Plant Archives Vol. 26, Supplement 2, 2026 pp.1095-1106 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.128

CRISPR/CAS-MEDIATED GENOME EDITING IN FRUIT CROPS: ADVANCES, CHALLENGES AND FUTURE DIRECTIONS: A COMPREHENSIVE REVIEW

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(Date of Receiving : 03-05-2026; Date of Revision : 19-06-2026; Date of Acceptance : 07-07-2026)

ABSTRACT

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system has transformed plant biotechnology, offering unprecedented precision for modifying complex polyploid fruit crop genomes. This review comprehensively examines the deployment of CRISPR/Cas9, Cas12a, Cas13 and base-editing variants across major fruit crops including strawberry (*Fragaria* × *ananassa*), banana (*Musa spp*.), apple (Malus × domestica), citrus (*Citrus spp*.) and grape (*Vitis vinifera*). We discuss key applications encompassing disease resistance engineering, yield improvement, fruit quality enhancement, abiotic stress tolerance and accelerated domestication. Landmark achievements include powdery mildew resistance in strawberry via *MLO* gene disruption, Fusarium wilt resistance in banana through *eIF4E* editing and improved lycopene accumulation in tomato by targeting the SlPSY1 locus. We critically evaluate major challenges including efficient delivery systems for recalcitrant species, off-target editing, regulatory hurdles and germplasm access issues. Emerging frontiers prime editing, epigenome editing, RNA base editing and AI-assisted guide RNA design are discussed in the context of next-generation fruit crop improvement. This review highlights CRISPR/Cas technology as a cornerstone of sustainable fruit crop breeding, while emphasizing the need for robust regulatory frameworks and equitable technology access.

Keywords **:** CRISPR/Cas9, genome editing, fruit crops, disease resistance, fruit quality, plant biotechnology, base editing, prime editing

Introduction

The importance of fruits in agriculture around the world cannot be overstated due to their nutritional value through providing vitamins, minerals, fibers and other beneficial compounds to many people. The global fresh fruit industry accounted for around USD 780 billion in 2023 and is anticipated to exceed USD 1.1 trillion by 2030, owing to growing awareness about health and growing population (FAO, 2023). Nevertheless, the cultivation of fruits confronts several problems, among which are abiotic stress induced by climate change, emergence of pathogens, more than 30–40% post-harvest losses and consumer preference for higher quality fruits (Savary *et al*., 2019; Zhao *et al*., 2022).

Classic plant breeding has resulted in impressive advancements in fruits throughout thousands of years; nevertheless, classical plant breeding faces limitations

posed by extended juvenile periods, difficult polyploidy genomes, limited genetic variation and undesirable linkage drag that comes with the introduction of wild relatives' genetics (Schaart *et al*., 2021). On the other hand, transgenic technology, which emerged in the 1980s, helped to overcome some of these limitations; however, it was widely distrusted by the public, heavily regulated and boycotted on commercial markets (Barta *et al*., 1986; Slater *et al*., 2003).

The emergence of programmable nucleases including zinc finger nucleases (ZFNs), TALEN and eventually CRISPR/Cas systems marked a paradigm shift in genome targeting technologies. The CRISPR/Cas9 technology which is based on the type II CRISPR-Cas defense system from S. pyogenes by Doudna, Charpentier *et al*. (Jinek *et al*., 2012) emerged very quickly as the premier genome editing tool due to its simplicity and efficiency. Unlike the ZFNs and TALENs which need costly and time-consuming protein engineering for each target site, CRISPR/Cas9 requires a programmable sgRNA to target the Cas9 endonuclease to genomic targets containing a protospacer adjacent motif (PAM) sequence 5'-NGG-3' for SpCas9 (Ran *et al*., 2013).

CRISPR/Cas in fruit crops has gone through the evolution of moving from simple proof-of-principle experiments to complex traits engineering including disease resistance (Tripathi *et al*., 2021; Malnoy *et al*., 2016), quality improvement of the fruit (Nonaka *et al*., 2017; Li *et al*., 2018), fruit ripening regulation (Ito *et al*., 2015) and stress tolerance (Zhang *et al*., 2019). In this review, we summarize CRISPR/Cas applications in fruit crops in detail. Our goal is to provide insights into the biological and biochemical properties of engineered traits, compare editing tools, analyze delivery methods for difficult-to-transform plants and speculate on future trends.

CRISPR/Cas Systems: Mechanisms and Variants

Type II CRISPR/Cas9

Typically, the standard SpCas9 system comprises two RNA molecules: the CRISPR RNA (crRNA) and the transactivating crRNA (tracrRNA). However, most laboratories prefer to combine them into one RNA molecule called the guide RNA (sgRNA), which is around 100 nucleotides long. The 5'-spacer sequence (20 nt) of the sgRNA determines the target site via Watson-Crick binding, whereas the 3'-scaffold region interacts with Cas9. Additionally, the target site needs to be followed by a PAM sequence (5'-NGG-3') on the complementary strand. SpCas9 creates blunt-end DSBs within the targeted sequence, which can be repaired by either NHEJ or HDR using provided donor templates (Doudna and Charpentier, 2012; Jinek *et al*., 2012; Ran *et al*., 2013).

In recent years, several SpCas9 variants have been developed for specific purposes. High-fidelity variants (eSpCas9, SpCas9-HF1) reduce off-target effects (Slaymaker *et al*., 2016; Kleinstiver *et al*., 2016); PAM-variant SpCas9 derivatives (SpRY, SpG) expand targetable sites (Walton *et al*., 2020); smaller orthologs, including SaCas9 and CjCas9, make the use of viral vectors possible for delivery in mammals and plasmids for plants.

Type V CRISPR/Cas12a (Cpf1)

There are several key differences between Cas12a, initially discovered in Acidaminococcus species (AsCas12a) and Lachnospiraceae bacterium (LbCas12a) and SpCas9: Cas12a requires a single crRNA (without the need for tracrRNA), is compatible with T-rich PAM sequence (5'-TTTV-3'), produces 5' staggered cuts to aid HDR and possesses endogenous RNase activity, which facilitates multiple gene edits from pre-crRNA arrays (Zetsche *et al*., 2015; Zhong *et al*., 2018). These benefits are especially pertinent in the context of fruit.

Base Editors

Base editors carry out efficient single-base substitutions without creating double-strand breaks and employing any donor templates. CBEs perform the conversion from C•G to T•A through the fusion of cytidine deaminase with a catalytically inactive Cas9 nuclease (nCas9), while ABEs induce the change from A•T to G•C through an engineered adenosine deaminase enzyme (Komor *et al*., 2016; Gaudelli *et al*., 2017). Generation four versions of CBE (CBE4max) and ABE (ABE8e) base editors exhibit high efficiencies with minimal side effects such as off-target RNA editing (Anzalone *et al*., 2020). In fruit plants, base editors have been used for the generation of gain-of-function alleles and regulation of elements without regenerating steps related to DSBs (Veillet *et al*., 2019).

