

Review

Improving Horticultural Crops via CRISPR/Cas9: Current Successes and Prospects

Bed Prakash Bhatta ^{1,2}  and Subas Malla ^{2,*}

¹ Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA; bhatabp18@tamu.edu

² Texas A&M AgriLife Research and Extension Center, Uvalde, TX 78801, USA

* Correspondence: subas.malla@ag.tamu.edu; Tel.: +1-830-278-9151

Received: 9 September 2020; Accepted: 12 October 2020; Published: 14 October 2020



Abstract: Horticultural crops include a diverse array of crops comprising fruits, vegetables, nuts, flowers, aromatic and medicinal plants. They provide nutritional, medicinal, and aesthetic benefits to mankind. However, these crops undergo many biotic (e.g., diseases, pests) and abiotic stresses (e.g., drought, salinity). Conventional breeding strategies to improve traits in crops involve the use of a series of backcrossing and selection for introgression of a beneficial trait into elite germplasm, which is time and resource consuming. Recent new plant breeding tools such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein-9 (Cas9) technique have the potential to be rapid, cost-effective, and precise tools for crop improvement. In this review article, we explore the CRISPR/Cas9 technology, its history, classification, general applications, specific uses in horticultural crops, challenges, existing resources, associated regulatory aspects, and the way forward.

Keywords: CRISPR; horticulture

1. Introduction

The current population of the world is 7.7 billion and is projected to increase to 8.5 billion by 2030, 9.7 billion in 2050, and 10.9 billion by 2100 [1]. The demand for food has gone up and continues to surge with the ever-increasing human population, and as a result, agricultural production has to keep up with the constantly rising demand [2].

However, increased challenges to agricultural production have emerged in recent years, such as the evolution of new races of pests and diseases, increased incidences of drought, heatwaves, changing climates, and other abiotic stresses [3]. Developing high yielding crops that are resistant to biotic and abiotic stresses is one way to address the increasing pressures on agriculture and answer the growing demand for food, feed, and fuel. Conventional breeding and mutation breeding have historically been a successful approach to introduce important genetic variations for crop improvement [4]. However, the diversity of favorable genes or alleles in plants in nature is finite. Additionally, crop improvement via conventional breeding requires extensive time, space, and funding [5]. Transgenic crops have potential as a solution to the limitations of traditional breeding however, the problems associated with them are numerous. These crops are subject to strict regulations regarding their use, import, and export. Ultimately, the potential for positive impact by transgenic foods in global food security is dependent on how the public and governing bodies view the technologies [6]. Currently, there are significant numbers of people advocating against the use of transgenic crops, with some international markets not accepting transgenic crops at all. The availability of transgene-free, genome editing tools using site-directed nucleases (SDN) has opened many paths of opportunity in the field of agriculture. The multifaceted impact of gene-editing tools includes its benefits for human

health (e.g., therapeutics, regenerative medicine), and opportunities to improve production qualities and disease resistance of crops and livestock.

Specifically, CRISPR/Cas9 is one of the most recent and widely adopted gene-editing techniques [7]. While it was first reported in the 1980s, the full potential of this method began to be harnessed just a decade ago. During this relatively short period, much interest and debate occurred regarding its use in human, animal, and plant applications. The technique is involved in forward as well as reverse genetics [8]. In humans, particular interest has arisen in managing age-related diseases such as Huntington's disease [9] and colon cancer [10], and control of heritable diseases such as sickle cell anemia [11]. In animals, several types of research have been carried out, such as increasing body mass in goats [12] and developing avian leukosis virus resistance in chicken [13]. In plants, CRISPR/Cas9 has been extensively used to improve crop disease resistance, which involves knocking-out susceptibility genes and overexpression of resistance genes. Some crops improved for pathogen/disease resistance include powdery mildew resistant wheat [14], cucumber vein yellowing virus-resistant cucumber [15], powdery mildew resistant apple, and grapes [16], blast-resistant rice [17], and canker resistant citrus [18]. However, like every innovation, the technology has been controversial at times and generated public outcry due to the gene-editing of a human embryo by a research group [19].

1.1. Reaching the CRISPR Age

Gene or genome editing refers to changes in an organism's deoxyribonucleic acid (DNA), either by adding, replacing or modifying the genetic material [20]. Gene editing involves the use of SDN through transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and CRISPR/Cas9 [21]. Genome editing tools such as mega nucleases, ZFNs, TALENs, and CRISPR/Cas9 are based on artificially engineered SDN and have been used to introduce mutations through DNA modification in many plant species along with food crops [22]. DNA modifications could be in the form of single-nucleotide polymorphism (SNPs), deletions, insertions, or substitutions. All these gene-editing tools primarily rely on the double-strand breaks (DSBs), which are repaired by the cell's repair mechanism.

In less than four decades (Table 1), gene-editing technology has undergone tremendous development and transformation. An early example of gene editing is the replacement of the yeast chromosomal segments constructed in vitro by genetic transformation [23]. Similarly, correction of a defective gene having point and deletion mutations were done in mammalian cells using a mutated gene [24].

Table 1. Timeline of events in clustered regularly interspaced short palindromic repeats (CRISPR) discovery and use.

1987–1995
Short direct repeats observed in <i>Escherichia coli</i> , <i>Haloferax mediterranei</i> , and <i>Haloferax volcanii</i> [25–27]
2002
The term “CRISPR” coined, CRISPR components identified/named [28]
2005
CRISPR speculated as a defense mechanism in bacteria [29]
2007–2008
CRISPR/Cas genes confirmed to provide resistance to phages/ explanation of antiviral defense mechanism [30,31]
2010
CRISPR/Cas system can specifically cleave double-strand DNA [32]

Table 1. Cont.

2012 Cas9 endonuclease guided by RNA for gene editing [7]
2013 Human genome-edited by CRISPR/Cas9 system [33,34] First use of CRISPR/Cas9 in plants [14,35–37]
2014– Routine application of CRISPR/Cas9 for crop improvement [38–41]

Several novel tools for either editing or silencing genes evolved in course of time. Mega-nucleases are the oldest SDN based tool used for gene editing. Both ZFNs and ribonucleic acid interference (RNAi) were the groundbreaking technologies developed in the late 20th century, which were used extensively for cutting DNA sequences at specific sites and silencing gene expression. Initial work on RNAi involved an effective interference using double-stranded RNA, which led to the manipulation of gene expression in *Caenorhabditis elegans*, a nematode [42]. Likewise, the earliest research on ZFNs showed that linking of zinc finger proteins with *FokI* endonuclease enables cutting DNA at predetermined sites [43]. Furthermore, TALENs were used to create DSBs at specific, targeted sites [44] and this technique is widely popular as a gene-editing tool in crops.

Many studies have utilized these tools: mega nucleases in maize (*Zea mays*) [45], ZFNs in maize, *Arabidopsis*, and soybean (*Glycine max*) [46–48], and TALENs in rice (*Oryza sativa*) [49,50], wheat (*Triticum aestivum*) [14], maize [51], tomato (*Solanum lycopersicum*) [52], and *Arabidopsis* [53]. However, ZFNs and TALENs require complex protein engineering which has limited their broad application in plants [54].

Due to the intensive protein engineering requirement limitations, researchers continued the quest to develop a gene-editing technique to eliminate this challenge. CRISPR/Cas9 technology was first reported as an adaptive immune response in bacteria and archaea to defend against invading viral and plasmid DNA [29,55]. When viruses (bacteriophages) and plasmids infect bacteria, the host chromosome integrates short fragments of phage and plasmid's nucleic acid as a repetitive element known as CRISPR [56]. Utilizing this nucleic-acid-based immunity stored in its molecular memory, bacteria recognizes new invading phages and plasmids and protect themselves [57]. The main advantage of CRISPR/Cas9 over prior gene-editing techniques is that it does not require complex protein engineering.

1.2. CRISPR/Cas9, Novel Variants and Challenges

The recently proposed classification and nomenclature of CRISPR/Cas systems utilize the information obtained from phylogeny and comparative genomic analyses. Based on the associated unique signature Cas proteins (endonucleases or cleaving proteins), there are two major classes of CRISPR/Cas systems. Under Class 1, there are three types of systems: type I, type III, and type IV. These types are more common in archaea than in bacteria [58]. Except for rudimentary Cas-protein for type IV, both type I and type III utilize more than one Cas protein and the effector module is complex. Under Class 2, there are two types of systems: type II and type V. The effector module for Class 2 is comparatively simpler and utilizes a single Cas protein, which is Cas9 the signature protein for type II, which was first discovered as a novel, large protein [59].

