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GENOME EDITING IN PLANTS

Review

Genome editing in fruit, ornamental, and industrial crops

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Abstract The advent of genome editing has opened new avenues for targeted trait enhancement in fruit, ornamental, industrial, and all specialty crops. In particular, CRISPR-based editing systems, derived from bacterial immune systems, have quickly become routinely used tools for research groups across the world seeking to edit plant genomes with a greater level of precision, higher efficiency, reduced off-target effects, and overall ease-of-use compared to ZFNs and TALENs. CRISPR systems have been applied successfully to a number of horticultural and industrial crops to enhance fruit ripening, increase stress tolerance, modify plant architecture, control the timing of flower development, and enhance the accumulation of desired metabolites, among other commercially-important traits. As editing technologies continue to advance, so too does the ability to generate improved crop varieties with non-transgenic modifications; in some crops, direct transgene-free edits have already been achieved, while in others, T-DNAs have successfully been segregated out through crossing. In addition to the potential to produce non-transgenic edited crops, and thereby circumvent regulatory impediments to the release of new, improved crop varieties, targeted gene editing can speed up trait improvement in crops with long juvenile phases, reducing inputs resulting in faster market introduction to the market. While many challenges remain regarding optimization of genome editing in ornamental, fruit, and industrial crops, the ongoing discovery of novel nucleases with niche specialties for engineering applications may form the basis for additional and potentially crop-specific editing strategies.

Keywords Genome editing · CRISPR/Cas · Targeted mutations · Crop improvement · Non-model crops · Precision breeding · Non-transgenic genome editing

Significance Statement

Improvement of fruit, ornamental, industrial, and other specialty crops traditionally requires long breeding cycles and significant resources. The innovations

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in genome editing now make it possible to rapidly and efficiently improve these crops that support society at a much larger scale. This review highlights several examples of editing demonstrated in such crops, and discusses various opportunities and challenges that remain to be addressed.

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Introduction

Transgenic approaches have dramatically improved our understanding of the underlying molecular and environmental control of desirable traits in crops. For model species like Arabidopsis and tobacco, the transformation process and associated protocols are well-documented. In non-model horticultural crops, however, the establishment of equally reliable transformation methods remains a challenge. One primary concern is transgenic approaches could result in random genomic insertions disrupting important genes and producing undesirable phenotypes. Despite its inherent challenges and its diverse applications, efficiency, and accuracy, genome editing has become an essential and powerful tool to improve different crops (Jaganathan et al. 2018).

Gene-editing approaches have been extensively documented in plants. Transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) were among the first editing technologies to be developed. In the last decade, clustered regularly interspaced short palindromic repeats (CRISPR) and associated proteins (CRISPR/Cas) have surpassed their predecessors to become the most reliable and cost-effective approach for gene-editing. First discovered for its role in the adaptive immune response of the bacteria *Streptococcus pyogenes*, the CRISPR/Cas9 system was subsequently adopted as a gene-editing technology, with its first application in plants described in 2014. The basic CRISPR/Cas system comprises a designed guide RNA that targets the Cas endonuclease to the desired location in the DNA, creating a double-strand break. The repair of the DNA, through insertions or deletions (indels), allows a change in the expression of a specific gene (Doudna and Charpentier 2014). In plants, genome editing efficiency is calculated based on the number of regenerated plants with mutations in the target gene divided by the total number of regenerated plants. The calculation of gene editing efficiency in a cultivar of a given species is essential as it provides information regarding the efficiency of the nuclease used and can serve as a guide for future experiments (Mao et al. 2019).

In commercial crops such as tomato, rice, maize, soy, carrots, barley, potato, and wheat, CRISPR editing has led to improved resistance against biotic and abiotic stress, as well as enhanced fruit and grain quality production, among other beneficial outcomes (Nekrasov et al. 2017; Wang et al. 2017b, 2019a; Ghogare et al. 2019; Ortigosa et al. 2019). The diversity of crops that have now been successfully modified, along with the variety of traits targeted, supports the broad application and relative ease-of-use of CRISPR for crop modification. This review will summarize CRISPR's use to modify three classes of horticultural crops of commercial importance; furthermore, it will discuss different traits targeted to improve a broad spectrum of fruit, ornamental, and industrial crops.

Genome editing in fruits

Fruits are a vital component of a healthy diet through their high vitamin content, antioxidants, and dietary fiber. In addition to nutritional value, fruit crops have high economic importance globally; in 2018, fruit production reached 800 million tonnes (FAOSTAT 2018). With the global population projected to reach 9 billion by 2050, the ability to produce enough food (a large portion of which will be fruit crops) to meet the projected future demands is a significant social challenge. Most fruit crops are perennial, requiring specific production methods, handling, and transportation (Radha and Mathew 2007). Many of these crops' susceptibility to biotic and abiotic stresses necessitates careful production and highlights several target traits for improvement. Approaches such as breeding, mutagenesis, interspecific hybridization, and genetic engineering have been used to produce varieties with desirable traits. In apple, natural hybridization led to improved sweetness, color, firmness, and size (Cornille et al. 2014; Kaiser et al. 2020); interspecific hybridization facilitated introduction of scab resistance (Crosby et al. 1992); mutagenesis enhanced fruit color (Joint FAO IAEA Division 1999); and genetic engineering facilitated production of non-browning apples (Xu 2013). In banana (*Musa spp.*), cucumber (*Cucumis sativus*), mandarin (*Citrus reticulata*), peach (*Prunus persica*), and strawberry (*Fragaria* × *ananassa*), conventional breeding has led to the improvement of various traits of commercial value (Bouis 2002; Moose and Mumm 2008; Omura and Shimada 2016; Feng et al. 2020). Through mutagenesis with gamma rays, higher sugar content has been achieved in elderberries (*Sambucus spp.*), self-fertility in sweet cherry (*Prunus avium*), larger bunch size and enhanced precocity in banana (*Musa spp.*) and grape (*Vitis spp.*), dwarfing in papaya (*Carica papaya*), and disease-resistance in pear (*Pyrus communis*) and strawberry (*Fragaria x ananassa*) (Joint FAO IAEA Division 1999; Mikulic-Petkovsek et al. 2016).

Most recently, transgenic technologies have been applied to improve a large number of essential traits in fruit crops. The first transgenic fruit approved was the "Flavr Savr" tomato developed by Calgene, which had an extended shelf-life. Even though "Flavr Savr" was approved by the US Food and Drug Administration (FDA) in 1994, it was withdrawn in 1997 (Klopper 2018). After Flavr Savr tomato, the virus-resistant 'Rainbow' papaya was approved for consumption by the USDA (Gonsalves 2006). Other genetically modified crops include pineapple and banana, wherein fruit quality, growth habit, and tolerance to abiotic stress were improved (Gonsalves 2006; Sreedharan et al. 2013; Igarashi et al. 2016; Yabor et al. 2020). The ringspot virus-resistant papaya, plum-pox virus-resistant plum, non-browning apple, and the pink-colored Rosé pineapple are currently among the few transgenic fruit crops approved for release and consumption in the US (Gonsalves 2006; Scorza et al. 2012; Xu 2013; Gurin et al. 2014; Young and Firoozabady 2010). Some of the first reports of precise genome editing using Zinc-finger nucleases (ZFN) was reported in apple and fig (Peer et al. 2015), followed by CRISPR/Cas9-mediated genome editing in sweet orange (Zhang et al. 2017), kiwifruit (Wang et al. 2018b), and melon (Hooghvorst et al. 2019). With the addition of gene editing to the crop engineering toolset, the development and timely release of new cultivars with enhanced traits will become increasingly achievable.

Tomato

Although efficiencies of TALEN and CRISPR/Cas9 or LbCpf1 for gene editing in tomato were comparable in the early studies (and were higher for ZFNs), the CRISPR/Cas9 system has far surpassed the others to become the preferred methodology for tomato genome editing. CRISPR/Cas9 has been used to edit over 70 different genes, including using a multiplexed guide RNA approach to target multiple genes at the same time (Table 1).

As a model fruit with a diploid genome, high-quality genome sequence (e.g. few high-frequency *k*-mers, full-length long terminal repeat retrotransposons with old average insertion ages, evenly distributed miRNA throughout the genome), high transformation efficiency, and economic importance, tomato is an ideal crop in which to test different genome editing technologies. Shortly after CRISPR was first employed in plants, its editing efficiency and accuracy were compared to TALENs in tomato. The evaluation of these editing technologies revealed the CRISPR advantage (Brooks et al. 2014; Lor et al. 2014). While both TALEN- and CRISPR/Cas9-induced mutations were found to be heritable, the efficiency of TALEN-mediated gene editing was lower than that of CRISPR/Cas9 (15% vs. 48%, respectively) (Brooks et al. 2014; Lor et al. 2014). Furthermore, the CRISPR/Cas9 approach produced homozygous mutations at a rate of approximately 3.4% (Brooks et al. 2014). Due to its greater ease of synthesis and its high efficiency, CRISPR/Cas9 has become the preferred approach for genome editing in tomato.

