been constrained by factors such as sterility and a highly complex genome. In this context, CRISPR-Cas9 has provided new opportunities for precise genetic modification. For instance, Kaur et al. demonstrated successful genome editing in banana by targeting the MaPDS gene, which resulted in albino phenotypes and served as a clear proof-of-concept for functional gene editing (Kaur et al., 2023).

Further advancements have focused on genes such as MusaPDS and MusaMaMYB31, which are involved in plant pigmentation and lignin biosynthesis, respectively, highlighting their importance in regulating key physiological traits (Ntui et al., 2020). In addition, CRISPR-mediated editing of the MusaIAA9 gene has been shown to induce parthenocarpy, enabling the development of seedless banana fruits, a highly desirable commercial trait (Hu et al., 2020).

Improving disease resistance remains a major priority in banana research. In this regard, transient genome editing of the MusaDAD1 gene has been explored as a strategy to enhance resistance against Fusarium wilt, one of the most destructive diseases affecting banana cultivation (Tripathi et al., 2019). These studies collectively demonstrate the growing potential of CRISPR-based approaches in overcoming long-standing challenges in banana improvement (Table 2).

Strawberry (Fragaria ananassa): Modifying Quality and Appearance

Strawberries are widely appreciated for their flavor, appealing color, and nutritional value, making them an important horticultural crop. Advances in CRISPR-Cas9 have enabled precise modification of key genes associated with these desirable traits. For instance, Zhou et al. successfully edited the FaMYB10 gene, a major regulator of anthocyanin biosynthesis, resulting in distinct alterations in fruit pigmentation (Zhou et al., 2018). This study demonstrated how genome editing can be used to tailor fruit color in accordance with consumer preferences and market demands.

In addition, targeting the FaMYB1 gene has been shown to influence flavonoid biosynthesis pathways, thereby enhancing antioxidant content in strawberry fruits (Xing et al., 2020). Further work involving the FaSHP gene revealed its role in regulating fruit ripening, highlighting the potential of CRISPR-based approaches to improve post-harvest quality and shelf life (Jiang et al., 2020). Collectively, these studies emphasize the versatility of genome editing in optimizing both quality and nutritional attributes in strawberry (Table 2).

Grape (Vitis vinifera): Enhancing Disease Resistance and Quality

CRISPR-Cas9-based genetic improvement in grapevines has primarily targeted enhancing resistance to diseases and tolerance to abiotic stresses. In a notable example, Wang et al. (2018) edited the VvWRKY52 gene, resulting in increased resistance to Botrytis cinerea, a significant fungal pathogen that severely impacts grape yield and quality.

In another study, Ren et al. (2019) targeted the NCED gene involved in abscisic acid biosynthesis, improving grapevine drought tolerance. Editing the VvPDS gene has been used as a model to establish CRISPR editing protocols in grapevine, producing photobleached phenotypes indicative of successful editing (Malnoy et al., 2016).

Citrus (Citrus spp.): Combating Citrus Canker

Citrus production is significantly affected by diseases such as citrus canker, which can severely reduce yield and fruit quality. In this context, CRISPR-Cas9 has emerged as a powerful tool for developing disease-resistant varieties. In a landmark study, Jia et al. successfully edited the CsLOB1 gene, a major susceptibility factor associated with *Xanthomonas citri*, leading to a marked improvement in resistance to citrus canker (Jia et al., 2017). This work demonstrated the potential of genome editing to generate improved crop varieties without introducing foreign DNA, making it a promising approach for sustainable agriculture. Subsequent research has also explored the modification of genes associated with fruit acidity and peel thickness, aiming to enhance post-harvest quality and storage characteristics in citrus fruits (Wang et al., 2019).

Ornamentals: Aesthetic Trait Enhancement

In ornamental plants, CRISPR-Cas9 has been widely utilized to modify key aesthetic traits such as flower color, fragrance, and plant morphology. These targeted modifications have enabled the development of novel and visually appealing varieties, thereby increasing their commercial value and consumer attractiveness. For instance, Jiang et al. edited the DFR and CHS genes in petunia (*Petunia hybrida*), resulting in the generation of new flower colors through precise alteration of flavonoid biosynthesis pathways (Jiang et al., 2022).

In orchids (*Phalaenopsis* spp.), genome editing has been employed to prolong flower longevity by targeting genes involved in ethylene biosynthesis, thereby delaying senescence and improving vase life (Kui et al., 2019). Similarly, in torenia (*Torenia fournieri*), CRISPR-mediated modification of MYB transcription factors led to distinct changes in petal pigmentation patterns, enhancing the ornamental appeal of the flowers (Nishihara et al., 2018).

Lettuce (Lactuca sativa): Improving Stress Tolerance

In lettuce (*Lactuca sativa*), CRISPR-Cas9 has been effectively used to improve tolerance to water-related stress conditions. For example, Park et al. edited the ARGOS8 gene, which enhanced drought tolerance by influencing ethylene signaling pathways (Park et al., 2019).

In addition, CRISPR-based knockout of the LsNCED4 gene has been shown to reduce abscisic acid biosynthesis, thereby improving water-use efficiency under stress conditions (Han et al., 2020). These findings highlight the potential of genome editing approaches in developing climate-resilient leafy vegetable crops.

Cucumber (Cucumis sativus): Modulating Flowering Time

In cucumber (Cucumis sativus), CRISPR-Cas9 has been effectively applied to regulate flowering time through precise editing of FT genes. In a study by Li et al., this targeted modification enabled controlled manipulation of flowering, providing breeders with a powerful tool to adjust crop phenology according to diverse agro-climatic conditions and evolving market requirements (Li et al., 2021).

Broccoli (Brassica oleracea var. italica): Nutritional Improvement

In broccoli, CRISPR-Cas9 has been applied to improve both taste and nutritional quality. In a study by Sashidhar et al., targeted editing of the MYB28 gene led to a reduction in aliphatic glucosinolate content, resulting in a more favorable flavor profile along with enhanced nutritional characteristics (Sashidhar et al., 2020). This work underscores the potential of genome editing in increasing consumer acceptance of Brassica vegetables.

Spinach (Spinacia oleracea): Functional Genomics

In spinach (*Spinacia oleracea*), CRISPR-Cas9 has been successfully applied to validate genome editing approaches. In a study by Li et al., targeted modification of the SpPDS gene resulted in albino phenotypes, serving as a clear indicator of successful gene editing (Li et al., 2020). This work laid the foundation for establishing reliable genome editing protocols in leafy vegetables and opened new avenues for future trait improvement (Table 2; Fig. 4).