Prime Editors

Prime editing, described by Anzalone *et al*. (2019), involves the use of nCas9 fused with a reverse transcriptase domain along with a prime editing guide RNA (pegRNA) that encodes both the spacer and an edit-specific template reverse transcriptase RNA. It makes all 12 types of base transitions, additions and deletions possible without creating DSBs or adding foreign donor DNA and causes considerably fewer off-target effects than SpCas9. Prime editing has been

successfully used in rice (Tang *et al*., 2020) and wheat (Lin *et al*., 2021). It represents an extremely promising technique for precision allele engineering in fruit crops, especially for knocking out specific genes.

Comparison of CRISPR Platforms

Table 1 : Comparison of major CRISPR/Cas platforms used in fruit crop genome editing.

System	PAM	Cut Type	Edit Types	Multiplex	Off-target Risk	Key Reference
SpCas9	NGG	Blunt DSB	Indels, HDR	Limited	Moderate	Jinek *et al*., 2012
SaCas9	NNGRRT	Blunt DSB	Indels, HDR	Limited	Low–Mod.	Ran *et al*., 2015
Cas12a	TTTV	5′ overhang DSB	Indels, HDR	Excellent	Low	Zetsche *et al*., 2015
CBE4max	NGG	Nick (non-target)	C→T	Moderate	Low–Mod.	Komor *et al*., 2016
ABE8e	NGG	Nick (non-target)	A→G	Moderate	Very Low	Gaudelli *et al*., 2017
PE3	NGG	Nick (target)	All 12 SNVs, indels	Limited	Very Low	Anzalone *et al*., 2019
Cas13d	None (RNA)	RNA cleavage	RNA knockdown	Yes	Low	Konermann *et al*., 2018

Delivery Strategies in Fruit Crops

A major limitation in the efficient editing of fruit crops is the effective introduction of CRISPR/Cas elements as either DNA (plasmid or T-DNA), RNA (mRNA + sgRNA), or ribonucleoproteins (RNP). This is because some of these crops do not take kindly to Agrobacterium-based transformation or regenerative methods.

Agrobacterium-Mediated Transformation

T-DNA delivery through Agrobacterium tumefaciens is the most commonly employed technique for the introduction of stable CRISPR/Cas into fruits. CRISPR cassette vectors driven by constitutive promoters such as CaMV 35S or tissue-preferred promoters are transformed in leaf discs, cotyledon, hypocotyl explants, or embryogenic calluses. Success stories reported involve tomato (Brooks *et al*., 2014), strawberry (Martín-Pizarro *et al*., 2019), citrus (Peng *et al*., 2017), banana (Tripathi *et al*., 2021) and grape (Malnoy *et al*., 2016). Nonetheless, this approach depends on genotype, is time-intensive (6-18 months) and leads to random integration of T-DNA, thereby requiring proper segregation for editing without the transgene.

Protoplast Transfection

PEP-mediated transfection of isolated protoplasts with CRISPR RNP complexes or DNA plasmids constitutes a quick way to perform editing free of any transgene. Because RNPs are transient in nature, it is possible to recover edited plants that contain indels but not Cas9 transgenes. PEP-mediated gene editing in CRISPR technology has been employed in tomatoes (Scheben *et al*., 2017), grapevines (Malnoy *et al*., 2016) and apples (Nishitani *et al*., 2016). The main limitations of this process include poor efficiency of protoplast regeneration in several woody perennial plants.

Particle Bombardment (Biolistics)

The gene-gun technique utilizes a machine such as Bio-Rad PDS-1000/He or its equivalents that bombard particles such as gold or tungsten that have DNA/RNP attached to them on embryogenic calli or immature embryos. Gene-gun technology does not have host range restrictions of Agrobacterium and is more suitable for monocots. CRISPR delivery through biolistics has been performed on bananas (Dale *et al*., 2017), strawberries (Martín-Pizarro *et al*., 2019) and papayas (Ferreira *et al*., 2002). The major drawback with this approach is the occurrence of complicated integrations.

Virus-Based Delivery

TRV, BeYDV and geminivirus replicons have been modified to introduce sgRNAs or Cas9 protein into plant cells. Geminivirus replicons produce many copies, leading to an increase in HDR efficiency due to an increase in local DNA concentration (Wang *et al*., 2017). The genome-editing technology using viruses (VIGE) allows avoiding the need for tissue culture; however, VIGE is limited by the host specificity of viruses, insert size and silencing of viral constructs in plants over time.

Nanoparticle-Based Delivery

New platforms based on nanoparticle vectors like carbon nanotubes, LDH nanosheets, lipid-based nanoparticles and polymers have been proposed to deliver RNPs for CRISPR applications in plants. The successful foliar delivery of CRISPR RNPs through LDH nanosheets was demonstrated in intact wheat leaves (Liu *et al*., 2020). The advantages of using nanoparticle-based systems include their independence from tissue culture techniques, but the efficiency of delivery is relatively low and needs optimization.

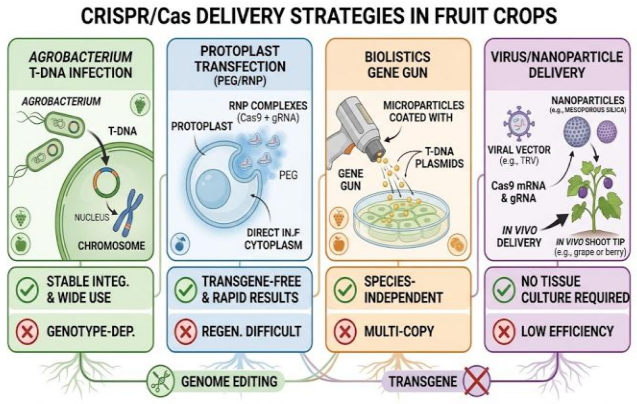

Fig. 1 : Overview of CRISPR/Cas delivery strategies in fruit crops, highlighting key advantages and limitations of each approach.

CRISPR/Cas Applications in Specific Fruit Crops

*Strawberry (*Fragaria × ananassa*)*

Cultivated strawberries are octoploids (2n = 8x = 56), presenting a great challenge for genome editing because of the existence of several homoeologous genes. The pioneering studies on CRISPR/Cas9 technology for editing octoploid strawberry have been performed by Martín-Pizarro *et al*. (2019) who targeted the *FaPG1* (polygalacturonase 1) gene for preventing the premature fruit softening and extending its shelf-life. Simultaneous editing of several copies (up to eight FaPG1 copies) was achieved by using appropriate guide RNA (sgRNA) design. Powdery mildew resistance has been attained via editing the *FaMLO2* gene, which is a homolog of barley mlo gene, whose absence leads to the resistance to a wide spectrum of *Blumeria graminis* and other powdery mildews (Pessina *et al*., 2016).

Recently, *FaRCO1* and FaMYB10 have also been edited via CRISPR technology in order to affect anthocyanins' synthesis and accumulation. It is necessary to mention another important aspect for commercializing strawberry products developed with the help of CRISPR technology. Namely, plants created with the help of protoplast transformation with RNA-protein complexes may be considered as non-genetically modified organisms (non-GMOs) in accordance with certain national regulations.