For both TALEN and CRISPR/Cas9 systems, the incorporation of geminivirus-based DNA replicons represents one strategy by which researchers have sought to increase double-strand break (DSB) repair frequency through homologous recombination (Čermák et al. 2015). Such replicons increase the number of DNA donor templates delivered to the plant cells, which is expected to contribute to an increased number of editing events. The process works as follows: first, the expression of the Cas9 nuclease from the viral genome induces a DSB at the target locus; next, the donor template is copied into the target site by homology-directed repair without the need for

Table 1 Summary of recent CRISPR, TALEN, and ZFN induced gene mutations in tomato and related species

Plant Species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	References
Solanum lycopersicum (Tomato)	AGL6	MADS-box transcription factor regulator of parthenocarpy	Parthenocarpy	Not available	CRISPR/Cas9	Klap et al. (2017)
	ER, SP5G, SP	Key regulators of stem length and flowering	Plant size, flowering	Not available	CRISPR/Cas9	Kwon et al. (2020)
	SlBZR2	Transcription factor regulating xylem differentiation in tomato	Xylem development	Not available	CRISPR/Cas9	Lee et al. (2019)
	SGR1, LCY-B1, LCY-B2, LCY-E, Blc (Multiplex)	Key enzymes of lycopene and β- and α-carotene biosynthesis	Lycopene content	96	CRISPR/Cas9	Li et al. (2018a, b, c)
	lncRNA1459	Long non-coding RNA involved in ripening	Fruit ripening	Not available	CRISPR/Cas9	Li et al. (2018a, b, c)
	PSY1, GABA-TP1, GABA-TP2, GABA-TP3, CAT9, SSADH (Multiplex for GABA)	Key enzymes involved in lycopene biosynthesis and γ-aminobutyric acid metabolism	Fruit color and γ-aminobutyric acid content	68	CRISPR/Cas9	Li et al. (2018a, b, c)
	CBF1	Transcriptional activator involved in cold response	Chilling tolerance	25–58	CRISPR/Cas9	Li et al. (2018a, b, c)
	NPR1	Key regulator of the salicylic acid responses	Increased drought tolerance	33–46	CRISPR/Cas9	Li et al. (2019)
	RIN	Transcription factor regulating ripening-related gene	Fruit ripening	Not available	CRISPR/Cas9	Li et al. (2020)
	MYC2	Transcription factor regulating jasmonic acid signaling	Jasmonic acid-mediated defense responses	Not available	CRISPR/Cas9	Liu et al. (2019)
	UVR8	UV-B photoreceptor regulating hypocotyl elongation, anthocyanin accumulation, and responses to UV-B stress	Increased tolerance to UV-B stress	Not available	CRISPR/Cas9	Liu et al. (2020)
	PRO	DELLA protein regulating gibberellic acid signaling	Plant size, leaf color	15	TALEN	Lor et al. (2014)
	Mlo1	Negative regulator of powdery mildew resistance	Increased resistance to powdery mildew	80	CRISPR/Cas9	Nekrasov et al. (2017)
	GAD2, GAD3	Key enzymes involved in γ-aminobutyric acid biosynthesis	γ-Aminobutyric acid content	Not available	CRISPR/Cas9	Nonaka et al. (2017)
	JAZ2	Control stomata dynamics during bacterial invasion	Resistance to *Pseudomonas syringae*	Not available	CRISPR/Cas9	Ortigosa et al. (2019)
	PDS, PIF4	A key enzyme in carotenoid biosynthesis, transcription factor negative regulating phyB-mediated red light responses	Albino explants	84	CRISPR/Cas9	Pan et al. (2016)
	HY5	Transcription factor regulating anthocyanin-related genes	Anthocyanin content	Not available	CRISPR/Cas9	Qiu et al. (2019)

Table 1 continued

Plant Species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	References
	MYB21	Transcription factor regulating fertilization and fruit initiation processes	Flower development	Not available	CRISPR/Cas9	Schubert et al. (2019)
	MYC2	Transcription factor regulating methyl jasmonate signaling pathway	Plant growth, fruit resistance to *Botrytis cinerea*	36	CRISPR/Cas9	Shu et al. (2020)
	SP5G	The key enzyme involved in controlling flowering gene	Harvest time	Not available	CRISPR/Cas9	Soyk et al. (2017)
	PROCERA	DELLA growth regulator	Control of plant height and parthenocarpy	0.2	CRISPR/Cas9	Tomlinson et al. (2019)
	IAA9	Negative regulator of parthenocarpy	Parthenocarpy	11–100	CRISPR/Cas9	Ueta et al. (2017)
	AN1, HKT1;2	Potassium transporter	Anthocyanin content and increased salinity tolerance	72	CRISPR/Cpf1-geminiviral replicon	Vu et al. (2020)
	ALS1	The key enzyme involved in the biosynthesis of branched-chain amino acids	Resistance to herbicides	71	CRISPR/Cas9	Veillet et al. (2019)
	eIF4E2	Translation initiation factor regulating plant susceptibility to RNA viruses	Decreased susceptibility to RNA virus	56	CRISPR/Cas9-NG	Veillet et al. (2020)
	MAPK3	Mitogen-activated protein kinase modulating the transcription of biotic and abiotic stress-related genes	Drought tolerance	42	CRISPR/Cas9	Wang et al. (2017a, b)
	AP2, NAC-NOR, FUL1, FUL2	Transcription factors regulating fruit ripening	Fruit ripening	Not available	CRISPR/Cas9	Wang et al. (2019a, b)
	BOP1, BOP2, BOP3, TMF	Transcriptional cofactors involved in meristem maturation	Inflorescence complexity	Not available	CRISPR/Cas9	Xu et al. (2016)
	AN2-like, MYBATV	Key regulators of anthocyanin-related genes	Anthocyanin content	Not available	CRISPR/Cas9	Yan et al. (2020)
	MET1	Enzyme regulating DNA methylation	DNA methylation leaf morphology, inflorescence, parthenocarpy	Not available	CRISPR/Cas9	Yang et al. (2019)
	MYB12	Transcription factor regulating anthocyanin accumulation	Change in fruit color	100	CRISPR/Cas9	Yang et al. (2019)
	ALC	NAC transcription factor regulating ripening	Long-shelf life	73–77	CRISPR/Cas9	Yu et al. (2017)
	lncRNA2155	Long non-coding RNA modulating the expression of ripening-related genes	Fruit ripening	Not available	CRISPR/Cas9	Yu et al. (2019a)
	MAPK3	Mitogen-activated protein kinase negative regulating thermotolerance	Increased tolerance to high temperature	Not available	CRISPR/Cas9	Yu et al. (2019b)

Table 1 continued

Plant Species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	References
	63 immunity-associated genes	Inmune-response related	Resistance to pathogens	68	CRISPR/Cas9	Zhang et al. (2020)
	PHO1;1	Phosphate transporter	Increased transport and accumulation of phosphate	Not available	CRISPR/Cas9	Zhao et al. (2019)
	ANT2	Transcription factor regulating anthocyanin-genes	Anthocyanin content	Not available	CRISPR/Cas9	Zhi et al. (2020)
	TGase	Key enzyme regulating photosynthesis by modulating cellular redox homeostasis	Impaired photosynthesis	Not available	CRISPR/Cas9	Zhong et al. (2019)
	CLV3 promoter	Signaling peptide regulating meristem development	Fruit size, inflorescence branching, plant architecture	67	CRISPR/Cas9	Rodríguez-Leal. et al. (2017)
	ACoS-AS1	Long-non coding RNA regulating carotenoid biosynthesis	Carotenoid content	Not available	CRISPR/Cas9	Xiao et al. (2020)
Solanum pennellii (Wild tomato)	ui10.1	A key regulator of pollen recognition and rejection in *Solanum*	Unilateral incompatibility	Not available	CRISPR/Cas9	Qin et al. (2018)
Physalis pruinosa (Ground cherry or husk tomato)	PprAGO7, PprSP, PprSP5G, PprCVL1	Key regulators of flowering and fruit locule number	Plant architecture, flower production, fruit size	Not available	CRISPR/Cas9	Lemmon et al. (2018)
Solanum pimpinellifolium (Wild tomato)	SP5G, SP, CLV3, WUS, GGP1 (Multiplex)	Key regulators of flowering and fruit locule number, a key enzyme involved in vitamin C biosynthesis	Shoot architecture, flower and fruit production, vitamin C content	57	CRISPR/Cas9	Li et al. (2018a, b, c)
	SP, O, FAS, FW2.2, MULT, CycB	Key regulators of flowering, fruit shape, fruit size, fruit number, a key enzyme involved in the conversion of lycopene into β-carotene	Morphology, fruit number, and size, lycopene content	Not available	CRISPR/Cas9	Zsögön et al. (2018)
Solanum lycopersicum x *Solanum pimpinellifolium*	ENO	Transcription factor regulating fruit size through the floral meristem development network	Fruit size	Not available	CRISPR/Cas9	Yuste-Lisbona et al. (2020)
Solanum pimpinellifolium (Wild tomato)	CLV3 promoter	Signaling peptide regulating meristem development	Fruit size, inflorescence branching, plant architecture	67	CRISPR/Cas9	Rodríguez-Leal. et al. (2017)

*All tomato-related reports represent Agrobacterium tumefaciens-mediated transformation stable integration of the transgene; the high copy number of donor templates leads to increased frequency of gene insertions (Čermák et al. 2015).