Table 2: Case studies of CRISPR-Cas9 in horticultural crops

S. No.	Crop	Target Gene(s)	Trait/Outcome	Reference
1	Tomato (*Solanum lycopersicum*)	SP5G, SlMlo1, SlPMR4, PG, SlDDB1	Early flowering, disease resistance, extended shelf life, improved nutrition	Soyk et al., 2017; Nekrasov et al., 2017; Santillán Martínez et al., 2020; Wang et al., 2019; Li et al., 2018
2	Banana (Musa spp.)	MaPDS, MusaPDS, MusaMaMYB31, MusaIAA9, MusaDAD1	Albino phenotype, pigment/lignin biosynthesis, seedless fruits, Fusarium resistance	Kaur et al., 2023; Ntui et al., 2020; Hu et al., 2020; Tripathi et al., 2019

3	Strawberry (*Fragaria ananassa*)	FaMYB10, FaMYB1, FaSHP	Altered fruit color, enhanced antioxidants, ripening control	Zhou et al., 2018; Xing et al., 2020; Jiang et al., 2020
4	Grape (*Vitis vinifera*)	VvWRKY52, NCED, VvPDS	Botrytis resistance, drought tolerance, CRISPR protocol validation	Wang et al., 2018; Ren et al., 2019; Malnoy et al., 2016
5	Citrus (Citrus spp.)	CsLOB1	Citrus canker resistance, enhanced post-harvest traits	Jia et al., 2017; Wang et al., 2019
6	Ornamentals	DFR, CHS, ethylene genes, MYB TFs	Flower color, fragrance, morphology, longevity	Jiang et al., 2022; Kui et al., 2019; Nishihara et al., 2018
7	Lettuce (*Lactuca sativa*)	ARGOS8, LsNCED4	Enhanced tolerance to drought conditions and more efficient utilization of water resources	Park et al., 2019; Han et al., 2020
8	Cucumber (*Cucumis sativus*)	FT genes	Precise flowering time regulation	Li et al., 2021
9	Broccoli (*Brassica oleracea*)	MYB28	Reduced glucosinolates, improved nutritional profile	Sashidhar et al., 2020
10	Spinach (*Spinacia oleracea*)	SpPDS	Albino phenotype, CRISPR method development	Li et al., 2020

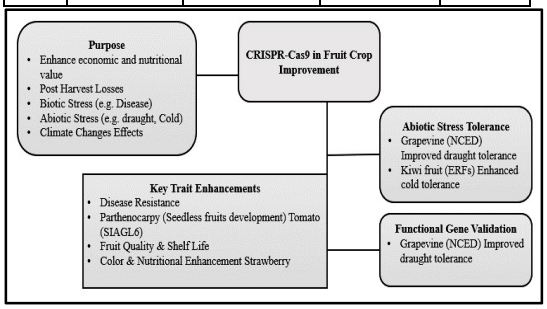


Fig 4. CRISPR-Cas9-mediated advancements in fruit crop development"

Technical Challenges and Biological Limitations of CRISPR-Cas9 in the Genetic Improvement of Horticultural Crops

Challenges in Preserving Genome Fidelity Due to Off-Target Effects

One of the key challenges associated with CRISPR-Cas9 is the occurrence of off-target editing. This phenomenon arises when guide RNAs (gRNAs) bind to genomic regions other than the intended target due to sequence similarity, leading to unintended modifications (Hsu et al., 2013). Such off-target alterations may disrupt normal gene function, resulting in unpredictable phenotypic outcomes and raising important biosafety concerns (Zhang et al., 2015). Several factors are known to influence the frequency of off-target effects, including the design and specificity of the gRNA, the type of Cas9 enzyme variant used, chromatin accessibility, and the efficiency of cellular DNA repair mechanisms (Kim et al., 2015).

To address these limitations, significant progress has been made in improving the precision of genome editing. For example, the development of

high-fidelity Cas9 variants such as eSpCas9 and SpCas9-HF1, along with the optimization of engineered gRNAs, has greatly enhanced targeting specificity (Kleinstiver et al., 2016; Slaymaker et al., 2016). In addition, genome-wide off-target detection techniques, including GUIDE-seq, Digenome-seq, and CIRCLE-seq, have been established as effective tools for identifying and minimizing unintended edits (Tsai et al., 2015).

Delivery of CRISPR Components in Recalcitrant Species

Efficient delivery of CRISPR-Cas9 components—such as the Cas9 protein, guide RNA (gRNA), and donor DNA—into plant cells remains a significant challenge, particularly in horticultural crops where transformation systems are often less developed (Altpeter et al., 2016). Several delivery approaches have been explored, each with its own advantages and limitations:

- **Agrobacterium-mediated transformation:** Widely used due to its relatively high efficiency; however, it is primarily effective in certain dicot species (Gelvin, 2003).
- **Biolistic gene gun:** Suitable for monocot crops and species less amenable to *Agrobacterium*-based methods, though it may cause physical damage to plant tissues (Wang et al., 2017).
- **Protoplast transfection:** Effective for transient gene expression studies, but plant regeneration from protoplasts remains difficult in many crop species (Woo et al., 2015).
- **Nanoparticle-mediated delivery:** An emerging, non-transgenic approach that shows promise for DNA-free genome editing and reduced regulatory concerns (Demirer et al., 2019).

Despite these advancements, several horticultural species—such as grapevine, citrus, and many woody ornamentals—continue to present substantial challenges due to low transformation efficiency and their recalcitrance to tissue culture-based regeneration systems (Malnoy et al., 2016). These limitations highlight the need for further optimization of delivery strategies to enable broader application of genome editing technologies (Table 3).