Among these CRISPR systems, the type II system from *Streptococcus pyogenes* bacteria has been extensively used for gene targeting purposes because of a single, unique effector protein. The CRISPR/Cas9 system utilizes a 20-bp DNA target (guide RNA or gRNA or spacer), followed by a short, trinucleotide (5'-NGG-3' or 5'-NAG-3') protospacer adjacent motif (PAM) in the host DNA. The gRNA is duplex in nature and constitutes of CRISPR RNA (crRNA) (homologous to target DNA), and trans-activating crRNA (tracrRNA) [7]. The single gRNA (sgRNA) directs the activity of Cas9 nuclease, thereby creating DSBs and mutations (insertions, deletions, and substitutions) at target-sites [60] (Figure 1). This process of successful DNA-binding and cleavage is only possible when the associated PAM sequence in the host is recognized [61]. The Cas9 endonuclease comprises of two connected lobes: a large globular recognition (REC) lobe and a small nuclease (NUC)

lobe [62–64]. The REC lobe constitutes of REC1 and REC2 domains, of which REC1 is critical for Cas9 function [64]. The NUC-lobe is a PAM-interacting lobe having two nuclease domains, HNH and RuvC-like, which respectively cleave the DNA strand complementary and non-complementary to the crRNA (target sequence) [7,64–66]. The blunt cleavage induced by Cas9 protein are repaired by two major pathways: error-prone non-homologous end joining (NHEJ) or high-fidelity homology-directed repair (HDR) [67]. NHEJ involves the mechanism where DNA ligase IV joins DSBs. During the repair, insertion/deletion of base pairs occurs, leading to a frameshift mutation and/or gene knockout [68]. Contrastingly, HDR involves either an endogenous, natural phenomenon involving sister chromatid as a repair template [63,69] or the introduction of an exogenous repair template DNA (single or double-stranded DNA) in the cut sites to facilitate precise gene editing [67,68,70]. Between the two repair mechanisms, the most common is NHEJ, as it leads to the creation of several mutations in the process of repairing and is a major source of genome rearrangement [69,71]. The difficulties in HDR-mediated DNA repair include competition of the exogenous repair template with the sister chromatid or insufficient delivery of the repair template via *Agrobacterium* or biolistic methods [63].

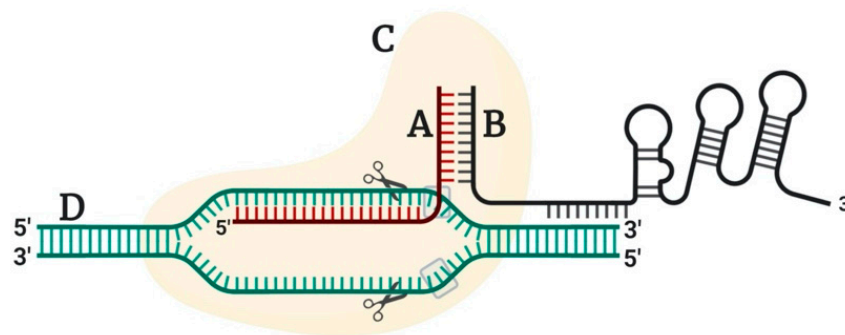


Figure 1. Components of a typical CRISPR/Cas9 construct; A: crRNA; B: tracrRNA; C: Cas9 endonuclease; D: double-stranded target DNA, a grey box with three nucleotides; protospacer adjacent motif (PAM) sequence. Created with BioRender.com.

Derivatives of CRISPR/Cas9 system include base editing that uses dCas9 (dead Cas9), and newly discovered CRISPR/Cpf1 (now CRISPR/Cas12a). dCas9 is also referred to as CRISPR interference (CRISPRi) and it utilizes a modified Cas9 protein. It is very effective in gene silencing by blocking transcription and can serve as an effective tool for targeted gene regulation without disrupting the target sequence [72]. The base editing technology (cytosine-based and adenine-based editors) utilizes dCas9 for the precise editing of a single base without double-strand breaks in the DNA. Using cytosine-based editors, a method has been engineered to convert C (Cytosine) to T (Thymine) and G (Guanine) to A (Adenine) [73]. The adenine-based editor converts A to C and C to G thus completing all four possible transitions [74].

CRISPR/Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) is a single RNA guided system with merits over the CRISPR/Cas9 system, such as a lower rate of off-target edits compared to CRISPR/Cas9 [75–78]. The T-rich PAM upstream of the target sequence in CRISPR/Cpf1 [79] would potentially increase the number of target regions and help in targeting AT-rich promoter regions [80]. CRISPR/Cpf1 endonuclease can cleave DNA without needing an RNA duplex structure, and the size of its RNA is smaller than the CRISPR/Cas9 guide RNA which makes it simpler and cheaper technology [75]. Using CRISPR/Cpf1 for gene editing makes it possible to insert a new DNA in the target site by the HDR repair pathway, while at the same time introducing random mutations near the target site. This is because Cpf1 cleaves far from the target site allowing multiple rounds of cleavage and repairs [75]. Sticky ends are produced via CRISPR/Cpf1, contrary to blunt ends by Cas9, leading to more precise editing [80].

One of several challenges and concerns associated with CRISPR/Cas9 is the significant problem of off-target effects, which may lead to undesired phenotypic changes in crops [81]. The unpredictable,

large on-site deletions created by this technology are also problematic, especially when used in human therapeutics [82]. Another important issue is the ethical concern that can arise with the use of this technology. Important ethical concerns include the potential use of CRISPR as a bioweapon [83] and its application to modify human germ cells/embryos [84] and the potential emergence of alleles overcoming CRISPR gene drives [85].

1.3. Applications of CRISPR/Cas9

Of all the available genome editing tools, CRISPR/Cas9 is popular in the plant community. Gene editing is evolving at a rapid pace but CRISPR/Cas9 is still an efficient, precise, and routinely used gene-editing platform. Crops edited with CRISPR/Cas9 have shown high efficiency. These include varying genome efficiencies: for instance, up to 91.6% in rice [86] and up to 79% in maize [87].

CRISPR/Cas9 has the potential to serve as an important plant breeding tool, which has been reflected in the level of interest generated in the plant breeding community. Part of its popularity is due to being simple to design and yet it allows multiplexing to edit multiple loci simultaneously by introducing multiple DSBs [33,88].

Several horticultural crops have been edited using CRISPR/Cas9 technology to meet a diverse array of research objectives including understanding gene function and several applied breeding objectives (Table 2). Some researchers also used CRISPR/Cas9 to lay the foundation for a breeding program by identifying genes contributing to a specific trait. This enables controlled crossing and introgression strategies. Similarly, novel mutations can be introduced directly into elite germplasm, thereby accelerating the breeding program [89].

Table 2. Examples of horticultural crops where CRISPR/Cas9 technology was used to meet research objectives.

Crop.	Research Objective Met Using CRISPR/Cas9	References
Tomato	Understand the role of a photoreceptor in seedling development/stress tolerance	[90]
	Bacterial speck resistance	[91]
	Combine desired traits with useful traits present in wild type	[92]
	Confirm function of a gene involved in Fusarium wilt tolerance	[93]
	Improve lycopene content	[94]
	Develop Tomato Yellow Leaf Curl Virus resistance	[95]
	Long shelf life, generate parthenocarp	[96]
	Transgene-free powdery mildew resistant plants	[97]
	Achieve ideotype	[98]
	Develop day-neutral and early yielding plants	[99]
Capsicum	Understand the role of a transcription factor in chloroplast development and fruit color	[100]
Carrot	Generate haploid plants	[101]
Potato	Reduce enzymatic browning	[102]
	Overcome self-incompatibility	[103,104]
Sweet potato	Reduce steroidal glycoalkaloids	[105]
	Develop amylopectin starch cultivars	[106]
	Enhance Fusarium wilt resistance	[107]
Watermelon	Validate function of vacuolar sugar transporter gene	[108]
	Obtain gynocious genotypes	[109]
	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>niveum</i> Race 1	[110]
	Functional characterization of a gene in fruit flesh sugar accumulation	[111]
	Herbicide resistance	[112]

Table 2. Cont.

Crop.	Research Objective Met Using CRISPR/Cas9	References
Pumpkin	Understanding the role of root apex in salt tolerance	[113]
Cucumber	Transgene-free gynocious plants	[114]
	Broad virus resistance	[15]
Cabbage	Compare delivery methods in model genes	[115]
	Target flowering-time regulator gene	[116]
	Generate early flowering phenotype	[117]
	Multiplex gene editing to overcome self-incompatibility and produce male-sterile lines	[118]
Lettuce	Generate seedlings capable of germinating at higher temperatures	[119]
Cassava	Brown streak resistance	[120]
Strawberry	Characterize a transcription factor involved in anther development	[121]
	Identify genes involved in auxin accumulation and biosynthesis	[122]
Citrus	Canker resistance	[18,123,124]
Apple	Reduce fire blight susceptibility	[125]
Banana	Inactivate banana streak virus	[126]
	Basis of generating dwarf and semi-dwarf cultivars	[127]
Grapes	Study editing efficiency	[128]
	Obtain biallelic mutations in the first generation	[129]
Papaya	Study the evolution of oomycetes in evading plant defense mechanism	[130]
Cacao	Edit gene involved in suppressing defense response	[131]
Coffee	Proof-of-concept to knock out genes of interest	[132]
Petunia	Understand genes involved in flower longevity and ethylene production	[133]
Orchid	Understand the MADS gene family expressed in floral organs	[134]
Chrysanthemum	First report of gene editing	[135]
Japanese morning glory	Flower longevity	[136]
	Understand the role of a gene in petal coloration	[137]

1.4. Existing Resources for CRISPR/Cas9

Multiple steps are involved in the gene-editing procedure using the CRISPR/Cas9 technique (Figure 2), including designing of gRNAs, the introduction of CRISPR vectors into plant systems, transformation, and analysis of edits in the transformed lines. First, the reference genome for the crop to be edited should be located. Some horticultural crops that have whole genome sequences available are cucurbits (melon, watermelon, cucumber, bottle/wax gourd): <http://cucurbitgenomics.org/>, solanaceous crops (tomato, potato, pepper, eggplant): <https://solgenomics.net/>, banana: <https://banana-genome-hub.southgreen.fr/>, citrus: <https://www.citrusgenomedb.org/>, apple: <https://iris.angers.inra.fr/gddh13/>, and spinach: <http://www.spinachbase.org/>. This is followed by identifying the gene(s) of interest, sequencing them, and ensuring their proper alignment with the reference genome. Next, the gRNAs are designed using several software such as CRISPR-P [138], CRISPR-PLANT [60], CRISPRdirect [139], Chop-Chop [140], and Benchling [141]. After this, a search for CRISPR vectors in plasmid repositories such as Addgene [142] should be completed to assemble the gRNAs and CRISPR/Cas9 cassette. For example, pHSN401, pHSN501, and pHSN401 are used for watermelon transformation [143] and pTC217 is used for tomato transformation [144]. The CRISPR constructs can be prepared using either ligation-dependent [145,146] or ligation-independent protocols [147], and sequenced for proper alignment of the constructs. The steps involved in vector construction can be simulated using software such as Benchling [141] and Snapgene [148].