Comparable editing efficiencies (7.3% vs. 8.8%) were observed for TALEN and CRISPR/Cas9 when geminiviral replicons were used to overexpress a key transcription factor controlling the anthocyanin biosynthesis pathway, *Anthocyanin mutant 1* (*ANT1*), in tomato. The targeted DNA insertions and corresponding purple phenotypes induced by both systems were heritable, and no off-target edits were detected (Čermák et al. 2015).

In addition to CRISPR/Cas9, the CRISPR/LbCpf with a geminiviral-delivery system has been utilized in tomatoes (Vu et al. 2020). In this instance, targeting of *ANT1* revealed an editing efficiency of 3.5%, 2.5 times lower than that reported by Čermák et al. (2015) for CRISPR/Cas9. Lower efficiency (0.66%) was reported by the same authors when the *High K Transporter 1;2* (*HKT1;2*) allele, which codes for a potassium ion transporter, was edited to increase tomato salinity tolerance (Vu et al. 2020). The replacement of A to G resulted in the substitution of Asn by Asp. This single amino-acid change increased the potassium selectivity in the HKT1-type transporter. The homology-directed repair (HDR) allele was stably inherited as both homozygous and heterozygous T1 plants showed increased salinity tolerance up to 100 mM NaCl at the germination stage (Vu et al. 2020). Overall, these studies demonstrate reduced editing efficiency of CRISPR/Cas9 and TALEN when geminiviral replicons are used.

Tomato has been used as a model system to study various biological processes. Of these, fruit ripening is the most studied one due to its direct impact on shelf life and preventing post-harvest losses. Much molecular work has been done in efforts to improve the regulation of ripening. One of the earliest genes edited in tomato with CRISPR/Cas9 system was *Ripening inhibitor* (*RIN*), which encodes a transcription factor thought to be a master regulator of fruit ripening (Ito et al. 2015). Three different sgRNAs were designed to target specific regions of *RIN,* including the start codon, the middle area of the coding region, and the central region of the C-terminal domain, required for transcription activation. The resulting editing efficiencies (50%, 16%, and 11%, respectively) varied according to the sgRNA used (Ito et al. 2015). *RIN* mutant fruits displayed incomplete ripening, with reduced red pigmentation compared to wild type fruits at five days after the breaker stage. In two different studies, the *RIN* mutation led to decreased carotenoid and ethylene content (97% and 80%), respectively, when compared to WT (Ito et al. 2017, 2020; Li et al. 2020).

Gene-editing of additional transcription factors involved in the regulation of fruit ripening has been achieved with high efficiency. Ripening was inhibited entirely when *Alcobaca (ALC)* and *Non-Ripening-like 1* were edited with efficiencies of 77% and 31%, respectively (Yu et al. 2017; Gao et al. 2018). GC content was determined to be an essential factor in the editing efficiency of the target sequence, as low (< 45%) or high GC content (> 75%) tend to be edited with a lower frequency than sequences with intermediate GC content (Yu et al. 2017).

While targeting transcription factors has proven useful for modulating the global ripening response in tomato, other editing efforts have directly targeted individual ripening-associated traits. Genes associated with fruit color, for example, have been modified successfully, with efficiencies of up to 100%. Editing of the *MYB12* gene resulted in a shift in color from red to pink (Deng et al. 2018; Yang et al. 2019). Furthermore, *MYB12* loss-of-function led to a 99% reduction in the naringenin chalcone content (a yellow flavonoid) in the peel compared to WT (Deng et al. 2018; Yang et al. 2019). Loss-of function of the *Phytoene synthase 1* (*PSY1*) gene encodes for a key enzyme in lycopene biosynthesis, resulting in a 55% reduction in lycopene content and produced yellow-fleshed fruits (Hayut et al. 2017; D'Ambrosio et al. 2018). Similarly, loss-of-function of the *PHYTOENE DESATURASE* (*PDS)* gene, encoding the enzyme catalyzing the conversion of phytoene into lycopene, generated plants with an albino phenotype with an editing efficiency of 84% (Pan et al. 2016).

Phytonutrient content in tomatoes is another ripening-associated trait of interest that has been modulated through gene editing. Multiplex editing of the γ-aminobutyric acid (GABA) shunt involved simultaneous targeting of *Gamma Aminobutyrate Transaminase 1* to *3* (*GABA-TP1 to3*), *Cationic Amino Acid Transpoerter 9 (CAT9)*, and *Succinate Semialdehyde Dehydrogenase* (*SSADH*) (Li et al. 2018a, b, c). The GABA content in the quadruple mutant increased 19-fold in leaves and 3.5-fold in fruits relative to WT;

however, GABA accumulation corresponded to a 67.5% decrease in plant height.

CRISPR/Cas9 was used to generate point mutations in the *Acetolactate synthase 1* (*ALS1*) gene. *ALS1*-mutants display sulfonylurea herbicide resistance and can be directly selected in vitro (Danilo et al. 2019; Veillet et al. 2019). Two studies have reported efficiencies ranging from 13 to 71% in the process of editing *ALS1* (Danilo et al. 2019; Veillet et al. 2019), with the use of a cytidine base editor contributing to the higher efficiency. Cytidine base editors induce specific C-to-T base conversion, leading to C to T editing by over 80% (Veillet et al. 2019).

Despite the high editing efficiencies reported, thus far, no significant improvements to abiotic-stress tolerance have been achieved using CRISPR/Cas9 in tomato. This is generally because editing of the target genes produces undesirable side effects on development. For example, knock-out mutants of *Mitogen-Activated Protein Kinase 3* (*MAPK3*) were generated with 42% efficiency (Wang et al. 2017a); however, these mutants displayed 15% higher ion leakage than WT, indicating significant membrane damage under drought stress. Similarly, a 46% editing efficiency of the *Non-Expressor of Pathogenesis Related Gene 1* (*NPR1*), a key regulator of salicylic acid-mediated responses that also play a role in mediating abiotic-stress responses, was achieved (Li et al. 2019); however, the mutants exhibited a 6% increase in ion leakage under drought stress than WT. Editing of *CBF1*, which encodes a C-repeat binding factor involved in chilling tolerance, was achieved with an efficiency of 58% (Li et al. 2018a, b, c). Still, the *CBF1* mutants exhibited more severe chilling-injury symptoms with higher ion leakage than WT (60% vs. 20%).

Targeted mutagenesis has facilitated the recreation of the quantitative trait loci (QTL) that contributed to domestication in wild tomato (*S. pimpinellifolium*). The use of eight gRNAs targeting different regions of *Clavata 3 (CLV3) cis*-regulatory elements, which encode a signal peptide controlling meristem size, produced edits with 67% efficiency (Rodríguez-Leal et al. 2017). Flowers of the T0 CLV3-mutants made more sepals, petals, stamen, and fruit locules than WT (100%, 60%, 140%, and 400% increases, respectively) resembling quantitative traits of domesticated tomato (Rodríguez-Leal et al. 2017). Moreover, a 57% efficiency was achieved through multiplex CRISPR/Cas9 editing of essential genes associated with plant development, flowering, fruit locule number, and vitamin C biosynthesis. These targeted mutations generated plants with domesticated phenotypes, including earlier flowering, increased fruit size and locule number, and reduced vitamin C content (Li et al. 2018a, b, c).

Grape

Recent studies have demonstrated successful CRISPR/Cas9-mediated gene editing in grapes. There are no reports of TALENs or ZFNs mediated gene editing in *Vitis spp.* likely due to the inherent recalcitrance of many cultivars to transformation. With an abundance of PAM sequences adjacent to possible target coding regions, the grape genome has up to 35 million target sites available for gene editing. This makes it possible for a single guide to potentially target multiple traits simultaneously (Wang et al. 2016b). In Chardonnay, the ability of CRISPR/Cas9 to edit the *L-idonate dehydrogenase gene* (*IdnDH*), which regulates biosynthesis of tartaric acid, was tested in cell suspension culture. Gene editing efficiency was ~ 100% in the cells, a promising finding; however, the regeneration of plants from cell cultures has been a challenging process (Ren et al. 2016).