*Banana (*Musa *spp.)*

As a rule, cultivated strawberries are octoploid plants (2n = 8x = 56), which makes the process of genome editing especially problematic due to the presence of several homoeologous genes. However, pioneering work aimed at developing CRISPR/Cas9 techniques for editing the octoploid strawberries has already been done by Martín-Pizarro *et al*. (2019), where the *FaPG1* (polygalacturonase 1) gene was targeted for prevention of fruit's premature softening. Simultaneous editing up to eight copies of the *FaPG1* gene was carried out with the use of proper guide RNA (sgRNA). Resistance to powdery mildew has been reached through genome editing of the *FaMLO2* gene, which is homologous to the barley mlo gene and its absence causes resistance to various Blumeria graminis and other powdery mildews (Pessina *et al*., 2016).

The *FaRCO1* and *FaMYB10* genes were also edited recently in order to control anthocyanins biosynthesis. However, there is one more issue to mention in relation to CRISPR technology usage for commercialization of strawberries products. Indeed, plants, created through the method of protoplast transformation with RNA-protein complex, can be classified as non-GMOs according to some national regulations.

Citrus (Citrus spp.)

Citrus Huanglongbing (HLB), a result of Candidatus Liberibacter asiaticus (CLas), is the most destructive citrus pathogen in the world, endangering the nine billion dollar US citrus industry and millions of hectares of citrus crops around the globe. CRISPR/Cas9 technology has been employed to disrupt the CsLOB1 (lateral organ boundaries 1) susceptibility factor utilized by Xanthomonas citri pv. citri (Xcc), the causative agent of citrus canker (Peng *et al*., 2017). Inhibition of *CsLOB1* in Duncan grapefruit and Hamlin sweet orange cultivars produced strong resistance against citrus canker without phenotypical changes in plants.

The CsWRKY22 and CsBZR1 transcription factor genes were targeted by Wang *et al*. (2019) to regulate defense signaling pathways after phytopathogenic infection by Phytophthora spp. CsDREB and CsERF transcription factors were knocked out by CRISPR/Cas9 in mandarin plants to increase cold resistance by Li *et al*. (2020). The regulation of citric acid production pathways via editing of the CitVHA-c4 subunit of the vacuolar H^+-ATPase complex (Liu *et al*., 2021) was studied as an approach to alter fruit flavor characteristics in citrus fruits.

*Apple (*Malus × domestica*)*

Apple is a significant temperate fruit crop with a sophisticated heterozygous diploid genome (742 Mb) consisting of about 57,000 predicted genes. Fire blight, caused by the bacterium Erwinia amylovora, is the most economically significant bacterial disease of apples and pears. Malnoy *et al*. (2016) showed the ability to knockout the disease-inducing protein from the Mlo protein family (MdDIPM4) with

CRISPR/Cas9 in apple protoplasts with a maximum editing efficiency rate of 2.4%. The non-browning trait, of great commercial interest regarding fresh cut apples, was achieved via CRISPR/Cas9 targeting of the polyphenol oxidase (PPO) gene family, similar to the already commercialized TALEN Arctic® apples from Okanagan Specialty Fruits. Nishitani *et al*. (2016) were the first to show CRISPR-based editing of the MdPDS (phytoene desaturase) marker locus in apples, thereby establishing base editing efficiencies in the difficult-to-edit species. Fruit quality characteristics, including MdMYB1-mediated anthocyanin production, ethylene biosynthesis through MdACS1/MdACO1 and MdABI8-mediated sugar accumulation, are current targets of CRISPR.

Grape (*Vitis vinifera*)

Grapevine (*Vitis vinifera*, 2n = 2x = 38) is one of the most commercially important fruit crops in the world, generating an economic output value of more than USD 50 billion per year worldwide. Disease resistance and fruit quality improvement are considered the two major applications for CRISPR technology in grapevine. These mainly include resistance to powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*) and Botrytis bunch rot. In 2016, Malnoy *et al*. successfully achieved targeted gene mutagenesis in grapevine by introducing RNPs into protoplasts and developed edited plants without any stable transformation, which was an outstanding example of gene-free editing in the field of fruit tree crop breeding.

Wang *et al*. (2018) targeted the transcription factor VvWRKY52, aiming to make grapevine resistant to Botrytis cinerea. Grapevine fruit quality can be improved by editing the VvDXS and VvDXR biosynthetic genes involved in terpenoid synthesis, thereby controlling the aromatic terpene composition of muscats (Sunitha and Rock, 2020). The editing process for VvGI (GIGANTEA) and VvCO (CONSTANS) orthologues in grapevine has also been started.

Other Fruit Crops

CRISPR/Cas9 applications have expanded to various fruits. For example, editing eIF4E was studied in papaya (*Carica papaya*) for the resistance against papaya ringspot virus (Ferreira *et al*., 2002). In mango (*Mangifera indica*), a CRISPR protocol was established for targeting MiPDS but regeneration remains difficult. In blueberry (*Vaccinium corymbosum*), regulation of *MYB* genes controlling anthocyanins for enhancing flavonoids content was investigated. In kiwi (*Actinidia chinensis*), targeting of *AcMLO* genes for resistance against powdery mildew was conducted by Agrobacterium transformation method (Wang *et al*., 2018). Regarding pomegranate (*Punica granatum*), CRISPR protocols have been established with limited information in literature.

Table 2 : Summary of major CRISPR/Cas9 applications in fruit crops.

Crop	Target Gene(s)	Trait Modified	CRISPR Tool	Delivery Method	Reference
Strawberry	FaPG1	Fruit softening (shelf life)	SpCas9	Biolistics/Agro.	Martín-Pizarro *et al*., 2019
Strawberry	FaMLO2	Powdery mildew resistance	SpCas9	Agrobacterium	Pessina *et al*., 2016
Banana	MaEIF4E	Banana streak virus resistance	SpCas9	Biolistics	Tripathi *et al*., 2021
Citrus	CsLOB1	Citrus canker resistance	SpCas9	Agrobacterium	Peng *et al*., 2017
Citrus	CsDREB, CsERF	Cold stress tolerance	SpCas9	Agrobacterium	Li *et al*., 2020
Apple	MdDIPM4	Fire blight resistance	SpCas9	Protoplast (RNP)	Malnoy *et al*., 2016
Grape	VvMLO3, VvMLO4	Powdery mildew resistance	SpCas9	Protoplast (RNP)	Malnoy *et al*., 2016
Grape	VvWRKY52	Botrytis resistance	SpCas9	Agrobacterium	Wang *et al*., 2018
Papaya	CpEIF4E	Ringspot virus resistance	SpCas9	Biolistics	Ferreira *et al*., 2002

Key Trait Improvement Area

Disease Resistance

S genes, which control susceptibility to diseases, are excellent targets for CRISPR due to the fact that a lack of function of such genes leads to broad-spectrum resistance in plants. For instance, the *MLO* gene family encodes the seven-transmembrane-domain plant-specific protein. Loss-of-function mutations in *mlo* genes have been demonstrated to be highly effective in conferring broad-spectrum resistance to powdery mildew caused by *Blumeria graminis* f. sp. hordei in

barley. The orthologues of MLO genes were successfully edited in tomato, strawberry, apple and grape by CRISPR (Büschges *et al*., 1997; Nekrasov *et al*., 2017; Pessina *et al*., 2016; Malnoy *et al*., 2016).