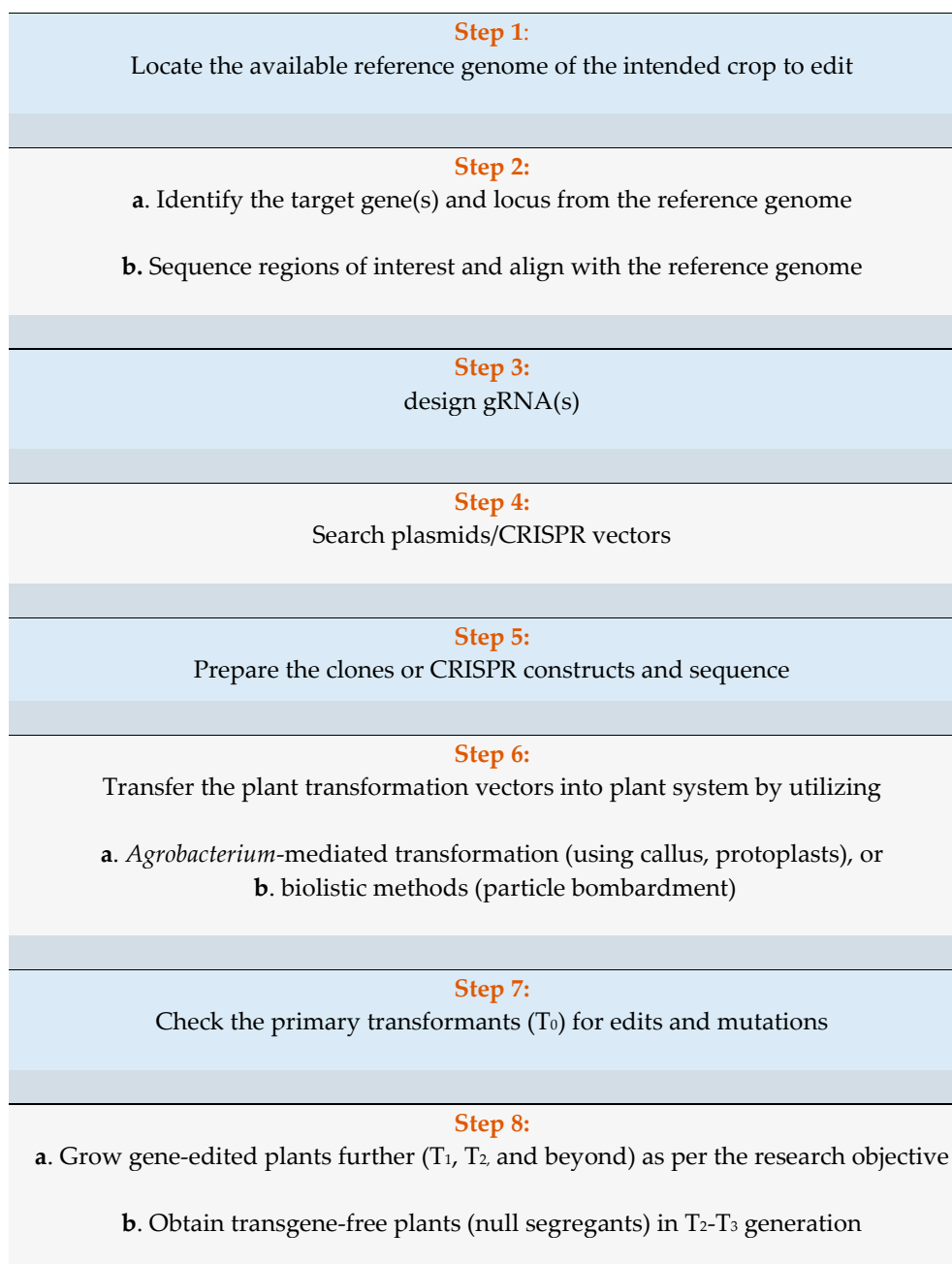


Figure 2. A typical workflow in a CRISPR/Cas9-based gene edit.

The transformation vectors are introduced into the plant using either *Agrobacterium*-mediated transformation [145,149–158] or biolistics [71,159,160]. RNA or DNA viruses have also been used to introduce the gRNA into the plant system [161,162]. Similarly, carbon nanotube nanoparticles have also been used as an efficient plasmid DNA delivery mechanism [163,164]. Researchers have also used protoplasts to first test the cleaving ability of sgRNAs in vivo, thereby determining their efficiency prior to entering into full-scale transformation [165,166]. Primary transformants (T₀) are checked for edits and mutations via polymerase chain reaction (PCR), Sanger sequencing, restriction digestion, and T7 endonuclease I (T7EI) assay [146,167–171] via software such as TIDE [172] and Snappgene [148] for analysis. The gene-edited plants are advanced one or more generations and confirmed for the absence of transgenic elements via PCR and whole genome sequencing [97,173–175]. Specifically, in seed-propagated crops, the T-DNA with the CRISPR/Cas9 construct segregates out in further

generations, potentially leading to transgene-free, null segregants. However, in clonally or vegetatively propagated crops, it is challenging to obtain null segregants [176].

1.5. The Regulatory Status of Gene-Edited Crops

Multiple crop improvements could be achieved using CRISPR/Cas9 by engineering crops with higher productivity [168,177], improved resistance to diseases [17], resistance to abiotic stresses [178–180], and better nutritional quality [94]. This technique has been utilized to create small insertions and deletions that are identical to natural genetic variation [181], which later repair either via the NHEJ pathway or through a donor template-based HDR pathway. Selection against transgenic elements such as the Cas9 and selection markers identifies the null segregants. Whole-genome sequencing can also be done to ensure that there are no traces of CRISPR/Cas9 elements [182] and also to assess the associated off-target effects.

Research in CRISPR/Cas9-based gene editing has focused mostly on NHEJ pathway-based gene editing, as it is a predominant repair pathway in plants [183]. The NHEJ pathway helps create transgene-free plants that do not undergo any regulatory scrutiny [184], as they do not contain foreign elements present in GMOs, and the mutations induced are similar to what would occur naturally as random mutagenesis. Therefore, plants generated from NHEJ-based editing are not currently regulated once null segregants are obtained. NHEJ enables rapid crop breeding (less than five years) [185,186] as compared to traditional breeding where it typically takes more than 10 years to develop a variety [187]. Similarly, in HDR, exogenously provided homologous DNA sequences (template) are used to precisely repair the DSBs in DNA [188]. Gene addition using HDR, to some extent, may deem it transgenic [189]. However, if the repair template is cisgenic, where genes from the same species, related interbreeding, or wild species are used, the resultant crops may not be considered transgenic. Nevertheless, the regulatory framework regarding NHEJ and HDR-mediated gene editing is perceived and defined in different respective contexts depending on the country.

In the United States, the United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA) are the agencies that oversee the regulation of genetically modified organisms (GMOs) [190]. Due to the removal of transgenic elements in plants, regulatory agencies in the USA do not consider CRISPR-edited plants as GMOs for regulatory purposes. CRISPR-edited plants can be deployed widely in less time and at a lower cost than conventional plant breeding [191].

In 2016, regulatory approval was provided by USDA to a waxy corn null segregant line [192], as it was not a plant pest and did not contain transgenes owing to selection in subsequent segregations. In the same year, the USDA stated that the CRISPR/Cas9 edited, anti-browning mushrooms need not be regulated as they contain no foreign DNA integrated to the mushroom genome [193]. Lines approved by regulatory agencies can be directly tested in the field, benefitting researchers and biotechnology companies [194], and help in the development of new cultivars in a limited timeframe with reduced costs.

In the USA, genome editing has been allowed as a potential expansion of traditional plant breeding tools for crop improvement [195]. A catalog of regulatory inquiries and approvals for gene-edited crops in the USA is available [196]. Several letters of inquiries have been received for gene-edited crops/organisms, including tomato, citrus, pennycress, soybean, sugarcane, camelina, petunia, flax, rice, and orchid—all of which received the regulatory waiver. Similarly, in May 2020, an updated biotechnology framework has been developed by USDA-APHIS and has been defined as SECURE (Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient) rule [197]. The framework provides three exemptions to make a single genetic modification to any plant species: (i) changes resulting after DSB in DNA in absence of an external repair template, (ii) targeted single base pair substitution, and (iii) introduction of a known gene that exists in the plant's gene pool [198].