Targeting the phytoene desaturase (*PDS)* gene was reported with an editing efficiency ranging from 2 to 70% (Nakajima et al. 2017). Subsequently, gRNAs with higher GC content were used to increase editing efficiency in Chardonnay, which generated 41 grape edits with an efficiency of 86% (Ren et al. 2019).

It has been hypothesized that grape anthocyanin production is linked to Pierce Disease (PD) and Grapevine Red Blotch Virus (GRBV) resistance. However, the specific nature of this relationship is mostly undescribed. To address this hypothesis, CRISPR/Cas9 editing of *Trans-Acting Small-interfering locus 4* (*TAS4*) genes, which code for siRNAs involved in anthocyanin production, was evaluated in the grapevine rootstock 101-14. Five *MYBA7* lines with bi-allelic edits were produced, including two *TAS4b* lines, one bi-allelic, and one heterozygous line. While these events demonstrated evidence of homologous recombination between *TAS4*a with *TAS4*b genes, the transgenic plants did not display alterations in pigment accumulation. Therefore, the goal of obtaining resistance against PD and GRBV was not achieved (Sunitha and Rock 2020). In another effort to test biotic stress responses, editing of the grape transcription factor encoded by *VvWRKy52* was evaluated in *V. vinifera* cv. 'Thompson Seedless'. This resulted in 15 biallelic and seven heterozygous lines with up to 64% editing efficiency (Wang et al. 2018a). When desired changes in a phenotype are not observed post-editing, it may indicate that either there is genetic redundancy or the environment may have a significant influence on the given trait.

Apple

Gene-editing has been demonstrated in apple where the PDS gene was mutated using CRISPR/Cas9 (Nishitani et al. 2016). Additional agronomically important targets include genes associated with the timing of flowering and disease resistance. The *Terminal Flower 1 (TFL1)*, a gene involved in floral repression, has been used in several proof-of-concept experiments for different gene silencing methods, including antisense, siRNA, and virus-induced-gene silencing. In all cases, its knock-down has resulted in accelerated flowering (Charrier et al. 2019). Consistent with these results, CRISPR-mediated knock-out of *TFL1* resulted in early flowering in 93% of edited apples.

Fire blight, caused by the bacteria *Erwinia amylovora,* is one of the main problems in commercial apple orchards. CRISPR/Cas9-mediated knock-out of putative Kinase Receptor *DIPM 4* in Gala and Golden Delicious by CRISPR/Cas9 significantly reduced fire blight susceptibility. The formation of necrotic tissue following *E. amylovora* infection was reduced by 50% in the edited plants (Pompili et al. 2020).

Pear

There is currently only a single report of successful gene editing in pear (*Pyrus communis L.).* After initial application in apple (*Malus x domestica* Borkh), CRISPR/Cas9 was tailored to target the *Phytoene Desaturas*e (*PDS*) gene and *Terminal Flower* 1 (*TFL*) genes in pear. Two different sgRNAs were used, resulting in an editing efficiency of 9%. Furthermore, edited plants displayed an early flowering phenotype, demonstrating that gRNAs used in apple were effective in pear, most likely due to the target region's similarity (Charrier et al. 2019).

Strawberry

The octoploid nature of commercial strawberry cultivars makes gene editing challenging (Zhou et al. 2018). In the wild diploid species, *Fragaria vesca,* CRISPR/Cas9, and two gRNAs have been used to edit auxin biosynthesis and signaling-associated genes FveTAA1 *FveARF8.* Mutations were more abundant in the T1 progeny than in T0 and even produced a homozygous knock-out of targeted genes. As auxin is an essential phytohormone for plant growth, the resulting homozygous or biallelic mutants appeared larger and grew faster than the heterozygous progeny. Successful editing may be attributed, in part, to the use of the U6 promoter, which shows enhanced expression of the guide sequences and increases mutation rates. The combination of regulatory elements used in this study can be utilized for future gene-editing approaches in the commercial octoploid strawberry (Zhou et al. 2018).

The first octoploid strawberry edit, targeting the floral developmental regulator *FaTM6*, was made in *Fragaria x ananassa Duch. cv. Camarosa*. Editing was highly efficient, and the resulting lines were chimeric with a high degree of heterozygosity in T0; full knock-outs are expected in later generations (Martín-Pizarro et al. 2019). These experiments demonstrated that gene editing is possible in both diploid and octoploid strawberry, establishing a foundation for subsequent work. Through transient CRISPR/Cas9 expression, the R2R3 MYB transcription factor 10 *(FvMYB10*) and chalcone synthase *(FvCHS)* genes were targeted in diploid and octoploid strawberry. However, no reduction in anthocyanin accumulation was observed. Nevertheless, in the same report, stable transformation with a CRISPR plasmid targeting *PDS*, *FvUF3GT* (anthocyanidin 3-O -glucosyltransferase), *FvF3H* (naringenin,2-oxoglutarate 3-dioxygenase), and *FvLDOX* (leucoanthocyanidin dioxygenase) produced edits with up to 10% of efficiency in *F. vesca* (Xing et al. 2018). In another report, *PDS* was targeted in *Fragaria vesca* ssp. *vesca* 'Hawaii 4' and octoploid *F.* × *ananassa* 'Calypso' producing successful edits with efficiencies of 80% and 50% in diploid and octoploid strawberry, respectively. As reported earlier, the utilization of U6 promoter with multiple sgRNAs resulted in higher efficiency. The albino phenotype, resulting from *PDS* edits, showed bi-allelic variants; this finding indicates that *PDS* is functionally diploid in 'Calypso'. For polyploids, it is crucial to continue to move to subsequent filial generations as bi-allelic gene edits are sometimes observed only in later generations (Wilson et al. 2019).

Banana

Conventional breeding of banana (*Musa* spp.) is challenging and sometimes not feasible due to low genetic variability of *Musa* germplasm, polyploidy, length of the production cycle, and sterility of a majority of the economically important cultivars. For instance, approximately half of commercially-grown bananas belong to the Cavendish group, a parthenocarpic cultivar with a triploid 'AAA' genome (D'hont et al. 2012; FAO 2020). Genetic engineering and gene editing are the most suitable strategies for the genetic improvement of most cultivars. The introduction of transgenes in fruits is still not widely accepted by consumers (Voytas and Gao 2014). To date, no genetically modified banana has been released for commercial purposes (Tripathi et al. 2019b). Therefore, gene editing represents a novel avenue for the banana industry since it can introduce changes to DNA intrinsic to the target species (Shew et al. 2018).

Half a dozen recent reports have demonstrated CRISPR/Cas9-mediated gene-editing in bananas (Kaur et al. 2018, 2020; Naim et al. 2018; Tripathi et al. 2019a; Ntui et al. 2020; Shao et al. 2020). The first study was reported in the cultivar 'Rasthali' (a triploid 'AAB' genome), where the *PDS* gene was successfully edited with an efficiency of 59% (Kaur et al. 2018). Shortly thereafter, a polycistronic gRNA was used to target *PDS* in the Cavendish cultivar (Naim et al. 2018), where 63% of the transgenic plants displayed full albinism from triallelic *PDS* knockouts, and 100% showed some form of Cas9-mediated editing. Ntui et al. (2020) reported similar editing rates as Naim et al. (2018) using the 'Sukali Ndiizi' (AAB) and 'Gonja Manjaya' (AAB) cultivar and two gRNAs to disrupt the *PDS* gene. The use of a polycistronic gRNA or multiple sgRNA, along with the CRISPR/Cas9 system, has thus far been highly successful in producing gene edits in *Musa* species.

The presence of the integrated endogenous Banana Streak Virus (eBSV) in the B genome of banana was targeted using CRISPR gene editing (Tripathi et al. 2019a). Under stress, this eBSV is activated to generate infectious viral particles that negatively impact overall plant health and ultimately yield losses. Inactivation of the eBSV by editing the corresponding viral sequences resulted in disruption of transcription or/and translation. Seventy-five percent of the edited plants remained asymptomatic compared to the non-edited control plants under water stress conditions. This study also reported a high mutation efficiency (95%) using multiple gRNAs.

In addition to pathogen resistance, CRISPR editing has also been used to impart desirable developmental characteristics and phytonutrient properties in bananas. CRISPR/Cas9 was utilized to edit five isoforms of the *MaGA20ox2* gene, which is involved in gibberellin biosynthesis (Shao et al. 2020). Single gRNAs were used to target each *MaGA20ox2* isoform resulting in the production of semi-dwarf mutants. Evaluation of growth characteristics and yield of these mutants at multiple locations and over multiple years is needed to determine the potential of these edited lines. A Cavendish banana cultivar enriched in β-carotene was developed via CRISPR/Cas9-mediated targeting of lycopene epsilon-cyclase (*LCYε*) (Kaur et al. 2020). Editing resulted in multiple types of indels with an efficiency of 83%, and metabolic profiling of the fruit pulp of selected, edited lines showed enhanced accumulation of β-carotene content up to sixfold (∼ 24 µg/g). In addition to the high efficiency of target edits, no significant effect on the agro-morphological parameters was reported making this the first report in banana to improve a nutritional trait using a genome editing approach (Kaur et al. 2020).