Another example includes eIF4E, the gene family that codes the translation initiation factor utilized by potyviruses during replication. Genes belonging to the eIF4E family have been edited in tomato, cucumber, cassava and banana to provide resistance to various viruses (Chandrasekaran *et al*., 2016; Tripathi *et al*., 2021). Finally, *S* genes coding for proteins hijacked by bacteria for infection are potential targets for editing. *LOB1* in citrus, *SWEET* sugar transporter genes hijacked by Xanthomonas for infecting rice and fruit trees and DIPM proteins in apple are among such examples (Peng *et al*., 2017; Malnoy *et al*., 2016).

Fruit Quality and Nutritional Enhancement

Quality attributes include color, flavor, aroma, texture, sugar/acid balance and nutritional content. CRISPR technology has successfully been used to alter biosynthetic pathways involved in controlling these quality parameters with impressive accuracy. Tomato fruit lycopene content was increased via disruption of lycopene β-cyclase and lycopene ε-cyclase, diverting flux into lycopene synthesis instead of α- and β-carotenoids (Nonaka *et al*., 2017). Anthocyanin accumulation, which is controlled by *MYB-bHLH-WD40* transcription factors, has been improved through targeted disruption of transcriptional repressors.

Modification of flavor has been done by altering the biosynthesis of active flavor compounds. GABA-enriched tomatoes (Nonaka *et al*., 2017) and high-GABA sweet pepper have been obtained through disrupting GABA shunt enzymes. Modification of terpene-based aroma content in grapes and strawberries through terpenoid biosynthesis genes will provide opportunities for development of novel flavors not possible in conventional crossing breeding programs restricted by the flavor wheel concept. Cell wall modifying enzymes include PGs, PMEs and expansins. Editing FaPG1 in strawberries resulted in improved post-harvest storage life without compromising flavor.

Fruit Ripening and Post-Harvest Quality

Ethylene acts as a master regulator of ripening in climacteric fruits like tomatoes, apples, bananas, kiwis and mangoes. The use of CRISPR for disrupting ethylene biosynthesis pathway genes such as 1-aminocyclopropane-1-carboxylate synthase (ACS) and ACC oxidase (ACO) or ethylene signal transduction components such as *ETR1, EIN3 and EIL1* allows for precise regulation of fruit ripening initiation and development (Ito *et al*., 2015; Wang *et al*., 2020). Fruit ripening delayed mutant lines of bananas using CRISPR have already been generated and this technology shows huge potential for increasing fruit shelf-life in global markets. In contrast, cell wall hydrolases play an important role in softening of non-climacteric strawberries.

Abiotic Stress Tolerance

Due to climate change, there are increasing incidences of abiotic stresses in the form of drought, high temperature, salt stress and freezing that impact fruit crop production in favorable areas. Modification of negative regulators of stress responses using CRISPR technology can be one of the solutions to develop plants with high stress tolerance capacity. Tolerance to drought was developed in tomatoes through *SlMAPK3* modification (Zhang *et al*., 2019) and in *SlARF4* gene (Wang *et al*., 2020). For cold tolerance, modification in citrus trees using *CsDREB and CsERF* transcription factors was used (Li *et al*., 2020). Modulation of salt stress tolerance includes modification of *SlHKT1* (potassium transporter) and *SlSOS1* (salt overly sensitive 1) genes.

Yield and Plant Architecture

In tomatoes, stem cell identity and floral transition pathways that regulate yield-related traits have been well studied, thus making tomatoes an excellent crop for CRISPR-based yield improvement. Stem cell identity manipulation in the *CLAVATA3-WUSCHEL* pathway in tomato resulted in increases in locule number and size (Rodriguez-Leal *et al*., 2017). Manipulation of the SELFPRUNING (*SP)* growth habit gene and its association with the *SFT* (SINGLE FLOWER TRUSS), which is the florigen, was used to manipulate plant structure to make them suitable for mechanized harvesting. Parthenocarpy, where fruit develops without pollination or fertilization, has been achieved in tomatoes and grapes by suppressing the auxin/indole-3-acetic acid repressors *SlIAA9 and VvIAA9*.

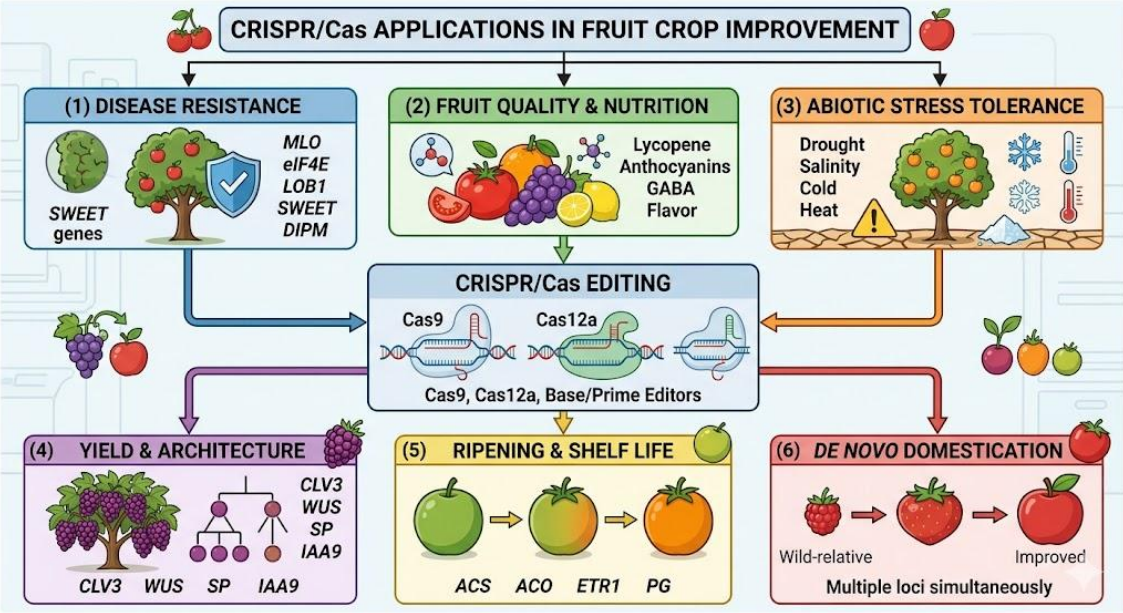

Fig. 2 : Overview of major CRISPR/Cas application domains in fruit crop improvement, illustrating the breadth of targeted traits and the central role of the editing platform.