The regulation of gene-edited crops differs based on the legal framework in each nation or group of nations. In Canada, only if the gene-edited product (plant, feed, or food) is novel, i.e., different from what is already available, it undergoes the pre-market assessment [199]. In Europe, there are stringent

regulations on CRISPR/Cas9 [200]. Recently, the Court of Justice of the European Union, the highest court of Europe, ruled that gene-edited crops be treated with the same set of regulations that are imposed on genetically modified (GM) organisms [201]. In contrast, Australia has taken the middle ground, allowing the use of gene-editing without introducing any foreign genetic material [202]. There is also a favorable environment for gene-edited crops in Asian countries such as China and Japan, where several field trials of gene-edited crops have been reported [203]. In India, there is a regulation mechanism for genetic engineering which includes “modification of an organism or in a cell by deletion and removal of parts of the heritable material” [204]. In South Africa, GMO regulations are in place but there is still a need for genome-editing-specific legislation [205]. In Argentina, the regulatory framework is on a case-by-case basis taking into consideration the process involved, i.e., the breeding methodology used, new trait or characteristic introduced, and evidence of the genetic changes present in the final product [206].

1.6. Way Forward

There is immense potential for gene editing techniques including novel plant breeding tools such as CRISPR/Cas9 in enhancing production, productivity, quality, and nutritional characteristics of horticultural crops. This technology is expected to contribute to resolving the food deficit issues prevailing in the world. Acceptance of plant innovation and a congenial regulatory atmosphere in the USA allows gene-edited plant products with the market potential to be extensively researched and appear on the grocery shelf [207]. This includes TALEN-edited Calyno™ high-oleic soybean oil [208]. The primary concern currently is producer and consumer acceptance to the CRISPR-edited products commercially available [209,210]. Proponents of gene editing argue that characteristics of the final product, not the process involved, should be considered for food safety assessment [211]. However, there will still be differing perceptions of gene-edited products, specifically due to the varying regulatory provisions in different countries. A global scientific consensus and uniform regulatory measures across countries might add to the usefulness of gene-editing technology beyond the domain of research.

Author Contributions: Writing—original draft preparation, B.P.B.; writing—review and editing, S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This review received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. United Nations (UN). *Probabilistic Population Projections based on the World Population Prospects 2019*; United Nations, Department of Economic and Social Affairs, Population Division: New York, NY, USA, 2019.
2. Ort, D.R.; Merchant, S.S.; Alric, J.; Barkan, A.; Blankenship, R.E.; Bock, R.; Croce, R.; Hanson, M.R.; Hibberd, J.M.; Long, S.P. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8529–8536. [[CrossRef](#)]
3. Velásquez, A.C.; Castroverde, C.D.M.; He, S.Y. Plant–Pathogen Warfare under Changing Climate Conditions. *Curr. Biol.* **2018**, *28*, R619–R634. [[CrossRef](#)] [[PubMed](#)]
4. Schaart, J.G.; Van De Wiel, C.C.; Lotz, L.A.; Smulders, M.J. Opportunities for Products of New Plant Breeding Techniques. *Trends Plant. Sci.* **2016**, *21*, 438–449. [[CrossRef](#)] [[PubMed](#)]
5. Abdelrahman, M.; Al-Sadi, A.M.; Pour-Aboughadareh, A.; Burritt, D.J.; Tran, L.-S.P. Genome editing using CRISPR/Cas9–targeted mutagenesis: An opportunity for yield improvements of crop plants grown under environmental stresses. *Plant. Physiol. Biochem.* **2018**, *131*, 31–36. [[CrossRef](#)] [[PubMed](#)]
6. Voytas, D.F.; Gao, C. Precision Genome Engineering and Agriculture: Opportunities and Regulatory Challenges. *PLoS Biol.* **2014**, *12*, e1001877. [[CrossRef](#)] [[PubMed](#)]
7. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science* **2012**, *337*, 816–821. [[CrossRef](#)]

8. Gurumurthy, C.B.; Grati, M.H.; Ohtsuka, M.; Schilit, S.L.; Quadros, R.M.; Liu, X.Z. CRISPR: A versatile tool for both forward and reverse genetics research. *Hum. Genet.* **2016**, *135*, 971–976. [[CrossRef](#)]
9. Xu, X.; Tay, Y.; Sim, B.; Yoon, S.-I.; Huang, Y.; Ooi, J.; Utami, K.H.; Ziaei, A.; Ng, B.; Radulescu, C.; et al. Reversal of Phenotypic Abnormalities by CRISPR/Cas9-Mediated Gene Correction in Huntington Disease Patient-Derived Induced Pluripotent Stem Cells. *Stem Cell Rep.* **2017**, *8*, 619–633. [[CrossRef](#)]
10. Li, W.; Cho, M.Y.; Lee, S.; Jang, M.; Park, J.; Park, R. CRISPR-Cas9 mediated CD133 knockout inhibits colon cancer invasion through reduced epithelial-mesenchymal transition. *PLoS ONE* **2019**, *14*, e0220860. [[CrossRef](#)]
11. Ye, L.; Wang, J.; Tan, Y.; Beyer, A.I.; Xie, F.; Muench, M.O.; Kan, Y.W. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: An approach for treating sickle cell disease and β -thalassemia. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 10661–10665. [[CrossRef](#)]
12. Wang, X.; Niu, Y.; Zhou, J.; Zhu, H.; Ma, B.; Yu, H.; Yan, H.; Hua, J.; Huang, X.; Qu, L.; et al. CRISPR/Cas9-mediated MSTN disruption and heritable mutagenesis in goats causes increased body mass. *Anim. Genet.* **2018**, *49*, 43–51. [[CrossRef](#)] [[PubMed](#)]
13. Koslová, A.; Kučerová, D.; Reinišová, M.; Geryk, J.; Trefil, P.; Hejnar, J. Genetic Resistance to Avian Leukosis Viruses Induced by CRISPR/Cas9 Editing of Specific Receptor Genes in Chicken Cells. *Viruses* **2018**, *10*, 605. [[CrossRef](#)] [[PubMed](#)]
14. Wang, Y.; Cheng, X.; Shan, Q.; Zhang, Y.; Liu, J.; Gao, C.; Qiu, J.-L. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* **2014**, *32*, 947–951. [[CrossRef](#)] [[PubMed](#)]
15. Chandrasekaran, J.; Brumin, M.; Wolf, D.; Leibman, D.; Klap, C.; Pearlsman, M.; Sherman, A.; Arazi, T.; Gal-On, A. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol. Plant. Pathol.* **2016**, *17*, 1140–1153. [[CrossRef](#)] [[PubMed](#)]
16. Malnoy, M.; Viola, R.; Jung, M.-H.; Koo, O.-J.; Kim, S.; Kim, J.-S.; Velasco, R.; Kanchiswamy, C.N. DNA-Free Genetically Edited Grapevine and Apple Protoplast Using CRISPR/Cas9 Ribonucleoproteins. *Front. Plant. Sci.* **2016**, *7*, 1904. [[CrossRef](#)]
17. Wang, F.; Wang, C.; Liu, P.; Lei, C.; Hao, W.; Gao, Y.; Liu, Y.-G.; Zhao, K. Enhanced Rice Blast Resistance by CRISPR/Cas9-Targeted Mutagenesis of the ERF Transcription Factor Gene OsERF922. *PLoS ONE* **2016**, *11*, e0154027. [[CrossRef](#)]
18. Peng, A.H.; Chen, S.C.; Lei, T.G.; Xu, L.Z.; He, Y.R.; Wu, L.; Yao, L.X.; Zou, X.P. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. *Plant. Biotechnol. J.* **2017**, *15*, 1509–1519. [[CrossRef](#)]
19. Cyranoski, D.; Ledford, H. Genome-edited baby claim provokes international outcry. *Nature* **2018**, *563*, 607–608. [[CrossRef](#)]
20. National Academies of Sciences, Engineering, and Medicine. Committee on Human Gene Editing: Scientific, Medical, and Ethical Considerations, the Basic Science of Genome Editing. In *Human Genome Editing: Science, Ethics, and Governance*; National Academies Press (US): Washington, DC, USA, 2017.
21. Čermák, T.; Curtin, S.J.; Gil-Humanes, J.; Čegan, R.; Kono, T.J.; Konečná, E.; Belanto, J.J.; Starker, C.G.; Mathre, J.W.; Greenstein, R.L.; et al. A Multipurpose Toolkit to Enable Advanced Genome Engineering in Plants. *Plant. Cell* **2017**, *29*, 1196–1217. [[CrossRef](#)]
22. Baltes, N.J.; Gil-Humanes, J.; Voytas, D.F. Genome engineering and agriculture: Opportunities and challenges. In *Progress in Molecular Biology and Translational Science*; Elsevier: Amsterdam, The Netherlands, 2017; Volume 149, pp. 1–26.
23. Scherer, S.; Davis, R.W. Replacement of chromosome segments with altered DNA sequences constructed in vitro. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 4951–4955. [[CrossRef](#)]
24. Thomas, K.R.; Folger, K.R.; Capecchi, M.R. High frequency targeting of genes to specific sites in the mammalian genome. *Cell* **1986**, *44*, 419–428. [[CrossRef](#)]
25. Ishino, Y.; Shinagawa, H.; Makino, K.; Amemura, M.; Nakata, A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J. Bacteriol.* **1987**, *169*, 5429–5433. [[CrossRef](#)] [[PubMed](#)]
26. Mojica, F.J.; Juez, G.; Rodriguez-Valera, F. Transcription at different salinities of Haloferax mediterranei sequences adjacent to partially modified PstI sites. *Mol. Microbiol.* **1993**, *9*, 613–621. [[CrossRef](#)] [[PubMed](#)]