A PEG-mediated CRISPR/Cas9 gene editing was reported in banana protoplasts (Wu et al. 2020). This system was further optimized for the successful delivery of CRISPR/Cas12a plasmids, as well as CRISPR/Cas9 ribonucleoproteins (RNPs). However, editing efficiency was relatively low (0–1% efficiency) compared to the gene-editing in stable lines.

To date, there are no reports of gene editing of *Musa* spp. with other gene-editing approaches. The CRISPR/Cas9 gene-editing system is well-established in banana, at least for knock out of specific genes by non-homologous end joining (NHEJ); however, other more complicated applications of CRISPR gene editing by homologous recombination have not been reported. This is probably due to the lingering difficulty of achieving efficient and precise gene editing by homologous recombination, for which the efficiencies are often below the 1% range (Ayar et al. 2013; Wang et al. 2015; Watanabe et al. 2016; Wolter et al. 2018).

Cacao

Similar to banana, commercially cultivated cacao is derived from a narrow genetic diversity, and many cultivars are susceptible to various diseases (Irish et al. 2010). CRISPR gene-editing presents an avenue to breed disease-resistant cacao varieties and also to broaden the genetic pool. So far, only one study has been conducted in *Theobroma cacao* (Fister et al. 2018). A transient transformation approach was used to show the efficacy of CRISPR/Cas9 in detached leaf tissue and to evaluate the resistance against *Phytophthora tropicalis* via an in vitro pathogen bioassay targeting *TcNPR3*, a regulator of cacao's defense response. Editing of this gene was achieved with an efficiency of ~ 27% and resulted in enhanced resistance to *P. tropicalis*. Stable transformation of somatic embryos was also attempted; however, the use of embryos generated chimeras. Further studies need to be performed to demonstrate stable genome editing of *T. cacao* and improve the efficiency of CRISPR/Cas9 (Fister et al. 2018).

Citrus

Citrus canker, caused by *Xanthomonas citri* (Xcc), is one of the most commercially-impactful diseases of citrus (Gottwald et al. 2002). The *Citrus sinensis Lateral Organ Boundary* gene (*CsLOB1*) is a susceptibility gene for citrus canker disease (Hu et al. 2014); its promoter contains an effector binding element (EBE_{Pth4A}), which is recognized by the main transcription activator-like (TAL) effector PthA4 of Xcc. TAL PthA4 binds the EBE_{Pth4A} domain and activates *CsLOB1* to promote disease development (Jia et al. 2017; Peng et al. 2017). In grapefruit (*Citrus paradisi*), pumelo (*Citrus maxima*), and Wannjinchenng orange (*Citrus sinensis*), the coding region and EBE_{Pht4A} promoter regions of *CSLOB1* have been mutated via CRISPR in efforts to enhance resistance to citrus canker disease (Jia et al. 2016, 2017, 2019; Peng et al. 2017; Jia and Wang 2020). Mono-allelic, biallelic (homozygous and heterozygous), and/or chimeric mutations were reported in all the above studies, and the corresponding mutation rates were assessed using next-generation sequencing. A direct correlation between plant resistance and the ratio of mutated cells was proposed (Jia et al. 2016, 2017, 2019; Peng et al. 2017; Jia and Wang 2020). Conclusions from these studies, however, are constrained by different experimental limitations. There was no evidence that mutations remained stable once the plants reached maturity. Importantly, no details were provided in any of these reports, or references therein, to confirm the effectiveness of the genetic transformation based on epicotyl explants. Thus, effective canker resistance in mature, field-grown plants remains to be demonstrated.

Furthermore, multiplex genome editing proof-of-concept experiments have been conducted in citrus using Csy-4 and tRNA-based approaches. The latter has been shown to be more efficient for obtaining biallelic and homozygous mutants (44.4% mutation rate) (Huang et al. 2020).

Plant immunity-associated *WRKY22* transcription factor has also been targeted in citrus to impart canker resistance, as its expression level is inversely correlated with the disease (Zhou et al. 2017). Wanjincheng orange has two Cs*WRKY22* alleles ($CsWRKY22^{G}$ and $CsWRKY22^{-}$), the ratio of which is 2:1. The first exon of the *CsWRKY22* alleles was mutated using CRISPR/Cas9. Consequently, reduced disease symptoms were observed in the leaves of two mutant plants, although mutations were only statistically significant in $CsWRKY22^{G}$ (Wang et al. 2019b).

Cucurbits

Cucumber is one of several crops that are particularly susceptible to major viruses including potyviruses and ipomoviruses. These viruses require plant host factors to maintain their virulence cycle. For this to occur, viruses associate with the eukaryotic translation initiation factor complex (eIF4F) host factors (including eIF4e, eIF4G, and eIF4A) through the viral-encoded protein. The host factors bind to the viral RNA 5′, a process that is essential for the translation of the virus. One way of preventing this association is by inducing mutations to the host factors (Chandrasekaran et al. 2016). In cucumber, CRISPR/Cas9 was used to introduce indels and SNPs in *eIF4E*. The resulting mutant plants exhibited resistance to two potyviruses (Zucchini yellow mosaic virus [ZWMV] and Papaya ringspot mosaic virus *W* [PRSV-W], and one ipomovirus (Cucumber vein yellowing virus [CVYN]). In the case of CVYC, total resistance was demonstrated, whereas, for ZYMV and PRSV-W, the mutant plants exhibited mild symptoms, and their development was not compromised.

In addition to virus resistance, traits associated with flower development have also been targeted. Wild-type cucumbers bear both male and female flowers. Gynoecious cucumber bearing hermaphroditic and female flowers was obtained through indel mutations in the *CmWIP1* transcription factor, which is involved in the inhibition of carpel development in melon and cucumber. The edited plants bore seven times more female flowers than wild-type. Such a flowering phenotype would eliminate the need for manual emasculation procedures used in hybrid production and reduce labor costs (Hu et al. 2017).

PDS was successfully mutated in watermelon in proof-of-concept experiments using CRISPR/Cas9 (Tian et al. 2017). Weeds are a severe threat to watermelon production because of their short canopy. Mutations of the acetolactate synthase (*ALS*) gene impart herbicide resistance in non-cultivated plant species, such as *Amaranthus hybridus* (Yu and Powles 2014). Subsequently, CRISPR-mediated C to T base-editing was achieved in Pro190 (CCG) of the watermelon *ALS* gene (Yu and Powles 2014; Tian et al. 2018). Genome edited, and wild type plants were treated with the recommended levels of tribenuron (17 g ai/ha), a sulfonylurea herbicide for broad weed control. The yield of edited plants was not compromised, whereas all wild-type plants were severely damaged 14 days after the treatment (Tian et al. 2018) (Table 2).

Ornamental crops

Ornamental crops, such as roses, chrysanthemums, orchids, and tulips, are economically important, with a combined worldwide market of over a billion dollars (Chandler and Brugliera 2011). As ornamental plants are mainly cultivated for aesthetic purposes, there is a high demand for new traits associated with desirable plant architecture, smell, and color. Traditionally, techniques such as hybridization breeding and mutation breeding were used to achieve such traits (Xiong et al. 2015). However, the long time required for breeding, difficulty in attaining homozygous mutation in polyploids, lack of precision, and low frequency in obtaining desired results necessitate implementing other technologies, such as transgenic and genome editing technologies.

The availability of genome sequences for major ornamental crops such as *Chrysanthemum nankingense, Ipomoea nil, Rosa chinensis, Dendrobium officinale,* and *Petunia hybrid*, along with classic transgenic technologies (gene overexpression and silencing), has led to the production of several new varieties. Examples include an orange pigment-producing petunia, developed via the introduction of a gene encoding dihydroflavonol reductase from maize, or an early flowering chrysanthemum and orchid, produced by introducing genes from the MADS-box gene family (Shulga et al. 2011; Sawettalake et al. 2017). The development of genome editing technologies, such as ZFN, TALENs, and CRISPR/Cas9, has opened a new avenue for their rapid improvement. These approaches also enable an in-depth understanding of the metabolic function of annotated genes in recently sequenced and assembled genomes. Furthermore, the introduction of transgenes is less of an issue in ornamental plants than it is for food crops, and it is expected that the public acceptance rate might be higher (Nishihara and Nakatsuka 2011).