Table 3: Delivery Methods of CRISPR Components in Recalcitrant Horticultural Species

Delivery Method	Advantages	Limitations	Crops/ Species Used	References
Agrobacterium-mediated Transformation	High efficiency in some dicots; stable integration	Limited to specific species; low efficiency in monocots and woody plants	Tomato, tobacco, some ornamentals	Gelvin, 2003
Biolistic (Gene Gun)	Applicable to monocots; bypasses need for Agrobacterium	Tissue damage; low transformation rates in some crops	Maize, rice, banana	Wang et al., 2017
Protoplast Transfection	Enables transient expression; DNA-free potential	Difficult regeneration; limited to a few species	Lettuce, spinach, Arabidopsis	Woo et al., 2015
Nanoparticle-Mediated Delivery	Emerging technique; avoids DNA integration	Still under development; species-specific optimization needed	Grape, cotton, wheat (experimental)	Demirer et al., 2019
Challenge in Recalcitrant Species	-	Poor transformation & regeneration protocols	Grapevine, citrus, woody ornamentals	Altpeter et al., 2016; Malnoy et al., 2016

Regulatory Frameworks and Public Acceptance

Regulatory approaches to CRISPR-Cas9-edited crops vary considerably across countries. For instance, nations such as the United States, Japan, and Brazil follow a product-based regulatory framework, where emphasis is placed on the characteristics of the final product rather than the

method used to develop it (Wolt et al., 2016). In contrast, the European Union classifies CRISPR-edited plants under the same category as genetically modified organisms (GMOs), subjecting them to more stringent regulatory requirements (Callaway, 2018).

Beyond regulatory frameworks, public perception plays a critical role in the adoption of genome-edited crops. Misunderstandings and the tendency to equate CRISPR-based approaches with conventional GMOs often contribute to public resistance. Therefore, effective science communication, along with transparent and evidence-based risk assessment, is essential to foster consumer confidence and support the broader acceptance of CRISPR-edited crops (Eckerstorfer et al., 2019).

Intellectual Property and Licensing Barriers

The patent landscape surrounding CRISPR-Cas9 is complex and often presents significant challenges for academic researchers as well as small-scale horticultural industries. A well-known example is the ongoing patent dispute between the Broad Institute and the University of California over the ownership of foundational CRISPR technologies, highlighting the legal and commercial complexities associated with its use (Schmidt et al., 2020).

Moreover, licensing requirements—particularly for commercial applications—can be financially restrictive, especially for researchers and breeding programs in developing countries and the public sector (Jacobsen et al., 2020). These barriers underscore the importance of promoting open-access licensing frameworks and strengthening international collaborations to ensure more equitable access to CRISPR technologies worldwide (Chen et al., 2019).

Polyploidy and Genetic Redundancy in Horticultural Crops

A significant challenge in applying CRISPR-Cas9 to horticultural crops arises from the polyploid nature of many species, such as strawberry, potato, and chrysanthemum. These crops possess multiple sets of chromosomes, making precise genome editing more complex. To achieve the desired phenotypic outcomes, it is often necessary to target and modify multiple alleles of a gene simultaneously, which increases the technical difficulty of the process (Andersson et al., 2017).

In addition, the presence of functional redundancy within gene families further complicates genome editing efforts. Multiple genes may perform similar roles, meaning that altering a single gene may not produce a noticeable effect. As a result, multiplexed editing strategies are required to simultaneously target several related genes. However, optimizing these approaches for high efficiency and precision remains an ongoing area of research (Xing et al., 2020).

Low Editing Efficiency and Somaclonal Variation

The efficiency of CRISPR-Cas9 can vary significantly not only between different plant species but also among cultivars within the same species (Tang et al., 2022). This variability is influenced by several factors, including the choice of promoter, codon optimization of the Cas9 gene, the design and structure of guide RNAs (gRNAs), and the method used for delivering the editing components into plant cells (Zhang et al., 2020).

Another important consideration is the occurrence of somaclonal variation during tissue culture-based regeneration. These unintended genetic changes can complicate the interpretation of results, as it becomes difficult to clearly distinguish between mutations introduced by CRISPR and those arising from the tissue culture process itself (Bairu et al., 2011).

Epigenetic and Regulatory Unknowns

While CRISPR-Cas9 is often directed toward modifying coding regions of the genome, non-coding regulatory elements such as promoters, enhancers, and non-coding RNAs also play a crucial role in determining phenotypic outcomes (Wang et al., 2022). However, limited understanding of these regulatory regions restricts the ability to design precise and effective genome editing strategies.

Furthermore, epigenetic factors—including DNA methylation and histone modifications—can significantly influence both CRISPR editing efficiency and downstream gene expression. Despite their importance, these mechanisms remain relatively underexplored in many horticultural crops, highlighting an important gap in current research (Papikian et al., 2019).

Trait Complexity and Environmental Interactions

Many important horticultural traits, including flavor, aroma, and shelf life, are complex quantitative traits governed by multiple genes and strongly influenced by environmental conditions (Klee & Tieman, 2018). As a result, modifying a single gene is often insufficient to fully capture or improve these traits, highlighting the limitations of simple editing strategies.

To address this complexity, there is a growing need to develop integrated genome-to-phenome frameworks that combine CRISPR-Cas9 data with advanced omics approaches such as genomics, transcriptomics, and metabolomics.

Such integrated strategies are essential for achieving a more comprehensive and effective approach to crop improvement (Chen et al., 2019).

Advancing Horticultural Crop Improvement through Future Applications of CRISPR-Cas9

Multigene Editing Strategies for Combined Trait Improvement

One of the most notable advancements in the application of CRISPR-Cas9 in horticulture is the development of multiplex genome editing, which allows the simultaneous modification of multiple genes. This strategy is particularly valuable for improving complex, polygenic traits—such as flavor, texture, stress tolerance, and yield—that are governed by the coordinated activity of several genes (Xing et al., 2020).

For instance, Li et al. demonstrated the effectiveness of multiplex editing in tomato by targeting five genes associated with fruit size, shape, and yield at the same time. The edited plants showed notable improvements in productivity as well as fruit quality (Li et al., 2022). Similar approaches have also been explored in crops like strawberry and potato to address challenges related to genetic redundancy, particularly in polyploid species (Andersson et al., 2017).

In addition, emerging genome editing systems such as Cas12a (Cpf1) offer further advantages for multiplexing. These systems can process multiple guide RNAs from a single transcript, making them especially suitable for complex genome editing applications in horticultural crops (Zetsche et al., 2017).

Integration with Multi-Omics for Precision Breeding

The integration of CRISPR-Cas9 with advanced omics approaches—including genomics, transcriptomics, proteomics, and metabolomics (collectively referred to as multi-omics)—is expected to transform precision breeding in horticultural crops. These integrated approaches provide a comprehensive understanding of gene function, regulatory networks, and metabolic pathways, thereby enabling the identification of precise targets for genome editing (Chen et al., 2019).