27. Mojica, F.J.; Ferrer, C.; Juez, G.; Rodriguez-Valera, F. Long stretches of short tandem repeats are present in the largest replicons of the Archaea *Haloferax mediterranei* and *Haloferax volcanii* and could be involved in replicon partitioning. *Mol. Microbiol.* **1995**, *17*, 85–93. [[CrossRef](#)] [[PubMed](#)]
28. Jansen, R.; Van Embden, J.D.A.; Gaastra, W.; Schouls, L.M. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol. Microbiol.* **2002**, *43*, 1565–1575. [[CrossRef](#)] [[PubMed](#)]
29. Pourcel, C.; Salviñol, G.; Vergnaud, G. CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology* **2005**, *151*, 653–663. [[CrossRef](#)] [[PubMed](#)]
30. Barrangou, R.; Fremaux, C.; Deveau, H.; Richards, M.; Boyaval, P.; Moineau, S.; Romero, D.A.; Horvath, P. CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. *Science* **2007**, *315*, 1709–1712. [[CrossRef](#)] [[PubMed](#)]
31. Brouns, S.J.J.; Jore, M.M.; Lundgren, M.; Westra, E.R.; Slijkhuis, R.J.H.; Snijders, A.P.L.; Dickman, M.J.; Makarova, K.S.; Koonin, E.V.; Van Der Oost, J. Small CRISPR RNAs Guide Antiviral Defense in Prokaryotes. *Science* **2008**, *321*, 960–964. [[CrossRef](#)]
32. Garneau, J.E.; Dupuis, M.-È.; Villion, M.; Romero, D.A.; Barrangou, R.; Boyaval, P.; Fremaux, C.; Horvath, P.; Magadán, A.H.; Moineau, S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* **2010**, *468*, 67–71. [[CrossRef](#)]
33. Cong, L.; Ran, F.A.; Cox, D.; Lin, S.; Barretto, R.; Habib, N.; Hsu, P.D.; Wu, X.; Jiang, W.; Marraffini, L.; et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science* **2013**, *339*, 819–823. [[CrossRef](#)]
34. Jinek, M.; East, A.; Cheng, A.; Lin, S.; Ma, E.; Doudna, J. RNA-programmed genome editing in human cells. *eLife* **2013**, *2*, e00471. [[CrossRef](#)] [[PubMed](#)]
35. Belhaj, K.; Chaparro-Garcia, A.; Kamoun, S.; Nekrasov, V. Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant. Methods* **2013**, *9*, 39. [[CrossRef](#)]
36. Shan, Q.; Wang, Y.; Li, J.; Gao, C. Genome editing in rice and wheat using the CRISPR/Cas system. *Nat. Protoc.* **2014**, *9*, 2395–2410. [[CrossRef](#)] [[PubMed](#)]
37. Brooks, C.; Nekrasov, V.; Lippman, Z.B.; Van Eck, J. Efficient Gene Editing in Tomato in the First Generation Using the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-Associated9 System. *Plant. Physiol.* **2014**, *166*, 1292–1297. [[CrossRef](#)] [[PubMed](#)]
38. Barrangou, R.; Doudna, J.A. Applications of CRISPR technologies in research and beyond. *Nat. Biotechnol.* **2016**, *34*, 933–941. [[CrossRef](#)] [[PubMed](#)]
39. Jaganathan, D.; Ramasamy, K.; Sellamuthu, G.; Jayabalan, S.; Venkataraman, G. CRISPR for Crop Improvement: An Update Review. *Front. Plant. Sci.* **2018**, *9*, 985. [[CrossRef](#)] [[PubMed](#)]
40. Xu, J.; Hua, K.; Lang, Z. Genome editing for horticultural crop improvement. *Hortic. Res.* **2019**, *6*, 113–116. [[CrossRef](#)]
41. Ghogare, R.; Williamson-Benavides, B.; Ramírez-Torres, F.; Dhingra, A. CRISPR-associated nucleases: The Dawn of a new age of efficient crop improvement. *Transgenic Res.* **2019**, *29*, 1–35. [[CrossRef](#)]
42. Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **1998**, *391*, 806–811. [[CrossRef](#)]
43. Kim, Y.G.; Cha, J.; Chandrasegaran, S. Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 1156. [[CrossRef](#)]
44. Christian, M.; Cermak, T.; Doyle, E.L.; Schmidt, C.; Zhang, F.; Hummel, A.; Bogdanove, A.J.; Voytas, D.F. Targeting DNA Double-Strand Breaks with TAL Effector Nucleases. *Genetics* **2010**, *186*, 757–761. [[CrossRef](#)] [[PubMed](#)]
45. Gao, H.; Smith, J.; Yang, M.; Jones, S.; Djukanovic, V.; Nicholson, M.G.; West, A.; Bidney, D.; Falco, S.C.; Jantz, D.; et al. Heritable targeted mutagenesis in maize using a designed endonuclease. *Plant. J.* **2010**, *61*, 176–187. [[CrossRef](#)] [[PubMed](#)]
46. Shukla, V.K.; Doyon, Y.; Miller, J.C.; DeKolver, R.C.; Moehle, E.A.; Worden, S.E.; Mitchell, J.C.; Arnold, N.L.; Gopalan, S.; Meng, X.; et al. Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. *Nature* **2009**, *459*, 437–441. [[CrossRef](#)] [[PubMed](#)]
47. Zhang, F.; Maeder, M.L.; Unger-Wallace, E.; Hoshaw, J.P.; Reyon, D.; Christian, M.; Li, X.; Pierick, C.J.; Dobbs, D.; Peterson, T.; et al. High frequency targeted mutagenesis in *Arabidopsis thaliana* using zinc finger nucleases. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12028–12033. [[CrossRef](#)] [[PubMed](#)]

48. Curtin, S.J.; Zhang, F.; Sander, J.D.; Haun, W.J.; Starker, C.; Baltes, N.J.; Reyon, D.; Dahlborg, E.J.; Goodwin, M.J.; Coffman, A.P.; et al. Targeted Mutagenesis of Duplicated Genes in Soybean with Zinc-Finger Nucleases. *Plant. Physiol.* **2011**, *156*, 466–473. [[CrossRef](#)] [[PubMed](#)]
49. Li, T.; Liu, B.; Spalding, M.H.; Weeks, D.P.; Yang, B. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat. Biotechnol.* **2012**, *30*, 390–392. [[CrossRef](#)] [[PubMed](#)]
50. Shan, Q.; Zhang, Y.; Chen, K.; Zhang, K.; Gao, C. Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. *Plant. Biotechnol. J.* **2015**, *13*, 791–800. [[CrossRef](#)] [[PubMed](#)]
51. Liang, Z.; Zhang, K.; Chen, K.; Gao, C. Targeted Mutagenesis in Zea mays Using TALENs and the CRISPR/Cas System. *J. Genet. Genom.* **2014**, *41*, 63–68. [[CrossRef](#)]
52. Lor, V.S.; Starker, C.G.; Voytas, D.F.; Weiss, D.; Olszewski, N.E. Targeted Mutagenesis of the Tomato PROCERA Gene Using Transcription Activator-Like Effector Nucleases. *Plant. Physiol.* **2014**, *166*, 1288–1291. [[CrossRef](#)]
53. Joung, J.K.; Sander, J.D. TALENs: A widely applicable technology for targeted genome editing. *Nat. Rev. Mol. Cell Biol.* **2012**, *14*, 49–55. [[CrossRef](#)]
54. Mishra, R.; Joshi, R.K.; Zhao, K. Genome Editing in Rice: Recent Advances, Challenges, and Future Implications. *Front. Plant. Sci.* **2018**, *9*, 1361. [[CrossRef](#)] [[PubMed](#)]
55. Doudna, J.A.; Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. *Science* **2014**, *346*, 1258096. [[CrossRef](#)] [[PubMed](#)]
56. Sorek, R.; Lawrence, C.M.; Wiedenheft, B. CRISPR-Mediated Adaptive Immune Systems in Bacteria and Archaea. *Annu. Rev. Biochem.* **2013**, *82*, 237–266. [[CrossRef](#)] [[PubMed](#)]
57. Datsenko, K.A.; Pougach, K.; Tikhonov, A.; Wanner, B.L.; Severinov, K.; Semenova, E. Molecular memory of prior infections activates the CRISPR/Cas adaptive bacterial immunity system. *Nat. Commun.* **2012**, *3*, 945. [[CrossRef](#)]
58. Makarova, K.S.; Wolf, Y.I.; Alkhnbashi, O.S.; Costa, F.; Shah, S.A.; Saunders, S.J.; Barrangou, R.; Brouns, S.J.; Charpentier, E.; Haft, D.H.; et al. An updated evolutionary classification of CRISPR–Cas systems. *Nat. Rev. Genet.* **2015**, *13*, 722–736. [[CrossRef](#)]
59. Bolotin, A.; Quinquis, B.; Sorokin, A.; Ehrlich, S.D. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology* **2005**, *151*, 2551–2561. [[CrossRef](#)]
60. Xie, K.; Zhang, J.; Yang, Y. Genome-Wide Prediction of Highly Specific Guide RNA Spacers for CRISPR–Cas9-Mediated Genome Editing in Model Plants and Major Crops. *Mol. Plant.* **2014**, *7*, 923–926. [[CrossRef](#)]
61. Sternberg, S.H.; Redding, S.; Jinek, M.; Greene, E.C.; Doudna, J.A. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature* **2014**, *507*, 62–67. [[CrossRef](#)]
62. Jinek, M.; Jiang, F.; Taylor, D.W.; Sternberg, S.H.; Kaya, E.; Ma, E.; Anders, C.; Hauer, M.; Zhou, K.; Lin, S.; et al. Structures of Cas9 Endonucleases Reveal RNA-Mediated Conformational Activation. *Science* **2014**, *343*, 1247997. [[CrossRef](#)]
63. Belhaj, K.; Chaparro-Garcia, A.; Kamoun, S.; Patron, N.; Nekrasov, V. Editing plant genomes with CRISPR/Cas9. *Curr. Opin. Biotechnol.* **2015**, *32*, 76–84. [[CrossRef](#)]
64. Nishimasu, H.; Ran, F.A.; Hsu, P.D.; Konermann, S.; Shehata, S.I.; Dohmae, N.; Ishitani, R.; Zhang, F.; Nureki, O. Crystal Structure of Cas9 in Complex with Guide RNA and Target DNA. *Cell* **2014**, *156*, 935–949. [[CrossRef](#)] [[PubMed](#)]
65. Barrangou, R. RNA-mediated programmable DNA cleavage. *Nat. Biotechnol.* **2012**, *30*, 836–838. [[CrossRef](#)] [[PubMed](#)]
66. Shan, Q.; Wang, Y.; Li, J.; Zhang, Y.; Chen, K.; Liang, Z.; Zhang, K.; Liu, J.; Xi, J.J.; Qiu, J.-L.; et al. Targeted genome modification of crop plants using a CRISPR–Cas system. *Nat. Biotechnol.* **2013**, *31*, 686–688. [[CrossRef](#)] [[PubMed](#)]
67. Ran, F.A.; Hsu, P.D.; Wright, J.; Agarwala, V.; Scott, D.A.; Zhang, F. Genome engineering using the CRISPR–Cas9 system. *Nat. Protoc.* **2013**, *8*, 2281–2308. [[CrossRef](#)]
68. Voytas, D.F. Plant Genome Engineering with Sequence-Specific Nucleases. *Annu. Rev. Plant. Biol.* **2013**, *64*, 327–350. [[CrossRef](#)]
69. Knoll, A.; Fauser, F.; Puchta, H. DNA recombination in somatic plant cells: Mechanisms and evolutionary consequences. *Chromosome Res.* **2014**, *22*, 191–201. [[CrossRef](#)]
70. Hsu, P.D.; Lander, E.S.; Zhang, F. Development and Applications of CRISPR–Cas9 for Genome Engineering. *Cell* **2014**, *157*, 1262–1278. [[CrossRef](#)]