CRISPR/Cas9 has recently been implemented to develop desired traits in ornamental crops; however, there are no previous reports of the use of ZFNs and TALENs for genome editing. In *Lotus japonicas* symbiosis receptor-like kinase and three hemoglobin genes were targeted, responsible for nodule formation and reduction of nodule free oxygen concentration in the presence of rhizobia, effectively enhancing symbiotic nitrogen fixation. A mutation rate of 35% was obtained for gRNA targeting the symbiosis receptor-like kinase gene and 28.5% for the leghemoglobin genes (Wang et al. 2016a). Recently, transgenic *Chrysanthemum morifolium* with a multicopy GFP gene was targeted using CRISPR/Cas9 with different promoters to establish a gene-editing method in chrysanthemum. It was observed that the ubiquitin promoter from *Petriselinum crispum* was more effective at inducing mutations in chrysanthemum, and therefore producing more significant decreases in fluorescence, compared to CaMV35s (Kishi-Kaboshi et al. 2017). In another study, CRISPR/Cas9-mediated genome editing was established in the orchid species *Dendrobium officinale*. In *D. officinale,* an abundance of lignocellulose limits the use of this variety in

Table 2 Summary of recent studies of CRISPR, TALEN, and ZFN induced gene mutations in fruit crops

Plant species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
Actinidia deliciosa (kiwifruit)	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	7.14–91.67%	CRISPR/Cas9	Agrobacterium-mediated transformation	Wang et al. (2018a, b)
Cucumis sativus (cucumber)	Eukaryotic translation initiation factor 4E (eIF4E)	Plant immunity	Total resistance to Cucumber vein yellowing virus. Partial resistance to Zucchini yellow mosaic virus and Papaya ringspot mosaic virus-W	20%	CRISPR/Cas9	Agrobacterium-mediated transformation	Chandrasekaran et al. (2016))
Cucumis sativus (cucumber)	Transcription factor WIP1	Carpel development	Gynoecious	64.30%	CRISPR/Cas9	Agrobacterium-mediated transformation	Hu et al. (2017)
Cucumis melo (melon)	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	42–45%	CRISPR/Cas9	Agrobacterium-mediated transformation	Hooghvorst, López-Cristoffanini, and Nogués (2019)
Citrullus lanatus (watermelon)	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	100%	CRISPR/Cas9	Agrobacterium-mediated transformation	Tian et al. (2017)
	Acetolactate synthase (ALS)	Amino acid biosynthesis	Herbicide resistance	23%	CRISPR/Cas9	Agrobacterium-mediated transformation	Tian et al. (2018)
Citrus sinensis (sweet orange)	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	75%	CRISPR/Cas9	Agrobacterium-mediated transformation	Zhang et al. (2017)
	Transcription factor WRKY22	Plant immunity	Partial resistance to citrus canker	68.2–85.7%	CRISPR/Cas9	Agrobacterium-mediated transformation	Wang et al. (2019b)
	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	3.2–3.9%	CRISPR/Cas9	Agrobacterium-mediated transformation	Jia and Wang (2014)
	Lateral Organ Boundaries promoter (LOB1)	Plant immunity	Partial resistance to citrus canker	42%	CRISPR/Cas9	Agrobacterium-mediated transformation	Peng et al. (2017)

Table 2 continued

Plant species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
Citrus ×paradise (grapefruit)	Lateral Organ Boundaries promoter (LOB1)	Plant immunity	Partial resistance to citrus canker	15.63–81.25%	CRISPR/Cas9	Agrobacterium-mediated transformation	Jia et al. (2016)
	Lateral Organ Boundaries promoter (LOB1)	Plant immunity	Partial resistance to citrus canker	88.79–89.36%	CRISPR/Cas9	Agrobacterium-mediated transformation	Jia et al. (2017)
Fragaria vesca (wild strawberry)	FveTAA1	auxin biosynthesis and signaling	dwarf plants	NA	CRISPR/Cas9	Agrobacterium-mediated transformation	Zhou et al. (2018)
	FveARF8	auxin biosynthesis and signaling	dwarf plants	NA	CRISPR/Cas9	Agrobacterium-mediated transformation	
	FvMYB10	MYB transcription factor	Anthocyanin synthesis affected	10	CRISPR/Cas9	Agrobacterium-mediated transformation	Xing et al. (2018)
	FvCHS	chalcone synthase	Anthocyanin synthesis affected	NA	CRISPR/Cas9	Agrobacterium-mediated transformation	
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino explants	10	CRISPR/Cas9	Agrobacterium-mediated transformation	
	FvUF3GT	anthocyanidin 3-O -glucosyltransferase	Anthocyanin synthesis affected	10	CRISPR/Cas9	Agrobacterium-mediated transformation	
		naringenin,2-oxoglutarate 3-dioxygenase	Anthocyanin synthesis affected	10	CRISPR/Cas9	Agrobacterium-mediated transformation	
	FvLDOX	leucoanthocyanidin dioxygenase	Anthocyanin synthesis affected	10	CRISPR/Cas9	Agrobacterium-mediated transformation	
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	80	CRISPR/Cas9	Agrobacterium-mediated transformation	Wilson et al. (2019)

Table 2 continued

Plant species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
*Fragaria*cv. × *ananassa*(strawberry)	*FaTM6*	anther development and petal formation in strawberry	lack of flowers	NA	CRISPR/Cas9	Agrobacterium-mediated transformation	Martín-Pizarro, Triviño, and Posé (2019)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	50	CRISPR/Cas9	Agrobacterium-mediated transformation	Wilson et al. (2019)
Malus × *domestica*(apple)	Kinase receptor MdDIPM4	Plant immunity	Partial resistance to fire blight	73.3–77.8%	CRISPR/Cas9	Agrobacterium-mediated transformation	Pompili et al. (2020)
	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	84–90%	CRISPR/Cas9	Agrobacterium-mediated transformation	Charrier et al. (2019)
	Terminal Flower 1 (TFL)	Flower repressor	Early flowering			Agrobacterium-mediated transformation	
	B-glucuronidase (gus A)	GUS expression	Marker gene expression	10–40%	ZFN	Agrobacterium-mediated transformation	Peer et al. (2015)

Table 2 continued

Plant species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
Malus prunifolia × *Malus pumila* (apple)	Phytoene desaturase (PDS)	Carotenoid biosynthesis	Albino	13.60%	CRISPR/Cas9	Agrobacterium-mediated transformation	Nishitani et al. (2016)
Musa spp (Banana)	eBSV	Endogenous virus	Inactivation of eBSV into infectious viral particles	70–95	CRISPR/Cas9	Agrobacterium-mediated transformation	Tripathi et al. (2019a, b)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	59	CRISPR/Cas9	Agrobacterium-mediated transformation	Kaur et al. (2018)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	100	CRISPR/Cas9	Agrobacterium-mediated transformation	Naim et al. (2018)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	100	CRISPR/Cas9	Agrobacterium-mediated transformation	Ntui et al. (2020)
	MaGA20ox2	Participates in the biosynthesis of gibberellin	semi-dwarf plants	Not reported	CRISPR/Cas9	Agrobacterium-mediated transformation	Shao et al. (2020)
	LCYε	A key enzyme in the biosynthesis of β-carotene	β-carotene-enriched fruits	83	CRISPR/Cas9	Agrobacterium-mediated transformation	Kaur et al. (2020)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	0–1	CRISPR/Cas9, Cas12, CRISPR/Cas9-RNPs	Protoplast-PEG-mediated transformation	Wu et al. (2020)
Pyrus communis L	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino explants	9	CRISPR/Cas9	Agrobacterium-mediated transformation	Charrier et al. (2019)
	TFL	terminal flower development	early flowering	9	CRISPR/Cas9	Agrobacterium-mediated transformation	

Table 2 continued

Plant species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
Vitis spp (grape)	IdnDH	Regulates the biosynthesis of tartaric Acid	Estable accumulation of tartaric acid	~ 100	CRISPR/Cas9	Protoplast-PEG-mediated transformation	Ren et al. (2016)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	2 to 70	CRISPR/Cas9	Agrobacterium-mediated transformation	Nakajima et al. (2017)
	PDS	A key enzyme of the chlorophyll and carotenoid synthesis	Albino	86	CRISPR/Cas9	Agrobacterium-mediated transformation	Ren et al. (2019)
	TAS4	Trans-Acting Small-interfering locus4	anthocyanin production related to disease resistance against Pierce disease (PD) and Grapevine Red Blotch Virus (GRBV	Not reported	CRISPR/Cas9	Agrobacterium-mediated transformation	Sunitha and Rock (2020)
	VvWRKy52	WrKy transcription factors	biotic stress response	64	CRISPR/Cas9	Agrobacterium-mediated transformation	Wang et al. (2018a, b)

natural health products. Targeting of five genes associated with lignocellulose biosynthesis—coumarate 3-hydroxylase, cinnamate 4-hydroxylase, coumarate-coenzyme A ligase, cinnamoyl coenzyme A reductase, and irregular xylem 5—were targeted using multiple gRNAs, and mutation rates of 10–100% were achieved in different loci of these genes (Kui et al. 2017). Recently, in another orchid, *Phalaenopsis equestris,* multiple mutants for MADS genes (MADS8, MADS36, and MADS44) were obtained using CRISPR/Cas9. The triple mutants were achieved at a high rate of 97.9%; single and double mutants were also obtained to understand the effect of different gene mutations on floral development (Tong et al. 2020).