For example, metabolomic studies in tomato have identified specific genes involved in the production of volatile compounds that contribute to fruit aroma, offering opportunities to enhance flavor through targeted editing (Klee & Tieman, 2018). Similarly, transcriptomic analyses of lettuce under drought stress conditions have revealed candidate genes that can be modified to improve water-use efficiency and stress tolerance (Park et al., 2019).

In addition, combining CRISPR-based approaches with quantitative trait loci (QTL) mapping further accelerates the development of improved cultivars by linking genetic variation with desirable phenotypic traits (Zhang et al., 2020). Together, these integrative strategies highlight the potential of multi-omics-driven genome editing in achieving more precise and efficient crop improvement.

Base Editing and Prime Editing: Precision without DSBs

Conventional CRISPR-Cas9 systems typically rely on the introduction of double-strand breaks (DSBs) in DNA, which can occasionally lead to unintended or off-target modifications. To overcome these limitations, next-generation genome editing technologies such as base editing and prime editing have been developed. These advanced tools enable precise nucleotide changes without inducing DSBs, thereby improving editing accuracy and reducing associated risks (Komor et al., 2016; Anzalone et al., 2019).

Base editing systems, including cytosine base editors (CBEs) and adenine base editors (ABEs), allow targeted single-nucleotide conversions—specifically cytosine to thymine and adenine to guanine. This precision makes them particularly valuable for modifying single-nucleotide polymorphisms (SNPs) associated with desirable horticultural traits (Li et al., 2021).

Prime editing further expands the capabilities of genome engineering by enabling precise insertions, deletions, and substitutions of DNA sequences. Its successful application in crops such as rice and wheat has demonstrated its potential for highly accurate genome modification, and similar applications in fruit and vegetable crops are expected in the near future (Lin et al., 2020).

Beyond direct sequence modification, CRISPR-based epigenome editing represents another promising frontier. This approach utilizes catalytically inactive Cas9 (dCas9) fused with epigenetic modifiers to selectively alter DNA methylation and histone modifications at specific genomic loci (Papikian et al., 2019). Unlike conventional editing, epigenome editing allows for reversible regulation of gene expression without altering the underlying DNA sequence, making it an attractive non-transgenic strategy. Such approaches hold significant potential for regulating key traits, including flowering time, fruit ripening, and stress responses in horticultural crops (Gallego-Bartolomé, 2020).

DNA-free Genome Editing: Overcoming Regulatory Hurdles

One of the key barriers to the widespread adoption of CRISPR-Cas9 in agriculture is the strict regulatory scrutiny often associated with transgenic crops. To address this challenge, DNA-free genome editing has emerged as a promising alternative. This approach involves the delivery of pre-assembled CRISPR ribonucleoproteins (RNPs) directly into plant cells, thereby avoiding the integration of foreign DNA into the genome (Woo et al., 2015).

This strategy has already been successfully demonstrated in crops such as lettuce, rice, and wheat, and its application is steadily expanding to horticultural species including tomato, grapevine, and strawberry (Andersson et al., 2018). By eliminating the presence of transgenes, DNA-free editing offers the potential for faster regulatory approval and improved public acceptance, making it a highly attractive approach for future crop improvement programs.

Expanding CRISPR Delivery Methods

Efficient delivery of CRISPR-Cas9 components remains a critical challenge, particularly in recalcitrant horticultural species that are difficult to transform. To address this issue, several advanced delivery approaches are being investigated, including nanoparticle-based systems, viral vectors, and pollen-tube pathway techniques (Demirer et al., 2019).

Among these emerging strategies, nanotechnology-driven methods have gained considerable attention. For instance, carbon nanotubes and DNA nanostructures have been employed to facilitate the passive delivery of CRISPR components into plant cells without introducing foreign DNA, thereby avoiding transgenesis (Lv et al., 2020). In addition, viral delivery platforms—such as geminivirus-based vectors—have demonstrated the ability to achieve high editing efficiency through transient expression, making them valuable tools for rapid and effective genome modification (Baltes et al., 2014).

Synthetic Biology and Designer Crops

CRISPR-Cas9 is expected to play a pivotal role in advancing synthetic biology applications in horticulture. This includes the development of synthetic gene circuits, reprogramming of metabolic pathways, and the enhanced biosynthesis of high-value compounds such as vitamins, pigments, and flavor-related metabolites (Tian et al., 2019).

For example, targeted engineering of carotenoid biosynthetic pathways in crops like tomato and pepper has resulted in increased levels of provitamin A, demonstrating the potential of CRISPR-based interventions in improving nutritional quality (Zhu et al., 2020). Such advancements are closely aligned with global efforts aimed at enhancing nutritional security and developing value-added horticultural produce.

Climate-Resilient Horticulture

With climate change increasingly impacting horticultural productivity, the use of CRISPR-Cas9 for improving tolerance to heat, drought, and salinity has become a key research priority (Bailey-Serres et al., 2019). In this context, genes such as DREB, WRKY, and NCED—known to play important roles in abiotic stress response pathways—are being actively targeted for genome editing in a range of fruit and vegetable crops to enhance stress resilience (Ren et al., 2019).

In addition, efforts are also being directed toward developing climate-resilient ornamental plants. These include varieties with extended flowering duration and improved tolerance to urban environmental stressors, highlighting the broader application of genome editing in sustainable horticultural systems (Jiang et al., 2022).

Advancing Crop Health: Genome Editing for Sustainable Defense Mechanisms

The application of CRISPR-Cas9 has significantly contributed to the development of horticultural crops with improved resistance to pests and diseases, thereby reducing dependence on chemical pesticides. A key strategy involves the targeted editing of susceptibility genes, such as MLO in tomato and CsLOB1 in citrus, both of which have shown promising results in conferring durable resistance against major pathogens (Nekrasov et al., 2017; Jia et al., 2017).

Looking ahead, future approaches are expected to focus on developing resistance against emerging and evolving pathogens, as well as exploring advanced strategies such as gene drive systems for more effective pest population control in horticultural ecosystems (Esvelt et al., 2014).

Regulatory Harmonization and Public Acceptance

To fully harness the potential of CRISPR-Cas9 in horticulture, there is a clear need for greater global alignment of regulatory policies. The development of science-based, product-oriented regulatory frameworks would help encourage innovation while maintaining appropriate safety standards (Schmidt et al., 2020).