71. Svitashv, S.; Schwartz, C.; Lenderts, B.; Young, J.K.; Cigan, A.M. Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. *Nat. Commun.* **2016**, *7*, 13274. [[CrossRef](#)]
72. Qi, L.; Larson, M.H.; Gilbert, L.A.; Doudna, J.A.; Weissman, J.S.; Arkin, A.P.; Lim, W.A. Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. *Cell* **2013**, *152*, 1173–1183. [[CrossRef](#)]
73. Komor, A.C.; Kim, Y.B.; Packer, M.S.; Zuris, J.A.; Liu, D.R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **2016**, *533*, 420–424. [[CrossRef](#)]
74. Gaudelli, N.M.; Komor, A.C.; Rees, H.A.; Packer, M.S.; Badran, A.H.; Bryson, D.I.; Liu, D.R. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **2017**, *551*, 464–471. [[CrossRef](#)] [[PubMed](#)]
75. 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. Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. *Cell* **2015**, *163*, 759–771. [[CrossRef](#)] [[PubMed](#)]
76. Nishida, K.; Arazoe, T.; Yachie, N.; Banno, S.; Kakimoto, M.; Tabata, M.; Mochizuki, M.; Miyabe, A.; Araki, M.; Hara, K.Y.; et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science* **2016**, *353*, aaf8729. [[CrossRef](#)]
77. Kleinstiver, B.P.; Tsai, S.Q.; Prew, M.S.; Nguyen, N.T.; Welch, M.M.; Lopez, J.M.; McCaw, Z.R.; Aryee, M.J.; Joung, J.K. Genome-wide specificities of CRISPR-Cas Cpf1 nucleases in human cells. *Nat. Biotechnol.* **2016**, *34*, 869–874. [[CrossRef](#)] [[PubMed](#)]
78. Kim, D.; Kim, J.; Hur, J.K.; Been, K.W.; Yoon, S.-H.; Kim, J.-S. Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. *Nat. Biotechnol.* **2016**, *34*, 863–868. [[CrossRef](#)] [[PubMed](#)]
79. Zetsche, B.; Heidenreich, M.; Mohanraju, P.; Fedorova, I.; Kneppers, J.; DeGennaro, E.M.; Winblad, N.; Choudhury, S.R.; Abudayyeh, O.O.; Gootenberg, J.S.; et al. Multiplex gene editing by CRISPR–Cpf1 using a single crRNA array. *Nat. Biotechnol.* **2016**, *35*, 31–34. [[CrossRef](#)] [[PubMed](#)]
80. Scheben, A.; Wolter, F.; Batley, J.; Puchta, H.; Edwards, D. Towards CRISPR/Cas crops—Bringing together genomics and genome editing. *New Phytol.* **2017**, *216*, 682–698. [[CrossRef](#)]
81. Zhao, H.; Wolt, J.D. Risk associated with off-target plant genome editing and methods for its limitation. *Emerg. Top. Life Sci.* **2017**, *1*, 231–240. [[CrossRef](#)]
82. Lee, H.; Kim, J.-S. Unexpected CRISPR on-target effects. *Nat. Biotechnol.* **2018**, *36*, 703–704. [[CrossRef](#)]
83. Ouagrham-Gormley, S.B.; Fye-Marnien, S.R. Is CRISPR a security threat? In *Defense against Biological Attacks*; Springer: Berlin, Germany, 2019; pp. 233–251.
84. Caplan, A.L.; Parent, B.; Shen, M.; Plunkett, C. No time to waste—The ethical challenges created by CRISPR. *EMBO Rep.* **2015**, *16*, 1421–1426. [[CrossRef](#)]
85. Noble, C.; Olejarz, J.; Esvelt, K.M.; Church, G.M.; Nowak, M.A. Evolutionary dynamics of CRISPR gene drives. *Sci. Adv.* **2017**, *3*, e1601964. [[CrossRef](#)] [[PubMed](#)]
86. Miao, J.; Guo, D.; Zhang, J.; Huang, Q.; Qin, G.; Zhang, X.; Wan, J.; Gu, H.; Qu, L.-J. Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Res.* **2013**, *23*, 1233–1236. [[CrossRef](#)] [[PubMed](#)]
87. Wang, K.; Yang, B. A High-Efficiency CRISPR Platform for Maize Improvement. Available online: <https://vtechworks.lib.vt.edu/handle/10919/78865> (accessed on 2 October 2020).
88. Mao, Y.; Zhang, H.; Xu, N.; Zhang, B.; Gou, F.; Zhu, J.-K. Application of the CRISPR–Cas System for Efficient Genome Engineering in Plants. *Mol. Plant.* **2013**, *6*, 2008–2011. [[CrossRef](#)] [[PubMed](#)]
89. Scheben, A.; Yuan, Y.; Edwards, D. Advances in genomics for adapting crops to climate change. *Curr. Plant. Biol.* **2016**, *6*, 2–10. [[CrossRef](#)]
90. Liu, X.R.; Zhang, Q.W.; Yang, G.Q.; Zhang, C.L.; Dong, H.X.; Liu, Y.; Yin, R.H.; Lin, L. Pivotal roles of Tomato photoreceptor SIUVR8 in seedling development and UV-B stress tolerance. *Biochem. Biophys. Res. Commun.* **2020**, *522*, 177–183. [[CrossRef](#)]
91. Ortigosa, A.; Gimenez-Ibanez, S.; Leonhardt, N.; Solano, R. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SIJAZ2. *Plant. Biotechnol. J.* **2018**, *17*, 665–673. [[CrossRef](#)]
92. Zsögön, A.; Čermák, T.; Naves, E.R.; Notini, M.M.; Edel, K.H.; Weinl, S.; Freschi, L.; Voytas, D.F.; Kudla, J.; Peres, L.E.P. De novo domestication of wild tomato using genome editing. *Nat. Biotechnol.* **2018**, *36*, 1211–1216. [[CrossRef](#)]
93. Prihatna, C.; Barbetti, M.J.; Barker, S.J. A Novel Tomato Fusarium Wilt Tolerance Gene. *Front. Microbiol.* **2018**, *9*, 1226. [[CrossRef](#)]