CRISPR/Cas9 genome editing has been demonstrated in *Lilium pumilum* DC, *Lilium pumilum* Fisch, and *Lilium lonfiflorum* by targeting the *PDS* gene. Mutation rates of 69.57% and 63.64% were observed and in Fisch and White Heaven cultivars, respectively (Yan et al. 2019). As mentioned earlier, one of the desired traits for ornamental crops is flower color. Recently, a gene associated with flower color was targeted in *Torenia fournieri L.* Modification of flavanone-3-hydroxylase, which encodes for the main enzyme in the flavonoid biosynthesis pathway, was successfully edited to produce a pale-purple to almost-white flower color in 80% of all regenerated transgenic lines (Nishihara et al. 2018).

In petunia, an ornamental model crop, the endogenous nitrate reductase gene was edited using protoplasts (Subburaj et al. 2016a). The nitrate reductase enzyme is involved in nitrate uptake and assimilation, and loss-of-function mutants cannot assimilate nitrate efficiently (Subburaj et al. 2016b). In addition to nitrate reductase, the knock-out of *PDS* has also been demonstrated in petunia at 55.6–87.5% efficiency (Zhang et al. 2016). Self-incompatibility in petunia is regulated by polymorphic S-locus-comprising multiple pollen-specific genes (*SLF*), which are specifically expressed in late pollen developmental stages, and a single pistil-specific gene, which expresses in the pistil during fruit set. Each SLF protein is assembled into an Skp1-Cullin1-F-box (SCF) E3 ubiquitin ligase complex. The Cullin1 subunit (PiCUL1-P) and the Skp1 subunit (PiSSK1) in SCFSLF complexes are pollen-specific. PiSSK1 is involved in self-incompatibility, but its specific role remains to be elucidated. By inducing frame-shift indel mutations in the Skp1 subunit PiSSK1, it was established that this subunit is indispensable in the assembly of SCFSLF complexes because there was no fruit set in crosses between compatible plants and knock-out mutant lines (Sun and Kao 2018). Petunia flower longevity was also extended by knocking out 1-aminocyclopropane-1-carboxylate oxidase 1 (*ACO*). ACO encodes for the last enzyme in ethylene biosynthesis, and its partial knock-out caused a significant reduction in ethylene production in petals and pistils. As a result, flower longevity was extended from 6 to 10 days in the mutants (Xu et al. 2020).

In *Ipomoea nil* (Japanese morning glory), CRISPR/Cas9 was used to target flower longevity and color. NAC is a family of transcription factors involved in regulating hormone signaling to affect plant immunity (Yuan et al. 2019). One such transcription factor, Ephemeral 1 (EPH1), was knocked out in *I. nil* by CRISPR/Cas9. The mutations occurred at single or multiple target sites and involved insertions and/or deletions of one or more bases at the target sites resulting in an extension of senescence by up to 10.8 h, an increase in flower longevity of approximately 84% over that of wild type (Watanabe et al. 2017). Carotenoid cleavage dioxygenase 4 (*In*CCD4) cleaves carotenoids in petals during floral tissue development in *I. nil* (Watanabe et al. 2018). CRISPR/Cas9 InCCD4 knock-outs resulted in yellow midribs in flower petals. CRISPR/Cas9 null mutations in dihydroflavonol-4-reductase-B (DFR-B) impacted anthocyanin biosynthesis as expected, resulting in stems and leaves with no anthocyanins and in white, violet, and pale-violet, flowers (Watanabe et al. 2017) (Table 3).

Industrial crops

Industrial crops are primarily non-food crops grown for various raw materials they produce for industry or manufacturing. Exceptional biomass, lipids, cellulose, hydrocarbons, or metabolite accumulation are hallmarks of these crops. While there are industrial uses for the by-products of various crops, e.g., wheat straw as construction material (Bouasker et al. 2014), discussion within this review has been limited to non-food crops (Table 4).

Table 3 Summary of recent studies reporting genome editing in ornamental crops

Plant species	Target gene	Function of the encoded product	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
Lotus japonicus	Leghemoglobin loci (LjLb1, LjLb2, LjLb3), Symbiosis receptor-like kinase (LjSYMRK)	Symbiosis receptor kinase and Leghemoglobin genes	Nodule formation in the presence of rhizobia and establishing low free oxygen concentration in nodule	35	CRISPR/Cas9	Agrobacterium-mediated	Wang et al. (2016a, b)
Chrysanthemum morifolium	Yellowish green fluorescent protein (CpYGFP)	Fluorescence	Decrease in fluorescence	0–33.3	CRISPR/Cas9	Agrobacterium-mediated	Kishi-Kaboshi, Aida, and Sasaki (2017)
Dendrobium officinale	Coumarate 3-hydroxylase (C3H), Cinnamate 4-hydroxylase (C4H), 4-Coumarate:coenzyme A ligase (4CL), Cinnamoyl coenzyme A reductase (CCR), Cinnamoyl coenzyme A irregular xylem5 (IRX)	Lignocellulose biosynthesis	N/A	10–100	CRISPR/Cas9	Agrobacterium-mediated	Kui et al. (2017)
Lilium longiflorum, Lilium pumilum	Phytoene desaturase (LpPDS)	Chlorophyll biosynthesis	Albino	69.57 and 63.64	CRISPR/Cas9	Agrobacterium-mediated	Yan et al. (2019)
Phalaenopsis equestris	MADS genes (MADS44, MADS36 and MADS8)	Floral initiation and development	Long juvenile period	97.9	CRISPR/Cas9	Agrobacterium-mediated	Tong et al. (2020)
Torenia fournieri	Flavanone 3-hydroxylase (F3H)	Flavonoid biosynthesis	Pale blue flowers	80	CRISPR/Cas9	Agrobacterium-mediated	Nishihara et al. (2018)

Table 3 continued

Plant species	Target gene	Function of the encoded product	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	References
Petunia × *hybrid* (petunia)	Nitrate reductase (*NR*)	Nitrogen metabolism	Deficiency in nitrate assimilation	5.3–17.8%	CRISPR/Cas9	Protoplast transformation	Subburaj et al. (2016a, b)b
	Phytoene desaturase (*PDS*)	Carotenoid biosynthesis	Albino	55.6%–87.5%	CRISPR/Cas9	*Agrobacterium*-mediated transformation	B. Zhang et al. (2016)
	Skp1 subunit (*PiSSK1*)	Self-incompatibility	Self-incompatibility	Not determined	CRISPR/Cas9	*Agrobacterium*-mediated transformation	Sun and Kao (2018)
	1-aminocyclopropane-1- carboxylate oxidase1 (ACO)	Ethylene biosynthesis	Flower longevity	31.50%	CRISPR/Cas9	*Agrobacterium*-mediated transformation	J. Xu et al. (2020)
Ipomoea nil (Japanese morning glory)	Ephemeral 1 (EPH1)	Regulation of petal senescence	Flower longevity	100%	CRISPR/Cas9	*Agrobacterium*-mediated transformation	Shibuya et al. (2018)
	Carotenoid cleavage dioxygenase 4 (CCD4)	cleaves the 9, 10 double bond of C40-carotenoid	Flower color	55%	CRISPR/Cas9	*Agrobacterium*-mediated transformation	Watanabe et al. (2017)
	Dihydrofavonol4-reductase-B (DFR-B)	anthocyanin biosynthesis	Flower color	75%	CRISPR/Cas9	*Agrobacterium*-mediated transformation	Watanabe et al. (2018)

Table 4 Table summarizing literature reports of CRISPR-, TALEN-, and ZFN-induced gene mutations in industrial crops