Challenges in Fruit Crop Genome Editing

Polyploidy and Genome Complexity

Commercially significant fruit plants include strawberry (octoploid, 2n = 8x = 56), banana (triploid, 2n = 3x = 33) and blueberry (tetraploid, 2n = 4x = 48). They have numerous homologous gene copies that should all be modified in order to produce a phenotype. The result is an increased number of sgRNAs designed for modification of genes in different homologues and a lower efficacy of modification of each homologue in particular. High-fidelity *Cas9* and *Cas12*a are needed to avoid off-target events, due to repetitive DNA sequence composition of their genomes (Zetsche *et al*., 2015). Long read technologies (PacBio and Oxford Nanopore) will be indispensable for editing phase analysis.

Recalcitrant Transformation and Regeneration

There are many crops with fruits, especially woody perennial species (apple, citrus, grapes, mangoes, avocados), that are highly recalcitrant to transformation and regeneration, with the time taken ranging from 12-24 months. Transformation strategies that are specific to each genotype also make it difficult to modify commercially available varieties because model genotypes may lack desirable agronomic characteristics. The use of morphogenetic regulators, such as baby boom *(BBM), WUSCHEL (WUS) and GRF4-GIF1* fusion gene products, has greatly improved the efficiency of transformation in recalcitrant cereals (Lowe *et al*., 2016; Kong *et al*., 2020) and can now be used in fruit crops..

Off-Target Editing

The occurrence of off-target mutations at sites showing only partial homology with the guide RNA spacer creates the potential for undesired phenotypes and regulatory hurdles. Off-target mutation rates in plant species tend to be smaller compared to mammalian species as a result of structural differences in chromatin architecture and repair pathways, yet genome-wide off-target analyses using GUIDE-seq, Digenome-seq, or CIRCLE-seq are still necessary for regulatory applications (Kim *et al*., 2015). Strategies such as high fidelity Cas9 proteins, use of tru-gRNAs and the delivery of Cas9 RNPs help reduce the chances of off-targets. The future of requiring the sequencing of T0 and T1 plants in regulatory filings appears to be imminent.

Regulatory Landscape

Regulatory approaches vary widely in the case of CRISPR-modified crops, causing uncertainties with regards to their market entry. According to the USDA APHIS, most CRISPR-modified plants (especially those with small indel modifications that can be attained through conventional breeding techniques) are exempt from any form of regulation according to the SECURE guidelines (7 CFR Part 340). On the other hand, the FDA holds voluntary consultations. The European Court of Justice ruled in 2018 (Case C-528/16) that CRISPR-modified crops should be regulated as GMOs. However, the European Commission presented a proposal for regulations regarding new genomic technologies (NGT) in 2023.

Other nations such as Japan, Australia, Argentina, Brazil and the Philippines have opted for less stringent regulatory regimes in the cases of cisgenesis and small SDN-1 class modification.

Germplasm Access and Intellectual Property

Patent landscapes extending across the CRISPR enabling technologies including SpCas9 (University of California Berkeley/Broad Institute), Cas12a (Broad Institute) and many others, pose freedom-to-operate challenges with respect to the commercial exploitation of CRISPR-edited fruit cultivars. Licensing fees and obligations could disproportionately impact public crop breeding programs and plant breeders from developing nations which do not have the means to navigate the complicated intellectual property landscape. Public licensing models such as those currently in development through the Open CRISPR initiative could help address these issues, although much remains to be done in light of Nagoya and CBD requirements.

Table 3 : Major challenges in CRISPR/Cas-mediated fruit crop editing and potential mitigation strategies.

Challenge	Description	Mitigation Strategies
Polyploidy	Multiple homeologs require simultaneous editing; complex allele dosage effects	Multiplex sgRNA arrays; Cas12a; long-read genome sequencing for phasing
Recalcitrant regeneration	Low transformation efficiency in woody perennials; genotype dependency	Morphogenic regulators (BBM, WUS, GRF4-GIF1); hairy root systems; optimized culture media
Off-target editing	Unintended mutations at partially homologous sites; regulatory concerns	High-fidelity Cas9 variants; RNP delivery; truncated sgRNAs; whole-genome sequencing
Regulatory uncertainty	GMO regulations applied to CRISPR in many regions; lengthy approval timelines	Engagement with regulators; SDN-1 classification advocacy; public-private partnerships
IP and access barriers	Dense patent landscape; licensing costs for public breeders	Open licensing agreements; public sector platforms; international IP frameworks
Mosaicism	Chimeric editing in multicellular explants; inconsistent phenotypes	Single-cell-based regeneration; protoplast systems; early molecular screening
Delivery efficiency	Low Cas9/sgRNA delivery rates in recalcitrant species	Nanoparticles; virus-based delivery; optimized electroporation; lipofection

Regulatory Landscape Across Jurisdictions

Regulations concerning CRISPR edited plants have developed significantly since 2016 and are divergent internationally. In the US, there is a collaborative federal system comprising USDA, FDA and EPA where the status of edited plants is determined based on the type of edit, not the technique involved. With the implementation of USDA APHIS SECURE regulations (2021), SDN-1 class edits, which include small indels attainable through standard mutagenesis methods, are exempted from regulation if no plant pests are introduced. FDA still relies on its voluntary pre-market consultation program.

In Europe, the CJEU judgment of 2018 has extended all the provisions of Directive 2001/18/EC covering GMOs to apply equally to all organisms created using NGTs, thereby developing one of the most strict regulatory systems across the globe. However, in its recent legislative proposal for the NGT regulation of 2023, the European Commission is proposing a twofold system of regulation, separating NGT1 plants, similar to conventional mutations, from NGT2 plants.

Emerging Technologies and Future Directions

Prime Editing in Fruit Crops

Prime editing allows for targeted incorporation of defined mutations, small insertions and deletions without the need for DSBs or exogenous templates, providing a significant improvement compared to traditional HDR-based genome editing techniques. Integration of fruit crop-specific prime editors (PPE2, ePPE) and computational tools for pegRNA design would expedite prime editing implementation in fruit crops. Key applications encompass targeted insertion of disease resistance alleles that occur naturally (eIF4E^sat alleles in nightshades), manipulation of transcriptional promoters for temporal regulation of traits and repair of deleterious mutations within elite germplasm (Lin *et al*., 2021; Tang *et al*., 2020).

Epigenome Editing

Cas9 catalytically inactive enzyme (dCas9) tethered to epigenome modifiers such as DNA methylation transferase enzymes (DRM2), demethylation enzymes (ROS1), histone methyl transferases (CLF, SDG8) and histone acetyl transferases (HAT) allows heritable modulation of gene expression without sequence alteration. Fusion proteins dCas9-VPR and dCas9-KRAB for transcriptional activation and repression respectively have been reported in Arabidopsis thaliana and tomato plants (Lowder *et al*., 2018). For fruit species, epigenome editing may hold promise for activating gene expression from silencing alleles, manipulating imprinted regions of apomictic species and dosage

regulation of gene expression without sequence change.