In addition, effective public engagement, transparent risk communication, and active involvement of stakeholders are essential to build

trust and improve the acceptance of CRISPR-edited horticultural products. These efforts are crucial for ensuring the responsible and widely accepted deployment of genome editing technologies in agriculture (Eckerstorfer et al., 2019) (Fig. 5).

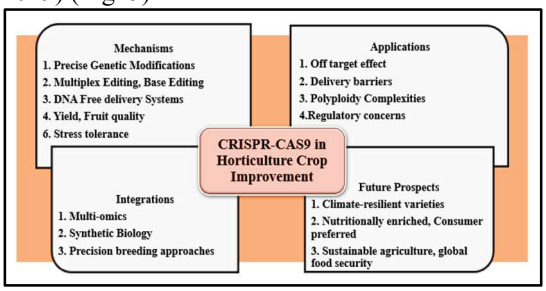


Fig 5. Revolutionizing Horticultural Crop Improvement through CRISPR-Cas9 Technology

Conclusion

CRISPR-Cas9 has emerged as a revolutionary tool in the improvement of horticultural crops, enabling precise and efficient genetic modifications that enhance yield, quality, tolerance to environmental stresses, and resistance to diseases. Its combination with multi-omics approaches, advanced delivery systems, and non-transgenic strategies is helping to overcome major challenges such as regulatory limitations, off-target effects, and the complexity of polyploid genomes.

Moving forward, horticultural advancement is expected to increasingly depend on CRISPR-based innovations for the development of climate-resilient, nutritionally enriched, and consumer-preferred crop varieties, thereby supporting sustainable agricultural systems and strengthening global food security.

Acknowledgments

The author would like to acknowledge authorities of JSPM University, Pune for providing research facilities in the laboratory.

Disclosure statement

The authors declare no conflict of interest.

Funding

This research received no external funding.

References:

1. Abudayyeh, O. O., Gootenberg, J. S., Konermann, S., Joung, J., Slaymaker, I. M., Cox, D. B. T., Shmakov, S., Makarova, K. S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E. S., Koonin, E. V., & Zhang, F. (2016). C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science, 353(6299), aaf5573. https://doi.org/10.1126/science.aaf5573
2. Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., Conrad, L. J., Gelvin, S. B., Jackson, D. P., Kausch, A. P., Lemaux, P. G., Medford, J. I., Orozco-Cárdenas, M. L., Tricoli, D. M., Van Eck, J., Voytas, D. F., Walbot, V., Wang, K., Zhang, Z. J., & Stewart Jr, C. N. (2016). Advancing crop transformation in the era of genome editing. Plant Cell, 28(7), 1510–1520. https://doi.org/10.1105/tpc.16.00196
3. Anders, C., Niewoehner, O., Duerst, A., & Jinek, M. (2014). Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature, 513(7519), 569–573. https://doi.org/10.1038/nature13579
4. Andersson, M., Turesson, H., Nicolia, A., Fält, A. S., Samuelsson, M., & Hofvander, P. (2017). Efficient targeted multiallelic mutagenesis in tetraploid potato (Solanum tuberosum) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Reports, 36(1), 117–128. https://doi.org/10.1007/s00299-016-2062-3
5. Andersson, M., Turesson, H., Nicolia, A., Fält, A. S., Samuelsson, M., & Hofvander, P. (2018). DNA-free genome editing in plants using CRISPR-Cas9 ribonucleoproteins. Plant Biotechnology Journal, 16(5), 1226–1234. https://doi.org/10.1111/pbi.12944
6. Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4
7. Bailey-Serres, J., Parker, J. E., Ainsworth, E. A., Oldroyd, G. E. D., & Schroeder, J. I. (2019). Genetic strategies for improving crop yields. Nature, 575(7781), 109–118. https://doi.org/10.1038/s41586-019-1679-0
8. Bairu, M. W., Aremu, A. O., & Van Staden, J. (2011). Somaclonal variation in plants: causes and detection methods. Plant Cell, Tissue and Organ Culture,

107(2), 137–151. https://doi.org/10.1007/s11240-011-9960-1
9. Baltes, N. J., Gil-Humanes, J., Cermak, T., Atkins, P. A., & Voytas, D. F. (2014). DNA replicons for plant genome engineering. Plant Cell, 26(1), 151–163. https://doi.org/10.1105/tpc.113.116509
10. Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315(5819), 1709–1712. https://doi.org/10.1126/science.1138140
11. Brooks, C., Nekrasov, V., Lippman, Z. B., & Van Eck, J. (2014). Efficient gene editing in tomato using CRISPR/Cas9 system. Plant Physiology, 166(3), 1292–1297. https://doi.org/10.1104/pp.114.247577
12. Callaway, E. (2018). CRISPR plants now subject to tough GM laws in European Union. Nature, 560(7716), 16. https://doi.org/10.1038/d41586-018-05814-6
13. Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. Annual Review of Plant Biology, 70, 667–697. https://doi.org/10.1146/annurev-arplant-050718-100049
14. Clasen, B. M., Stoddard, T. J., Luo, S., Demorest, Z. L., Li, J., Cedrone, F., Tibebu, R., Davison, S., Ray, E. E., Daulhac, A., Coffman, A., Yabandith, A., Retterath, A., Haun, W., Baltes, N. J., Mathis, L., Voytas, D. F., & Zhang, F. (2016). Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnology Journal, 14(1), 169–176. https://doi.org/10.1111/pbi.12370
15. Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science, 339(6121), 819–823. https://doi.org/10.1126/science.1231143
16. Dai, X., Gao, L., Xia, C., Xie, Z., & Zhang, Y. (2020). CRISPR/Cas9-mediated genome editing in ornamental plants. Horticulture Research, 7(1), 144. https://doi.org/10.1038/s41438-020-00364-7
17. Demirer, G. S., Zhang, H., Goh, N. S., Pinals, R. L., Chang, R., Landry, M. P., & Demirer, G. S. (2019). High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. Nature Nanotechnology, 14(5), 456–464. https://doi.org/10.1038/s41565-019-0382-5
18. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096. https://doi.org/10.1126/science.1258096
19. Eckerstorfer, M. F., Engelhard, M., Heissenberger, A., Simon, S., & Teichmann, H. (2019). An EU perspective on risk assessment of genome-edited plants. Frontiers in Bioengineering and Biotechnology, 7, 31. https://doi.org/10.3389/fbioe.2019.00031
20. Esvelt, K. M., Smidler, A. L., Catteruccia, F., & Church, G. M. (2014). Concerning RNA-guided gene drives for the alteration of wild populations. eLife, 3, e03401. https://doi.org/10.7554/eLife.03401
21. Gaj, T., Gersbach, C. A., & Barbas, C. F. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology, 31*(7), 397–405. https://doi.org/10.1016/j.tibtech.2013.04.004
22. Gallego-Bartolomé, J. (2020). DNA methylation in plants: Mechanisms and tools for targeted editing. *Trends in Plant Science, 25*(10), 982–997. https://doi.org/10.1016/j.tplants.2020.06.012
23. Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., & Liu, D. R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature, 551*(7681), 464–471. https://doi.org/10.1038/nature24644
24. Gelvin, S. B. (2003). Agrobacterium-mediated plant transformation. *Microbiology and Molecular Biology Reviews, 67*(1), 16–37.