Plant Species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	Reference
Microalgae	*Cre01.g000300*	Lipid catabolism	Enhanced Lipid productivity	13.7	CRISPR-Cas9	RNP Particle Bombardment	Nguyen et al. (2020)
	AGPL	Starch synthesis	Enhanced Lipid productivity	1.01	TALEN	Particle Bombardment	Takahashi et al. (2018)
Brassica napus (Rapeseed)	*BnALC*	Valve margin development	Shatter-resistant seeds	100	CRISPR-Cas9		Braatz et al. (2017)
	orf125	Cytoplasmic male sterility	Ferttile plants	100	TALEN	Agrobacterium	Kazama et al. (2019)
Brassica rapa (Field Mustard)	*Bra003491*	Pectin synthesis	NA	56	CRISPR-Cas9	Agrobacterium	Xiong et al. (2019)
	Bra007665			20			
	Bra014410			20			
Medicago sativa (Alfalfa)	*MsSGR*	Senescence	Delay of senescence	75	CRISPR-Cas9	Agrobacterium	Wolabu et al. (2020)
Jatropha curcas (Jatropha)	*JcCYP735A*			ND	CRISPR-Cas9	Agrobacterium	Cai et al. (2018)
	JcIPT (1–6)						
	JcCKX	Cytokinin synthesis	‘Decreased cytokinin synthesis				
Eucalyptus globulus (Eucalyptus)	*CCR1*	Lignin biosynthesis	Decreased lignification	100	CRISPR-Cas9	Agrobacterium	Dai et al. (2020)
	IAA9A	Auxin regulation	Decreased lignification	92.3			
Elaeis guineensis (Oil Palm)	*EgEMLP*	Disease resistance	*Ganoderma boninense* resistance	100	CRISPR-Cas9	Agrobacterium	Budiani et al. (2019)
Populus tomentosa (Poplar)	*PtoPDS*	Carotenoid biosynthesis	Albino plants	89	CRISPR-Cas9	Agrobacterium	Fan et al. (2015)
	LEAFY	Fertility	NA	2	ZFN	Heat shock	Lu et al. (2016)
	AGAMOUS		NA				

Table 4 continued

Plant Species (common names)	Target genes	Target pathway or trait	Phenotype	Genome editing efficiency (%)	Genome editing system	Transformation method	Reference
Panicum virgatum (Switchgrass)	*Pv4CL1, Pv4CL2, Pv4CL3*	Ligning synthesis	Decreased lignification	10	CRISPR-Cas9	Agrobacterium	Park et al. (2017)
	Tb1a	Plant architecture	Increased tillering	95.5, 11	CRISPR-Cas9	Agrobacterium	Liu et al. (2018)
	Tb1b		Increased tillering				
Gossypium hirsutum L. (Cotton)	*GhMYB25-like A*	Fiber development	NA	100, 98.8	CRISPR-Cas9	Agrobacterium	Li et al. (2017)
	GhMYB25-like D		NA				
Papaver somniferum (Opium Poppy)	*4′OMT2*	Benzylisoquinoline alkaloids (BIA) synthesis	Reduced BIA synthesis	85	CRISPR-Cas9	Agrobacterium	Alagoz et al. (2016)
Salvia miltiorrhiza (Red Sage)	*SmCPS1*	Tanshinone biosynthesis	Reduced tanshinone synthesis	11.5	CRISPR-Cas9	Agrobacterium	Li et al. (2017)
Hevea brasiliensis (Rubber Tree)	*HbFT1, HbFT2, HbTFL1-1, HbTFL1-2 HbTFL1-3*	Floral development	NA (protoplast edit)	5.56, 3.74, 4.29, 20.11 10.32	CRISPR-Cas9	Agrobacterium	Fan et al. (2020)
Taraxacum kok-saghyz (Rubber Dandelion)	*1-FFT*	Rubber synthesis	Increased alkaloid accumulation	80	CRISPR-Cas9	Agrobacterium	Iaffaldano et al. (2016)
Dendrocalamus latiflorus Munro (Bamboo)	*DlmPSY1-A, DlmPSY1-B DlmPSY1-C*	Phytoene synthesis	Albino phenotype	2.1	CRISPR	Polyethylene glycol	Ye et al. (2020)

Switchgrass

Switchgrass, a self-infertile allotetraploid (2n = 4x = 36) grass, is an ideal candidate for genetic engineering and gene function modification studies, as such experiments are difficult through conventional trait modification techniques. Genetic engineering projects in switchgrass target various traits, including ease of cell wall conversion to biofuels, plant architecture, and plant domestication (Nageswara-Rao et al. 2013).

To address recalcitrance to cell wall degradation during the fermentation process, editing was performed on one of the three genes coding for 4-Coumarate: coenzyme A ligase to disrupt monolignol's early stages biosynthesis. The *Pv4CL1* gene was selected as the target due to its preferential expression in lignified stem tissue. Mutation efficiencies of 10% were observed, along with reduced cell wall thickness and reduced total lignin content by 8–30%. (Park et al. 2017). Recently, successful CRISPR/Cas9 induced mutations were reported in *teosinte branched 1* (*tb1*) *a* and *b,* conferring increased tiller production and more desirable plant architecture at frequencies of 95.5% and 11%, respectively (Liu et al. 2018). Lignocellulosic crops like switchgrass are expected to become essential biofuel crops in the future (Ghadge et al. 2020). Lignin biosynthesis regulatory networks have been proposed, and genes associated with plant architecture identified, providing putative targets for future editing and trait improvement studies (Bahri et al. 2018; Rao et al. 2019).

Rubber tree

The Brazilian rubber tree (*Hevea brasiliensis* Muell. Arg.) is the only commercially available natural rubber source and, therefore, an exceptionally economically important crop. In 2016, the gross production value of natural rubber was over $18.5 billion (FAOSTAT 2016). Long juvenility in *Havea* spp. generally dictates breeding cycles of 25–30 years; therefore, contemporary rubber tree breeding programs are in their third or fourth-generation cycle (Souza et al. 2019). Modulation of the juvenile/reproductive cycle would be advantageous for breeders of rubber tree as it could allow for quicker selection of progeny. Recently, targeted edits were achieved in two gene subfamilies experimentally proven to modulate flowering time in higher plants. *FT* subfamily genes responsible for floral initiation (*HbFT1* and *HbFT2*) and *TFL1* subfamily genes responsible for floral suppression (*HbTFL1-1*, *HbTFL1-2,* and *HbTFL1-3*) were targeted using CRISPR/Cas9 ribonucleoprotein in rubber tree protoplast. Editing efficiencies of 5.56, 3.74, 4.29, 20.11, and 10.32% were observed in target sequences of *HbFT1, HbFT2, HbTFL1-1*, *HbTFL1-2,* and *HbTFL1-3,* respectively, with sgRNAs or multiple sgRNA combinations (Fan et al. 2020). Also, resistance to *Microcyclus ulei, the* causal agent of South American Leaf Blight, has been a breeding objective in *H. brasiliensis* for decades that could be rapidly achieved using genome editing (Van Beilen and Poirier 2007).

Dandelion

Despite its commercial popularity, the Brazilian rubber tree is vulnerable to factors that may impact its future viability. (Cornish 2017; Hafiz et al. 2018). Alternative natural rubber sources are being sought in anticipation of projected declines in Brazilian rubber tree production. One such source, Rubber Dandelion (*Taraxacum kok-saghyz*), has been the recipient of recent CRISPR/cas9 gene editing experiments. Rubber production and storage in dandelion roots is highly variable; therefore, a mechanism for normalizing rubber production is desirable (Cornish and Wenshuang 2012; Iaffaldano et al. 2016). The fructan 1-fructosyltransferase (*1-FFT*) gene was knocked out using editing, reducing the biosynthesis of the rubber production antagonist inulin with efficiencies as high as 80% (Iaffaldano et al. 2016). Evaluation of rubber synthesis was not conducted, as a deleterious hairy-root phenotype was imparted upon the regenerated dandelion plantlets as a consequence of transformation with *Agrobacterium rhizogenes.*

Hemp

Before the 1930s, Hemp (*Cannabis sativa*) was a predominant industrial crop with various commercial applications, including pulp and paper, building materials, textiles, cosmetics, food products, and nutritional supplements. There is emerging interest in its use as biomass fuel, seeds, and oil for human health, and its use as a wood alternative (Pal and Lucia 2019). However, there is no peer-reviewed study exploring the use of ZFN, TALEN, or CRISPR/Cas systems for genome editing in Cannabis. Given the economic importance and the sustainability potential, proposed changes in the regulatory landscape, availability of genetic engineering protocols, and genomic resources, this crop is primed for gene editing applications (van Bakel et al. 2011; Cherney and Small 2016; Deguchi et al. 2020).

Conclusions and future directions

The utility and feasibility of using CRISPR/Cas9 to study gene function and achieve desirable traits in horticulturally important crops have been demonstrated in fruits, ornamental, and industrial crops. Most horticultural crops are recalcitrant to in vitro manipulation; therefore, as advances are made in the regeneration of such crops, targeted gene editing via CRISPR/Cas9 will enable rapid trait improvement overcoming long breeding cycles. It is encouraging to note that the use of CRISPR/Cas9 combined with viral vectors or growth regulators has allowed the production of edited plants without the need for laborious tissue culture approaches (Ma et al. 2020; Maher et al. 2020). Unlike row crops, fruits, ornamental and industrial crops contribute much more to society than just subsistence. The application of targeted editing of genes that regulate desirable traits related to nutrition, novel organoleptic properties, and novel industrial uses can help develop value-added crops that provide food, feed, fiber, and fuel in abundance for the burgeoning population.

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