RNA Editing with CRISPR/Cas13

As opposed to Cas9, Cas13d targets single-strand RNAs via crRNA-guided RNA cleavage without making any changes to the genome. However, when paired with ADAR domains to form the REPAIR and RESCUE platforms, Cas13 allows precise RNA editing, which can be used for gene silencing without any permanent change to the DNA (Konermann *et al*., 2018). Such RNA editing platforms have proved very beneficial in applications involving pathogens and situations where gene modifications need to be temporary.

AI-Assisted Guide RNA Design and Off-Target Prediction

Models trained on genome-wide data from CRISPR screens (like CRISPOR, DeepCRISPR, Azimuth and CF-SAReg) significantly outperform rule-based methods in terms of guide RNA efficacy and off-target identification (Doench *et al*., 2016; Chuai *et al*., 2018). Using fruit-specific chromatin accessibility, DNA methylation maps and expression profiling for RNA guide design will further boost the prediction of editing efficiency. Large language models and graph neural networks will be trained on the results of CRISPR editing in plants in order to predict the effect of certain edits on phenotypes, making virtual editing strategy testing possible.

Speed Breeding and Accelerated Crop Improvement

Platforms of speed breeding, including prolonged photoperiod (22 hours of light), temperature optimization and somatic embryogenesis, have been shown to decrease generation time in long juvenile perennial fruit crops. The use of CRISPR gene editing technology with speed breeding and doubled haploid methods can enable fast fixation of the edited genes and minimize heterozygosity in highly heterozygous and cross-pollinating crops. De novo domestication using CRISPR is another approach being tested for tropical fruit crops that are nutritionally or climatically resilient yet poorly agronomically suited. This strategy would allow new crops to be developed for the future climate in less than a decade rather than millennia, as with conventional domestication.

Multiplex Editing and Stacking

Disruption of several genes or incorporation of several beneficial traits – disease resistance, better nutrition, modified ripening process – needs multiplexing sgRNAs delivery. The ability of Cas12a to process polycistronic arrays of crRNAs and tRNA-sgRNA arrays used for SpCas9 allow multiplex delivery of 6-12 sgRNAs on one vector. Complex CRISPR circuits incorporating logic gates, which can be controlled by specific cell stimuli like AND, OR and NOT logic gates, were constructed in mammalian cells and are now used for designing conditional expression in plants.

Table 4 : Emerging CRISPR/Cas-related technologies with potential applications in fruit crop improvement.

Technology	Mechanism	Fruit Crop Potential	Reference
Prime Editing	pegRNA + nick + reverse transcriptase; installs precise mutations	Precision allele engineering; no DSBs or donor DNA required	Anzalone *et al*., 2019
CBE/ABE Base Editing	Deaminase-nCas9 fusion; C→T or A→G conversion	Precise single-nucleotide variant introduction; regulatory element modification	Gaudelli *et al*., 2017
Epigenome Editing (dCas9)	dCas9-fused effectors modify DNA methylation or histone marks	Heritable expression changes without sequence alteration	Lowder *et al*., 2018
Cas13 RNA Editing	RNA-targeting CRISPR; post-transcriptional silencing or editing	Transient trait modification; antiviral strategies	Konermann *et al*., 2018
AI Guide RNA Design	ML models predict sgRNA activity and off-target from sequence/structure	Optimize editing in complex fruit crop genomes	Chuai *et al*., 2018
Speed Breeding + CRISPR	Extended photoperiod + genome editing for rapid cultivar development	Faster fixation of edits in long-juvenile fruit crops	Watson *et al*., 2018
Nanoparticle Delivery	Nanomaterial-mediated RNP or DNA delivery into intact plant cells	Transgene-free editing without tissue culture	Liu *et al*., 2020

Ethical, Social and Economic Considerations

Ethical, social and economic aspects of utilizing CRISPR to improve fruit crops should be also considered beyond mere technicalities and legal issues associated with genome editing. There is currently a highly polarized consumer perception of genome

edited food crops, where acceptability varies depending on framing (product attributes vs. technology used), trust in regulatory frameworks and socio-cultural value systems of food and nature (Lucht, 2015; Kolodinsky and Lusk, 2018).

The issue of corporate dominance over seed production industries and the impact of CRISPR patents on smallholder farmers in developing nations should receive special attention. Numerous fruit crops such as banana in Africa, mango in Asia and citrus in the Mediterranean region are essential to the existence of millions of smallholder farmers whose access to CRISPR fruits is limited due to the lack of relevant technology or capacity-building efforts (Adenle *et al*., 2018).

Conclusions

The advent of CRISPR/Cas technology revolutionized fruit crop improvement in terms of fast, inexpensive and accurate genome modifications which could not be achieved through conventional breeding programs or initial transgenic technologies. From CRISPR/Cas-mediated modification of tomatoes by Brooks *et al*. (2014) to the commercial production of GABA-enhanced tomatoes in Japan (Nonaka *et al*., 2017) and current field tests of bananas resistant to Fusarium wilt disease (Tripathi *et al*., 2021), CRISPR/Cas technology proved to be effective throughout fruit improvement strategies.

Disease resistance via disruption of susceptibility genes provided durable and broad resistance to the worst pathogens that threatened fruit industries — MLO-type of resistance against powdery mildew of strawberry, apple, tomato and grape; eIF4E resistance against viruses in tomatoes and bananas; LOB1-type of resistance against citrus canker. Enhancement of fruit quality and nutrition traits like high lycopene, anthocyanins, GABA and terpenes aroma components is in line with consumer demand for healthy fruits. Ripening control and extended shelf-life via gene editing of ACS/ACO and PG is profitable as it reduces food loss during transportation processes.

References

Adenle, A. A., Morris, E. J. and Parayil, G. (2018). Status of development and regulation of genetically modified crops in Africa and the implications for the future. *Agriculture & Food Security*, **7**(1), 1–15.

Anzalone, A. V., Koblan, L. W. and Liu, D. R. (2020). Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nature Biotechnology*, **38**(7), 824–844.

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. and Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, **576**(7785), 149–157.

Barta, A., Sommergruber, K., Thompson, D., Hartmuth, K., Matzke, M. A. and Matzke, A. J. M. (1986). The expression of a nopaline synthase–human growth hormone chimaeric gene in transformed tobacco and sunflower callus tissue. *Plant Molecular Biology*, **6**(5), 347–357.

Brooks, C., Nekrasov, V., Lippman, Z. B. and Van Eck, J. (2014). Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. *Plant Physiology*, **166**(3), 1292–1297.

Büschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., van Daelen, R., van der Lee, T., Diergaarde, P., Groenendijk, J., et al. (1997). The barley Mlo gene: A novel control element of plant pathogen resistance. *Cell*, **88**(5), 695–705.

Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., Sherman, A., Arazi, T. and Gal-On, A. (2016). Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Molecular Plant Pathology*, **17**(7), 1140–1153.

Chuai, G., Ma, H., Yan, J., Chen, M., Hong, N., Xue, D., Zhou, C., Zhu, C., Chen, K., Duan, B., et al. (2018). DeepCRISPR: Optimized CRISPR guide RNA design by deep learning. *Genome Biology*, **19**(1), 80.