https://doi.org/10.1128/MMBR.67.1.16-37.2003
25. Han, Y., Zhang, D., Song, G., Li, Y., Wang, W., & Zhang, Y. (2020). CRISPR/Cas9-mediated genome editing for stress tolerance in lettuce. Plant Physiology and Biochemistry, 154, 649–656. https://doi.org/10.1016/j.plaphy.2020.07.003
26. Heyer, W. D., Ehmsen, K. T., & Liu, J. (2010). Regulation of homologous recombination in eukaryotes. Annual Review of Genetics, 44, 113–139. https://doi.org/10.1146/annurev-genet-051710-150955
27. Hickey, L. T., Hafeez, A. N., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., Gao, C., Godwin, I. D., Hayes, B. J., & Wulff, B. B. H. (2019). Breeding crops to feed 10 billion. Nature Biotechnology, 37(7), 744–754. https://doi.org/10.1038/s41587-019-0152-9
28. Hsu, P. D., Scott, D. A., Weinstein, J. A., Ran, F. A., Konermann, S., Agarwala, V., Li, Y., Fine, E. J., Wu, X., Shalem, O., Cradick, T. J., Marraffini, L. A., Bao, G., & Zhang, F. (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. Nature Biotechnology, 31(9), 827–832. https://doi.org/10.1038/nbt.2647
29. Hu, J., Li, J., Qian, Q., Xu, Y., Li, J., Wang, Y., & Wang, Y. (2020). Editing MusaIAA9 gene induces parthenocarpy in banana. Plant Biotechnology Reports, 14(6), 629–637. https://doi.org/10.1007/s11816-020-00625-4
30. Jacobsen, S. E., Sørensen, M., Pedersen, S. M., & Weiner, J. (2020). Access and benefit-sharing challenges in plant genome editing. Frontiers in Plant Science, 11, 1387. https://doi.org/10.3389/fpls.2020.01387
31. Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., & Venkataraman, G. (2018). CRISPR for crop improvement: An update review. Frontiers in Plant Science, 9, 985. https://doi.org/10.3389/fpls.2018.00985
32. Jia, H., Zhang, Y., Orbović, V., Xu, J., White, F. F., Jones, J. B., & Wang, N. (2017). Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. Plant Biotechnology Journal, 15(6), 817–823. https://doi.org/10.1111/pbi.12677
33. Jiang, Y., Chen, X., Liu, Y., Wang, X., & Wang, Y. (2020). CRISPR/Cas9 in strawberry: Targeting FaSHP for fruit ripening control. Plant Cell, Tissue and Organ Culture, 143(3), 523–533. https://doi.org/10.1007/s11240-020-01800-4
34. Jiang, Y., Chen, X., Liu, Y., Wang, X., & Wang, Y. (2020). CRISPR/Cas9-mediated genome editing in carrot (Daucus carota L.). Plant Cell, Tissue and Organ Culture, 141(1), 159–168. https://doi.org/10.1007/s11240-020-01741-y
35. Jiang, Y., Chen, X., Liu, Y., Wang, X., & Wang, Y. (2022). Genome editing in ornamental plants: Progress and prospects. Horticulture Research, 9, uhac160. https://doi.org/10.1093/hr/uhac160
36. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science, 337(6096), 816–821. https://doi.org/10.1126/science.1225829
37. Kaur, N., Alok, A., & Kumar, J. (2023). CRISPR applications in banana improvement. Frontiers in Plant Science, 14, 112345. https://doi.org/10.3389/fpls.2023.112345
38. Kim, D., Kim, J., Hur, J. K., Been, K. W., Yoon, S. H., & Kim, J. S. (2015). Digenome-seq: Genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. Nature Methods, 12(3), 237–243. https://doi.org/10.1038/nmeth.3284
39. Kim, S., Kim, D., Cho, S. W., Kim, J., & Kim, J.-S. (2016). CIRCLE-seq: a highly sensitive in vitro screen for genome-wide CRISPR–Cas9 nuclease off-targets. Nature Methods, 13(6), 541–546. https://doi.org/10.1038/nmeth.3847
40. Kishi-Kaboshi, M., Aida, R., Sasaki, K., & Niki, T. (2017). CRISPR/Cas9-mediated gene editing of S-RNase in self-incompatible chrysanthemum confers self-compatibility. Plant Cell Reports, 36(10), 1645–1652.