94. Li, X.; Wang, Y.; Chen, S.; Tian, H.; Fu, D.; Zhu, B.; Luo, Y.; Zhu, H. Lycopene Is Enriched in Tomato Fruit by CRISPR/Cas9-Mediated Multiplex Genome Editing. *Front. Plant. Sci.* **2018**, *9*, 559. [[CrossRef](#)]
95. Tashkandi, M.; Ali, Z.; Aljedaani, F.; Shami, A.; Mahfouz, M.M. Engineering resistance against Tomato yellow leaf curl virus via the CRISPR/Cas9 system in tomato. *Plant. Signal. Behav.* **2018**, *13*, e1525996. [[CrossRef](#)]
96. Yu, Q.-H.; Wang, B.; Li, N.; Tang, Y.; Yang, S.; Yang, T.; Xu, J.; Guo, C.; Yan, P.; Wang, Q.; et al. CRISPR/Cas9-induced Targeted Mutagenesis and Gene Replacement to Generate Long-shelf Life Tomato Lines. *Sci. Rep.* **2017**, *7*, 11874. [[CrossRef](#)]
97. Nekrasov, V.; Wang, C.; Win, J.; Lanz, C.; Weigel, D.; Kamoun, S. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Sci. Rep.* **2017**, *7*, 482. [[CrossRef](#)] [[PubMed](#)]
98. Zsögön, A.; Cermak, T.; Voytas, D.; Peres, L.E.P. Genome editing as a tool to achieve the crop ideotype and de novo domestication of wild relatives: Case study in tomato. *Plant. Sci.* **2017**, *256*, 120–130. [[CrossRef](#)] [[PubMed](#)]
99. Soyk, S.; Müller, N.A.; Park, S.J.; Schmalenbach, I.; Jiang, K.; Hayama, R.; Zhang, L.; Van Eck, J.; Jiménez-Gómez, J.M.; Lippman, Z.B. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. *Nat. Genet.* **2016**, *49*, 162–168. [[CrossRef](#)] [[PubMed](#)]
100. Borovsky, Y.; Monsonego, N.; Mohan, V.; Shabtai, S.; Kamara, I.; Faigenboim, A.; Hill, T.; Chen, S.; Stoffel, K.; Van Deynze, A.; et al. The zinc-finger transcription factor Cc LOL 1 controls chloroplast development and immature pepper fruit color in Capsicum chinense and its function is conserved in tomato. *Plant. J.* **2019**, *99*, 41–55. [[CrossRef](#)]
101. Dunemann, F. New strategies for the development of haploid crop plants via genome elimination. *Jul.-Kühn-Arch.* **2016**, *457*, 40–45.
102. González, M.N.; Massa, G.A.; Andersson, M.; Turesson, H.; Olsson, N.; Fält, A.-S.; Storani, L.; Oneto, C.A.D.; Hofvander, P.; Feingold, S.E. Reduced Enzymatic Browning in Potato Tubers by Specific Editing of a Polyphenol Oxidase Gene via Ribonucleoprotein Complexes Delivery of the CRISPR/Cas9 System. *Front. Plant. Sci.* **2020**, *10*. [[CrossRef](#)]
103. Ye, M.; Peng, Z.; Tang, D.; Yang, Z.; Li, D.; Xu, Y.; Zhang, C.; Huang, S. Generation of self-compatible diploid potato by knockout of S-RNase. *Nat. Plants* **2018**, *4*, 651–654. [[CrossRef](#)]
104. Enciso-Rodriguez, F.; Manrique-Carpintero, N.C.; Nadakuduti, S.S.; Buell, C.R.; Zarka, D.; Douches, D. Overcoming Self-Incompatibility in Diploid Potato Using CRISPR-Cas9. *Front. Plant. Sci.* **2019**, *10*, 376. [[CrossRef](#)]
105. Nakayasu, M.; Akiyama, R.; Lee, H.J.; Osakabe, K.; Osakabe, Y.; Watanabe, B.; Sugimoto, Y.; Umemoto, N.; Saito, K.; Muranaka, T.; et al. Generation of α -solanine-free hairy roots of potato by CRISPR/Cas9 mediated genome editing of the St16DOX gene. *Plant. Physiol. Biochem.* **2018**, *131*, 70–77. [[CrossRef](#)]
106. Andersson, M.; Turesson, H.; Olsson, N.; Fält, A.-S.; Ohlsson, P.; Gonzalez, M.N.; Samuelsson, M.; Hofvander, P. Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. *Physiol. Plant.* **2018**, *164*, 378–384. [[CrossRef](#)] [[PubMed](#)]
107. Zhang, H.; Zhang, Q.; Zhai, H.; Gao, S.; Yang, L.; Wang, Z.; Xu, Y.; Huo, J.; Ren, Z.; Zhao, N.; et al. IbBBX24 Promotes the Jasmonic Acid Pathway and Enhances Fusarium Wilt Resistance in Sweet Potato. *Plant. Cell* **2020**, *32*, 1102–1123. [[CrossRef](#)]
108. Ren, Y.; Sun, H.; Zong, M.; Guo, S.; Ren, Z.; Zhao, J.; Li, M.; Zhang, J.; Tian, S.; Wang, J.; et al. Localization shift of a sugar transporter contributes to phloem unloading in sweet watermelons. *New Phytol.* **2020**, *227*, 1858–1871. [[CrossRef](#)]
109. Zhang, J.; Guo, S.; Ji, G.; Zhao, H.; Sun, H.; Ren, Y.; Tian, S.; Li, M.; Gong, G.; Zhang, H.; et al. A unique chromosome translocation disrupting CIWIP1 leads to gynoecey in watermelon. *Plant. J.* **2019**, *101*, 265–277. [[CrossRef](#)]
110. Zhang, M.; Liu, Q.; Yang, X.; Xu, J.; Liu, G.; Yao, X.; Ren, R.; Xu, J.; Lou, L. CRISPR/Cas9-mediated mutagenesis of Clpsk1 in watermelon to confer resistance to Fusarium oxysporum f.sp. niveum. *Plant. Cell Rep.* **2020**, *39*, 589–595. [[CrossRef](#)] [[PubMed](#)]
111. Guo, S.; Zhao, S.; Sun, H.; Wang, X.; Wu, S.; Lin, T.; Ren, Y.; Gao, L.; Deng, Y.; Zhang, J.; et al. Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. *Nat. Genet.* **2019**, *51*, 1616–1623. [[CrossRef](#)] [[PubMed](#)]

112. Tian, S.; Jiang, L.; Cui, X.; Zhang, J.; Guo, S.; Li, M.; Zhang, H.; Ren, Y.; Gong, G.; Zong, M.; et al. Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. *Plant. Cell Rep.* **2018**, *37*, 1353–1356. [[CrossRef](#)]
113. Huang, Y.; Cao, H.; Yang, L.; Chen, C.; Shabala, L.; Xiong, M.; Niu, M.; Liu, J.; Zheng, Z.; Zhou, L.; et al. Tissue-specific respiratory burst oxidase homolog-dependent H₂O₂ signaling to the plasma membrane H⁺-ATPase confers potassium uptake and salinity tolerance in Cucurbitaceae. *J. Exp. Bot.* **2019**, *70*, 5879–5893. [[CrossRef](#)] [[PubMed](#)]
114. Hu, B.; Li, D.; Liu, X.; Qi, J.; Gao, D.; Zhao, S.; Huang, S.; Sun, J.; Yang, L. Engineering Non-transgenic Gynoecious Cucumber Using an Improved Transformation Protocol and Optimized CRISPR/Cas9 System. *Mol. Plant.* **2017**, *10*, 1575–1578. [[CrossRef](#)] [[PubMed](#)]
115. Stajič, E.; Kiełkowska, A.; Murovec, J.; Bohanec, B. Deep sequencing analysis of CRISPR/Cas9 induced mutations by two delivery methods in target model genes and the CENH3 region of red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*). *Plant. Cell Tissue Organ. Cult.* **2019**, *139*, 227–235. [[CrossRef](#)]
116. Park, S.-C.; Park, S.; Jeong, Y.J.; Lee, S.B.; Pyun, J.W.; Kim, S.; Kim, T.H.; Kim, S.W.; Jeong, J.C.; Kim, C.Y. DNA-free mutagenesis of GIGANTEA in *Brassica oleracea* var. *capitata* using CRISPR/Cas9 ribonucleoprotein complexes. *Plant. Biotechnol. Rep.* **2019**, *13*, 483–489. [[CrossRef](#)]
117. Jeong, S.Y.; Ahn, H.; Ryu, J.; Oh, Y.; Sivanandhan, G.; Won, K.-H.; Park, Y.D.; Kim, J.-S.; Kim, H.; Lim, Y.P.; et al. Generation of early-flowering Chinese cabbage (*Brassica rapa* spp. *pekinensis*) through CRISPR/Cas9-mediated genome editing. *Plant. Biotechnol. Rep.* **2019**, *13*, 491–499. [[CrossRef](#)]
118. Ma, C.; Zhu, C.; Zheng, M.; Liu, M.; Zhang, D.; Liu, B.; Li, Q.; Si, J.; Ren, X.; Song, H. CRISPR/Cas9-mediated multiple gene editing in *Brassica oleracea* var. *capitata* using the endogenous tRNA-processing system. *Hortic. Res.* **2019**, *6*, 1–15. [[CrossRef](#)]
119. Bertier, L.D.; Ron, M.; Huo, H.; Bradford, K.J.; Britt, A.B.; Michelmore, R.W. High-Resolution Analysis of the Efficiency, Heritability, and Editing Outcomes of CRISPR/Cas9-Induced Modifications of nNCED4in Lettuce (*Lactuca sativa*). *G3 Genes Genomes Genet.* **2018**, *8*, 1513–1521. [[CrossRef](#)]
120. Gomez, M.A.; Lin, Z.D.; Moll, T.; Luebbert, C.; Chauhan, R.D.; Vijayaraghavan, A.; Kelley, R.; Beyene, G.; Taylor, N.J.; Carrington, J. Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 confers elevated resistance to cassava brown streak disease. *bioRxiv* **2017**, 209874. [[CrossRef](#)]
121. Martín-Pizarro, C.; Triviño, J.C.; Posé, D. Functional analysis of the TM6 MADS-box gene in the octoploid strawberry by CRISPR/Cas9-directed mutagenesis. *J. Exp. Bot.* **2019**, *70*, 885–895. [[CrossRef](#)]
122. Feng, J.; Dai, C.; Luo, H.; Han, Y.; Liu, Z.; Kang, C. Reporter gene expression reveals precise auxin synthesis sites during fruit and root development in wild strawberry. *J. Exp. Bot.* **2018**, *70*, 563–574. [[CrossRef](#)]
123. Jia, H.; Zhang, Y.; Orbović, V.; Xu, J.; White, F.F.; Jones, J.B.; Wang, N. Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. *Plant. Biotechnol. J.* **2017**, *15*, 817–823. [[CrossRef](#)]
124. Wang, L.; Chen, S.; Peng, A.; Xie, Z.; He, Y.; Zou, X. CRISPR/Cas9-mediated editing of CsWRKY22 reduces susceptibility to *Xanthomonas citri* subsp. *citri* in Wanjincheng orange (*Citrus sinensis* (L.) Osbeck). *Plant. Biotechnol. Rep.* **2019**, *13*, 501–510. [[CrossRef](#)]
125. Pompili, V.; Costa, L.D.; Piazza, S.; Pindo, M.; Malnoy, M. Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. *Plant. Biotechnol. J.* **2019**, *18*, 845–858. [[CrossRef](#)]
126. Tripathi, J.N.; Ntui, V.O.; Ron, M.; Muiruri, S.K.; Britt, A.; Tripathi, L. CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. *Commun. Biol.* **2019**, *2*, 1–11. [[CrossRef](#)] [[PubMed](#)]
127. Shao, X.; Wu, S.; Dou, T.; Zhu, H.; Hu, C.; Huo, H.; He, W.; Deng, G.; Sheng, O.; Bi, F.; et al. Using CRISPR/Cas9 genome editing system to create MaGA20ox2 gene-modified semi-dwarf banana. *Plant. Biotechnol. J.* **2019**, *18*, 17–19. [[CrossRef](#)]
128. Ren, F.; Ren, C.; Zhang, Z.; Duan, W.; Lecourieux, D.; Li, S.; Liang, Z. Efficiency Optimization of CRISPR/Cas9-Mediated Targeted Mutagenesis in Grape. *Front. Plant. Sci.* **2019**, *10*, 612. [[CrossRef](#)] [[PubMed](#)]
129. Wang, X.; Tu, M.; Wang, D.; Liu, J.; Li, Y.; Li, Z.; Wang, Y.; Wang, X. CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. *Plant. Biotechnol. J.* **2017**, *16*, 844–855. [[CrossRef](#)] [[PubMed](#)]