Dale, J., James, A., Paul, J. Y., Khanna, H., Smith, M., Peraza-Echeverria, S., Garcia-Bastidas, F., Kema, G., Waterhouse, P., Mengersen, K. and Harding, R. (2017). Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. *Nature Communications*, **8**(1), 1496.

Doench, J. G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E. W., Donovan, K. F., Smith, I., Tothova, Z., Wilen, C., Orchard, R., et al. (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature Biotechnology*, **34**(2), 184–191.

Doudna, J. A. and Charpentier, E. (2012). The new frontier of genome engineering with CRISPR-Cas9. *Science*, **346**(6213), 1258096.

Food and Agriculture Organization of the United Nations. (2023). *The state of food and agriculture 2023: Revealing the true cost of food*. Food and Agriculture Organization of the United Nations.

Ferreira, S. A., Pitz, K. Y., Manshardt, R., Zee, F., Fitch, M. and Gonsalves, D. (2002). Virus coat protein transgenic papaya provides practical control of papaya ringspot virus in Hawaii. *Plant Disease*, **86**(2), 101–105.

Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I. and Liu, D. R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, **551**(7681), 464–471.

Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, M. and Toki, S. (2015). CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. *Biochemical and Biophysical Research Communications*, **467**(1), 76–82.

Jiménez-Guerrero, I., Pérez-Montaño, F., Pérez-Vega, E., Wagner, V. E., Bhatt, M. A., Jishnu, A., Ramos, C. and Ollero, F. J. (2020). Show me your CRISPR toolkit and I

will tell you what you can do. *Current Issues in Molecular Biology*, **36**, 149–174.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A. and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**(6096), 816–821.

Kaur, N., Alok, A., Kumar, P., Kaur, N., Awasthi, P., Chaturvedi, S., Pandey, P., Pandey, A. K., Tiwari, S. and Singh, R. (2021). CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for beta-carotene biosynthesis in banana fruit. *Metabolic Engineering Communications*, **12**, e00168.

Kim, D., Bae, S., Park, J., Kim, E., Kim, S., Yu, H. R., Hwang, J., Kim, J. and Kim, J. S. (2015). Digenome-seq: Genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. *Nature Methods*, **12**(3), 237–243.

Kleinstiver, B. P., Pattanayak, V., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Zheng, Z. and Joung, J. K. (2016). High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, **529**(7587), 490–495.

Kolodinsky, J. and Lusk, J. L. (2018). Mandatory labels can improve attitudes toward genetically engineered food. *Science Advances*, **4**(6), eaaq1413.

Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. and Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, **533**(7603), 420–424.

Kong, J., Martin-Ortigosa, S., Finer, J., Orchard, N., Gunadi, A., Batts, L. A., Thakare, D., Rush, B., Schmitz, O., Stuiver, M., et al. (2020). Overexpression of the transcription factor GROWTH-REGULATING FACTOR5 improves transformation of dicot and monocot species. *Frontiers in Plant Science*, **11**, 572319.

Konermann, S., Lotfy, P., Brideau, N. J., Oki, J., Shokhirev, M. N. and Hsu, P. D. (2018). Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell*, **173**(3), 665–676.

Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Zhang, J., Liu, H., Qin, L., Rui, H., et al. (2019). Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or epigenetic variation in CRISPR/Cas9-edited cotton plants. *Plant Biotechnology Journal*, **17**(5), 858–868.

Li, M., Li, X., Zhou, Z., Wu, P., Fang, M., Pan, X., Lin, Q., Luo, W., Wu, G. and Li, H. (2016). Reassessment of the four yield-related genes Gn1a, DEP1, GS3 and IPA1 in rice using a CRISPR/Cas9 system. *Frontiers in Plant Science*, **7**, 377.

Li, T., Hu, J., Sun, Y., Li, B., Zhang, D., Li, W., Liu, J., Li, D., Gao, C., Zhang, Y. and Wang, Y. (2021). Highly efficient heritable genome editing in wheat using an RNA virus and bypassing tissue culture. *Molecular Plant*, **14**(11), 1787–1798.

Li, X., Wang, Y., Chen, S., Tian, H., Fu, D., Zhu, B., Luo, Y. and Zhu, H. (2018). Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. *Frontiers in Plant Science*, **9**, 559.

Li, Z., Liu, X., Xu, C., Li, J., Fang, D. and Chen, M. (2020). CRISPR/Cas9 editing of CsDREB and CsERF genes in *Citrus sinensis* under cold stress conditions. *Plant Cell Reports*, **39**(4), 563–576.

Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A. V., Raguram, A., Doman, J. L., et al. (2021). Prime genome editing in rice and wheat. *Nature Biotechnology*, **39**(4), 407–410.

Liu, Q., Yang, F., Zhang, J., Liu, H., Rahman, S., Islam, S., Ma, W. and She, M. (2021). Application of CRISPR/Cas9 in crop quality improvement. *International Journal of Molecular Sciences*, **22**(8), 4206.

Liu, Y., Zeng, Y., Liu, L., Zhuang, C., Fu, X., Huang, W. and Cai, Z. (2020). Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells. *Nature Communications*, **11**(1), 1–8.

Lowe, K., Wu, E., Wang, N., Hoerster, G., Hastings, C., Cho, M. J., Scelonge, C., Lenderts, B., Chamberlin, M., Cushatt, J., et al. (2016). Morphogenic regulators Baby boom and Wuschel improve monocot transformation. *The Plant Cell*, **28**(9), 1998–2015.

Lowder, L. G., Paul, J. W., Tang, X., Pham, T., Vitorello, V. A., Goud, D., Zheng, X., Voytas, D., Reed, J. W. and Qi, Y. (2018). Robust transcriptional activation in plants using multiplexed CRISPR-Act2.0 and mTALE-Act systems. *Molecular Plant*, **11**(2), 245–256.

Lucht, J. M. (2015). Public acceptance of plant biotechnology and GM crops. *Viruses*, **7**(8), 4254–4281.

Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., Velasco, R. and Nagamangala Kanchiswamy, C. (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. *Frontiers in Plant Science*, **7**, 1904.

Martín-Pizarro, C., Triviño, J. C. and Posé, D. (2019). Functional analysis of the TM6 MADS-box gene in the octoploid strawberry by CRISPR/Cas9-directed mutagenesis. *Journal of Experimental Botany*, **70**(3), 885–895.

Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C. and Wilhelm, R. (2019). What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects? A systematic map. *Environmental Evidence*, **8**(1), 27.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D. and Kamoun, S. (2017). Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Scientific Reports*, **7**(1), 482.

Nishitani, C., Hirai, N., Komori, S., Wada, M., Okada, K., Osakabe, K., Yamamoto, T. and Osakabe, Y. (2016). Efficient genome editing in apple using a CRISPR/Cas9 system. *Scientific Reports*, **6**(1), 31481.