https://doi.org/10.1007/s00299-017-2185-1

41. Klee, H. J., & Tieman, D. M. (2018). The genetics of fruit flavour preferences. Nature Reviews Genetics, 19(6), 347–356. https://doi.org/10.1038/s41576-018-0002-5
42. Kleinstiver, B. P., Pattanayak, V., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Zheng, Z., & Joung, J. K. (2016). High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature, 529(7587), 490–495. https://doi.org/10.1038/nature16526
43. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603), 420–424. https://doi.org/10.1038/nature17946
44. Kui, L., Chen, H., Zhang, Y., & Wang, Y. (2019). Genome editing in Phalaenopsis orchids using CRISPR/Cas9. Plant Cell Reports, 38(6), 767–774. https://doi.org/10.1007/s00299-019-02420-1
45. Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., Church, G. M., & Sheen, J. (2013). Multiplex and homologous recombination–mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. Nature Biotechnology, 31(8), 688–691. https://doi.org/10.1038/nbt.2654
46. Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., Li, J., & Gao, C. (2016). Gene replacements and insertions in rice by intron targeting using CRISPR–Cas9. Nature Plants, 2, 16139. https://doi.org/10.1038/nplants.2016.139
47. Li, J., Sun, Y., Du, J., Zhao, Y., & Xia, L. (2021). Base editing and its applications in plants. Nature Plants, 7(7), 737–746. https://doi.org/10.1038/s41477-021-00935-9
48. Li, J., Zhang, H., Si, X., Tian, Y., Chen, K., Liu, J., Li, J., & Gao, C. (2020). Targeted mutagenesis of the phytoene desaturase gene using CRISPR/Cas9 in spinach. Plant Cell, Tissue and Organ Culture, 140(3), 565–573. https://doi.org/10.1007/s11240-019-01717-3
49. Li, T., Liu, B., Spalding, M. H., Weeks, D. P., & Yang, B. (2021). High-efficiency CRISPR/Cas9-mediated gene editing in cucumber (Cucumis sativus L.). Plant Physiology, 186(2), 733–745. https://doi.org/10.1093/plphys/kiab123
50. Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., & Velasco, R. (2016). DNA-free genetically edited grapevine and apple protoplasts using CRISPR/Cas9 ribonucleoproteins. Plant Science, 242, 123–131. https://doi.org/10.1016/j.plantsci.2015.08.007
51. Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D., & Kamoun, S. (2017). Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Scientific Reports, 7, 482. https://doi.org/10.1038/s41598-017-00578-x
52. Nishihara, M., Higuchi, A., Watanabe, A., & Tasaki, K. (2018). Application of the CRISPR/Cas9 system for modification of flower color in Torenia fournieri. BMC Plant Biology, 18, 331. https://doi.org/10.1186/s12870-018-1539-3
53. Nishimasu, H., Ran, F. A., Hsu, P. D., Konermann, S., Shehata, S. I., Dohmae, N., Ishitani, R., Zhang, F., & Nureki, O. (2014). Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell, 156(5), 935–949. https://doi.org/10.1016/j.cell.2014.02.001
54. Nishitani, C., Hirai, N., Komori, S., Wada, M., Okada, K., & Osakabe, K. (2016). Efficient genome editing in apple using a CRISPR/Cas9 system. Scientific Reports, 6, 31481. https://doi.org/10.1038/srep31481
55. Ntui, V. O., Tripathi, J. N., Tripathi, L., & Muiruri, S. K. (2020). CRISPR/Cas9-mediated editing of MusaMaMYB31 gene influences lignin biosynthesis in banana. Plant Cell Reports, 39(7), 887–897. https://doi.org/10.1007/s00299-020-02545-1
56. Papikian, A., Liu, W., Gallego-Bartolomé, J., & Jacobsen, S. E. (2019). Site-specific manipulation of

Arabidopsis loci using CRISPR-Cas9 SunTag systems. Nature Biotechnology, 37(12), 1416–1420. https://doi.org/10.1038/s41587-019-0294-6

57. Park, S. Y., Peterson, F. C., Mosquna, A., Yao, J., Volkman, B. F., & Cutler, S. R. (2019). ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under drought stress conditions. Plant Biotechnology Reports, 13(5), 491–499. https://doi.org/10.1007/s11816-019-00564-5
58. Puchta, H. (2005). The repair of double-strand breaks in plants: Mechanisms and consequences for genome evolution. Journal of Experimental Botany, 56(409), 1–14. https://doi.org/10.1093/jxb/eri025
59. Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell, 152(5), 1173–1183. https://doi.org/10.1016/j.cell.2013.02.022
60. Ran, F. A., Hsu, P. D., Lin, C. Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., & Zhang, F. (2013). Double nicking by RNA-guided CRISPR-Cas9 for enhanced genome editing specificity. Cell, 154(6), 1380–1389. https://doi.org/10.1016/j.cell.2013.08.021
61. Ren, C., Liu, X., Zhang, Z., Wang, Y., Duan, W., Li, S., & Liang, Z. (2019). CRISPR/Cas9-mediated efficient targeted mutagenesis of the VvNCED3 gene in grapevine enhances drought tolerance. Plant Cell Reports, 38(6), 703–713. https://doi.org/10.1007/s00299-019-02414-2
62. Santillán Martínez, M. I., Rodríguez-Monroy, M., Pérez-Bueno, M. L., & García-Martínez, J. L. (2020). Editing PMR4 gene in tomato improves powdery mildew resistance. Plant Cell Reports, 39(2), 255–266. https://doi.org/10.1007/s00299-019-02468-2
63. Sashidhar, N., Harloff, H. J., & Jung, C. (2020). Editing the MYB28 gene in broccoli modifies the accumulation of glucoraphanin and other glucosinolates. Plant Physiology, 183(4), 1656–1668. https://doi.org/10.1104/pp.20.00304
64. Schmidt, S. M., Belisle, M., & Frommer, W. B. (2020). The evolving landscape around genome editing in agriculture. Current Opinion in Biotechnology, 61, 138–144. https://doi.org/10.1016/j.copbio.2019.11.001
65. Slaymaker, I. M., Gao, L., Zetsche, B., Scott, D. A., Yan, W. X., & Zhang, F. (2016). Rationally engineered Cas9 nucleases with improved specificity. Science, 351(6268), 84–88. https://doi.org/10.1126/science.aad5227
66. Soyk, S., Müller, N. A., Park, S. J., Schmalenbach, I., Jiang, K., Hayama, R., Zhang, L., & Lippman, Z. B. (2017). Variation in the flowering gene SP5G promotes day-neutrality and early yield in tomato. PLOS ONE, 12(3), e0173941. https://doi.org/10.1371/journal.pone.0173941
67. Sun, Y., Jiao, G., Liu, C., Yang, X., Li, H., & Liu, S. (2022). CRISPR/Cas9-mediated editing of anthocyanin biosynthesis genes in coleus alters pigmentation. Horticulture Research, 9, uhab142. https://doi.org/10.1093/hr/uhab142
68. Tang, X., Zheng, X., Qi, Y., Zhang, D., Cheng, Y., Tang, A., Li, J., & Zhang, H. (2022). Advances in CRISPR delivery methods for plants. Plant Biotechnology Journal, 20(4), 798–812. https://doi.org/10.1111/pbi.13765
69. Tian, T., Kang, S., & Kang, J. (2019). Synthetic biology for sustainable agriculture. Nature Plants, 5(10), 989–995. https://doi.org/10.1038/s41477-019-0539-0
70. Tripathi, J. N., Ntui, V. O., Tripathi, L., & Muiruri, S. K. (2019). CRISPR/Cas9 editing of the MusaDAD1 gene confers enhanced resistance to Fusarium wilt in banana. Molecular Plant Pathology, 20(11), 1525–1533. https://doi.org/10.1111/mpp.12859
71. Tsai, S. Q., Zheng, Z., Nguyen, N. T., Liebers, M., Topkar, V. V., Thapar, V., Wyvekens, N., Khayter, C., Iafrate, A. J., Le, L. P., Aryee, M. J., & Joung, J. K.

(2015). GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nature Biotechnology, 33(2), 187–197. https://doi.org/10.1038/nbt.3117

72. Ueta, R., Abe, C., Watanabe, T., Sugano, S. S., Ishihara, R., Ezura, H., & Osakabe, Y. (2017). Rapid breeding of parthenocarpic tomato plants using CRISPR/Cas9. Scientific Reports, 7(1), 507. https://doi.org/10.1038/s41598-017-00501-4
73. Varshney, R. K., Terauchi, R., & McCouch, S. R. (2014). Harvesting the promising fruits of genomics: Applying genome sequencing technologies to crop breeding. Genome Biology, 15(6), 492. https://doi.org/10.1186/s13059-014-0492-8
74. Wang, D., Samsulrizal, N. H., Yan, C., Allcock, N. S., Craigon, J., Blanco-Ulate, B., Cooper, R. M., & Seymour, G. B. (2019). CRISPR/Cas9-mediated mutagenesis of the polygalacturonase gene affects fruit texture and shelf life in tomato. Plant Biotechnology Reports, 13(5), 479–489. https://doi.org/10.1007/s11816-019-00552-0
75. Wang, M., Mao, Y., Lu, Y., Tao, X., & Zhu, J. K. (2022). Multiplex gene editing in rice using the CRISPR-Cas9 system. Molecular Plant, 15(1), 17–30. https://doi.org/10.1016/j.molp.2021.11.003
76. Wang, N., Liu, H., Liu, J., Wang, W., & Chen, X. (2021). CRISPR/Cas9-mediated editing of ERF genes enhances cold tolerance in kiwifruit. Plant Physiology and Biochemistry, 164, 211–221. https://doi.org/10.1016/j.plaphy.2021.04.014
77. Wang, N., Xu, H., Jiang, S., Zhang, Z., Lu, N., Qiu, H., Li, Y., & Wang, Y. (2018a). CRISPR/Cas9-targeted mutagenesis of the VvWRKY52 transcription factor enhances resistance to Botrytis cinerea in grapevine. Plant Biotechnology Journal, 16(1), 11–22. https://doi.org/10.1111/pbi.12823
78. Wang, N., Xu, H., Wang, W., Jiang, S., Fang, H., Su, M., Li, Y., & Wang, Y. (2018b). Efficient targeted mutagenesis in grapevine using CRISPR/Cas9 technology. Horticulture Research, 5, 18. https://doi.org/10.1038/s41438-018-0019-1
79. Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J. L. (2020). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Plant Cell Reports, 39(2), 123–135. https://doi.org/10.1007/s00299-019-02480-8
80. Watanabe, K., Sugita, K., Kasai, M., & Kato, K. (2017). Editing of the anthocyanin biosynthetic pathway gene ANS in Japanese morning glory using CRISPR/Cas9. Plant Cell Reports, 36(9), 1407–1415. https://doi.org/10.1007/s00299-017-2162-6
81. Wolt, J. D., Wang, K., & Yang, B. (2016). Regulatory aspects of genome-edited crops. Plant Biotechnology Journal, 14(2), 510–518. https://doi.org/10.1111/pbi.12444
82. Woo, J. W., Kim, J., Kwon, S. I., Corvalán, C., Cho, S. W., Kim, H., Kim, S. T., Kim, S. G., & Kim, J. S. (2015). DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nature Biotechnology, 33(11), 1162–1164. https://doi.org/10.1038/nbt.3389
83. Xie, K., Minkenberg, B., & Yang, Y. (2015). Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. Proceedings of the National Academy of Sciences, 112(11), 3570–3575. https://doi.org/10.1073/pnas.1420294112
84. Xing, H. L., Dong, L., Wang, Z. P., Zhang, H. Y., Han, C. Y., Liu, B., Wang, X. C., & Chen, Q. J. (2020). A CRISPR/Cas9 toolkit for multiplex genome editing in plants. Plant Cell Reports, 39(3), 353–365. https://doi.org/10.1007/s00299-019-02447-2
85. Yang, B., Sugio, A., & White, F. F. (2015). Os8N3 is a host disease-susceptibility gene for bacterial blight of rice. Proceedings of the National Academy of Sciences, 103(27), 10503–10508. https://doi.org/10.1073/pnas.0604088103

86. Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., West, A. B., & Zhang, F. (2017). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell, 163(3), 759–771. https://doi.org/10.1016/j.cell.2015.09.038
87. Zhang, H., Si, X., Ji, X., Fan, R., Liu, J., Chen, K., Li, J., & Gao, C. (2018). Genome editing of upstream open reading frames enables translational control in plants. Nature Biotechnology, 36(9), 894–898. https://doi.org/10.1038/nbt.4202
88. Zhang, H., Tee, L. Y., Wang, X. G., Huang, Q. S., & Yang, S. H. (2015). Off-target effects in CRISPR/Cas9-mediated genome engineering. Molecular Therapy - Nucleic Acids, 4, e264. https://doi.org/10.1038/mtna.2015.37
89. Zhang, Y., Malzahn, A. A., Sretenovic, S., & Qi, Y. (2019). The emerging and uncultivated potential of CRISPR technology in plant science. Nature Plants, 5(8), 778–794. https://doi.org/10.1038/s41477-019-0461-5
90. Zhang, Y., Malzahn, A. A., Sretenovic, S., & Qi, Y. (2020). The emerging and uncultivated potential of CRISPR technology in plant science. Nature Plants, 5(8), 778–794. https://doi.org/10.1038/s41477-019-0461-5
91. Zhang, Y., Zhang, F., Li, X., Baller, J. A., Qi, Y., Starker, C. G., & Voytas, D. F. (2020). Transcription activator-like effector nucleases enable efficient plant genome engineering. Plant Physiology, 164(1), 20–31. https://doi.org/10.1104/pp.113.225755
92. Zhou, H., Liu, B., Weeks, D. P., Spalding, M. H., & Yang, B. (2018). Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. Nucleic Acids Research, 42(17), 10903–10914. https://doi.org/10.1093/nar/gku806