Nonaka, S., Arai, C., Takayama, M., Matsukura, C. and Ezura, H. (2017). Efficient increase of γ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Scientific Reports*, **7**(1), 7057.

Pessina, S., Pavan, S., Catalano, C., Gallotta, A., Guarnaccia, V., Santilli, E., Morello, L., Ghidoli, G., Scozzoli, A., Poletti, V., et al. (2016). Characterization of the MLO gene family in Rosaceae and gene expression analysis in *Malus domestica*. *BMC Genomics*, ***17***, 40.

Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., Yao, L. and Zou, X. (2017). Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene *CsLOB1* promoter in citrus. *Plant Biotechnology Journal*, ***15***(12), 1509–1519.

Ran, F. A., Cong, L., Yan, W. X., Scott, D. A., Gootenberg, J. S., Kriz, A. J., Zetsche, B., Shalem, O., Wu, X., Makarova, K. S., et al. (2015). In vivo genome editing using *Staphylococcus aureus* Cas9. *Nature*, ***520***(7546), 186–191.

Ran, F. A., Hsu, P. D., Lin, C. Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., Scott, D. A., Inoue, A., Matoba, S., Zhang, Y. and Zhang, F. (2013). Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell*, ***154***(6), 1380–1389.

Rodriguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E. and Lippman, Z. B. (2017). Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, ***171***(2), 470–480.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N. and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, ***3***(3), 430–439.

Schaart, J. G., van de Wiel, C. C. M. and Smulders, M. J. M. (2021). Genome editing of polyploid crops: Prospects, achievements and bottlenecks. *Transgenic Research*, ***30***(2), 79–96.

Scheben, A., Wolter, F., Batley, J., Puchta, H. and Edwards, D. (2017). Towards CRISPR/Cas crops—Bringing together genomics and genome editing. *New Phytologist*, ***216***(3), 682–698.

Slater, A., Scott, N. and Fowler, M. (2003). *Plant biotechnology: The genetic manipulation of plants*. Oxford University Press.

Slaymaker, I.M., Gao, L., Zetsche, B., Scott, D.A., and Zhang, F. (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science*, ***351***(6268), 84–88.

Sunitha, S. and Rock, C. D. (2020). CRISPR/Cas9-mediated targeted mutagenesis of TAS4 and MYBA7 loci in grapevine rootstock 101–14. *Transgenic Research*, ***29***(3–4), 355–367.

Tang, X., Sretenovic, S., Ren, Q., Jia, X., Li, M., Fan, T., Yin, D., Xiang, S., Guo, Y., Liu, L., et al. (2020). Plant prime editors enable precise gene editing in rice cells. *Molecular Plant*, ***13***(5), 667–670.

Tripathi, J. N., Ntui, V. O., Ron, M., Muiruri, S. K., Britt, A. and Tripathi, L. (2021). CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. *Communications Biology*, ***4***(1), 1–11.

Tripathi, L., Ntui, V.O. and Tripathi, J. N. (2020).CRISPR/ Cas 9-based genome editing of banana for disease resistance. *Current Opinion in Plant Biology*, ***56***, 118–126.

Veillet, F., Perrot, L., Chauvin, L., Kermarrec, M. P., Guyon-Debast, A., Chauvin, J. E., Nogué, F. and Mazier, M. (2019). Transgene-free genome editing in tomato and potato plants using *Agrobacterium*-mediated delivery of a CRISPR/Cas9 cytidine base editor. *International Journal of Molecular Sciences*, ***20***(2), 402.

Walton, R. T., Christie, K. A., Whittaker, M. N. and Kleinstiver, B. P. (2020). Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. *Science*, ***368***(6488), 290–296.

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., Liu, Y. G. and Zhao, K. (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene *OsERF922*. *PLoS ONE*, ***11***(4), e0154027.

Wang, T., Zhang, H. and Zhu, H. (2019). CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. *Horticulture Research*, ***6***(1), 77.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C. and Qiu, J. L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, ***32***(9), 947–951.

Wang, Y., Liu, X., Ren, C., Zhong, G. Y., Yang, L., Li, S. and Liang, Z. (2016). Identification of genomic sites for CRISPR/Cas9-based genome editing in the *Vitis vinifera* genome. *BMC Plant Biology*, ***16***(1), 96.

Wang, Y., Zheng, X., Liu, G., Ren, C., Zhang, X., Yao, Q. and Liang, Z. (2018). Functional analysis of VvWRKY52 as a negative regulator in grapevine defense against *Botrytis cinerea*. *Plant Cell, Tissue and Organ Culture*, ***133***(1), 67–77.

Watson, A., Ghosh, S., Williams, M. J., Cuddy, W. S., Simmonds, J., Rey, M. D., Hatta, M. A. M., Hinchliffe, A., Steed, A., Reynolds, D., et al. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants*, ***4***(1), 23–29.

Xu, J., Hua, K. and Lang, Z. (2020). Genome editing for horticultural crop improvement. *Horticulture Research*, ***7***(1), 9.

Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., Volz, S. E., Joung, J., van der Oost, J., Regev, A., et al. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, ***163***(3), 759–771.

Zhang, H., Si, X., Ji, X., Fan, R., Liu, J., Chen, K., Wang, D. and Gao, C. (2018). Genome editing of upstream open reading frames enables translational control in plants. *Nature Biotechnology*, ***36***(9), 894–898.

Zhang, N., Roberts, H. M., Van Eck, J. and Martin, G. B. (2020). Generation and molecular characterization of CRISPR/Cas9-induced mutations in 63 immunity-associated genes in tomato reveals multiplexability and a low rate of off-target effects. *Plant Cell Reports*, ***39***(11), 1499–1511.

Zhang, X., Wang, L., Liu, M. and Li, D. (2019). CRISPR/Cas9 system: A powerful technology for in vivo and ex vivo gene therapy. *Science China Life Sciences*, ***62***(3), 289–314.

Zhao, G., Liu, Y., Lv, H., Han, F., Zhu, L., Zhu, B., Luo, Y. and Lim, S. H. (2022). Advances and challenges of CRISPR/Cas-based gene editing in horticultural crops. *Horticulture Research*, ***9***, uhac224.

Zhong, G. Y., Han, S., Qi, G., Zhou, Z., Zhong, G., Yi, Q. and Wang, F. (2018). CRISPR/Cpf1-based genome editing in crops. *Trends in Biotechnology*, ***36***(6), 570–572.

Zhou, J., Liu, G., Zhao, Y., Zhang, R., Tang, X., Li, L., Jia, X., Guo, Y., Wu, Y., Han, Y., et al. (2020). An efficient CRISPR-Cas12a promotion system for plant genome editing. *Nature Plants*, ***6***(3), 238–245.

Zsögön, A., Čermák, T., Naves, E. R., Notini, M. M., Edel, K. H., Weinl, S., Freschi, L., Voytas, D. F., Kudla, J. and Peres, L. E. P. (2018). De novo domestication of wild tomato using multiplex CRISPR/Cas9 genome editing. *Nature Biotechnology*, ***36***(12), 1211–1216.