130. Gumtow, R.; Wu, D.; Uchida, J.Y.; Tian, M. A Phytophthora palmivora Extracellular Cystatin-Like Protease Inhibitor Targets Papain to Contribute to Virulence on Papaya. *Mol. Plant.-Microbe Interact.* **2018**, *31*, 363–373. [[CrossRef](#)] [[PubMed](#)]
131. Fister, A.S.; Landherr, L.; Maximova, S.N.; Guiltinan, M.J. Transient Expression of CRISPR/Cas9 Machinery Targeting TeNPR3 Enhances Defense Response in Theobroma cacao. *Front. Plant. Sci.* **2018**, *9*, 9. [[CrossRef](#)]
132. Breitler, J.-C.; Dechamp, E.; Campa, C.; Rodrigues, L.A.Z.; Guyot, R.; Marraccini, P.; Etienne, H. CRISPR/Cas9-mediated efficient targeted mutagenesis has the potential to accelerate the domestication of Coffea canephora. *Plant. Cell Tissue Organ. Cult.* **2018**, *134*, 383–394. [[CrossRef](#)]
133. Xu, J.; Kang, B.; Naing, A.H.; Bae, S.; Kim, J.; Kim, H.; Kil Kim, C. CRISPR /Cas9-mediated editing of 1-aminocyclopropane-1-carboxylate oxidase1 enhances Petunia flower longevity. *Plant. Biotechnol. J.* **2019**, *18*, 287–297. [[CrossRef](#)] [[PubMed](#)]
134. Tong, C.; Wu, F.; Yuan, Y.; Chen, Y.; Lin, C. High-efficiency CRISPR/Cas-based editing of Phalaenopsis orchid MADS genes. *Plant. Biotechnol. J.* **2019**, *18*, 889–891. [[CrossRef](#)]
135. Kishi-Kaboshi, M.; Aida, R.; Sasaki, K. Generation of Gene-Edited Chrysanthemum morifolium Using Multi-Copy Transgenes as Targets and Markers. *Plant. Cell Physiol.* **2017**, *58*, 216–226. [[CrossRef](#)]
136. Shibuya, K.; Watanabe, K.; Ono, M. CRISPR/Cas9-mediated mutagenesis of the EPHEMERAL1 locus that regulates petal senescence in Japanese morning glory. *Plant. Physiol. Biochem.* **2018**, *131*, 53–57. [[CrossRef](#)] [[PubMed](#)]
137. Watanabe, K.; Oda-Yamamizo, C.; Sage-Ono, K.; Ohmiya, A.; Ono, M. Alteration of flower colour in Ipomoea nil through CRISPR/Cas9-mediated mutagenesis of carotenoid cleavage dioxygenase 4. *Transgenic Res.* **2017**, *27*, 25–38. [[CrossRef](#)] [[PubMed](#)]
138. Lei, Y.; Lu, L.; Liu, H.-Y.; Li, S.; Xing, F.; Chen, L.-L. CRISPR-P: A Web Tool for Synthetic Single-Guide RNA Design of CRISPR-System in Plants. *Mol. Plant.* **2014**, *7*, 1494–1496. [[CrossRef](#)]
139. Naito, Y.; Hino, K.; Bono, H.; Ui-Tei, K. CRISPRdirect: Software for designing CRISPR/Cas guide RNA with reduced off-target sites. *Bioinformatics* **2015**, *31*, 1120–1123. [[CrossRef](#)]
140. Labun, K.; Montague, T.G.; Gagnon, J.A.; Thyme, S.B.; Valen, E. CHOPCHOP v2: A web tool for the next generation of CRISPR genome engineering. *Nucleic Acids Res.* **2016**, *44*, W272–W276. [[CrossRef](#)] [[PubMed](#)]
141. Benchling Quick and Easy CRISPR Designs. Available online: <https://benchling.com/crispr> (accessed on 2 October 2020).
142. Addgene CRISPR Plasmids: Plants. Available online: <http://www.addgene.org/crispr/plant/> (accessed on 2 October 2020).
143. Xing, H.-L.; Dong, L.; Wang, Z.-P.; Zhang, H.-Y.; Han, C.-Y.; Liu, B.; Wang, X.-C.; Chen, Q.-J. A CRISPR/Cas9 toolkit for multiplex genome editing in plants. *BMC Plant. Biol.* **2014**, *14*, 327. [[CrossRef](#)] [[PubMed](#)]
144. Čermák, T.; Baltés, N.J.; Čegan, R.; Zhang, Y.; Voytas, D.F. High-frequency, precise modification of the tomato genome. *Genome Biol.* **2015**, *16*, 232. [[CrossRef](#)]
145. Ma, X.; Zhang, Q.; Zhu, Q.; Liu, W.; Chen, Y.; Qiu, R.; Wang, B.; Yang, Z.; Li, H.; Lin, Y.; et al. A Robust CRISPR/Cas9 System for Convenient, High-Efficiency Multiplex Genome Editing in Monocot and Dicot Plants. *Mol. Plant.* **2015**, *8*, 1274–1284. [[CrossRef](#)]
146. Xie, K.; Minkenberg, B.; Yang, Y. Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 3570–3575. [[CrossRef](#)]
147. Khan, A.A.; El-Sayed, A.; Akbar, A.; Mangravita-Novo, A.; Bibi, S.; Afzal, Z.; Norman, D.J.; Ali, G.S. A highly efficient ligation-independent cloning system for CRISPR/Cas9 based genome editing in plants. *Plant. Methods* **2017**, *13*, 86. [[CrossRef](#)]
148. Snapgene Tutorial Videos. Available online: <https://www.snapgene.com/support/tutorial-videos/> (accessed on 2 October 2020).
149. Jiang, W.; Zhou, H.; Bi, H.; Fromm, M.; Yang, B.; Weeks, D.P. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. *Nucleic Acids Res.* **2013**, *41*, e188. [[CrossRef](#)] [[PubMed](#)]
150. Mikami, M.; Toki, S.; Endo, M. Comparison of CRISPR/Cas9 expression constructs for efficient targeted mutagenesis in rice. *Plant. Mol. Biol.* **2015**, *88*, 561–572. [[CrossRef](#)] [[PubMed](#)]
151. Butler, N.M.; Atkins, P.A.; Voytas, D.F.; Douches, D.S. Generation and Inheritance of Targeted Mutations in Potato (*Solanum tuberosum* L.) Using the CRISPR/Cas System. *PLoS ONE* **2015**, *10*, e0144591. [[CrossRef](#)] [[PubMed](#)]

152. Endo, M.; Mikami, M.; Toki, S. Multigene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice. *Plant. Cell Physiol.* **2015**, *56*, 41–47. [[CrossRef](#)] [[PubMed](#)]
153. Pan, C.; Ye, L.; Qin, L.; Liu, X.; He, Y.; Wang, J.; Chen, L.; Lu, G. CRISPR/Cas9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations. *Sci. Rep.* **2016**, *6*, 24765. [[CrossRef](#)]
154. Char, S.N.; Neelakandan, A.K.; Nahampun, H.; Frame, B.; Main, M.; Spalding, M.H.; Becraft, P.W.; Meyers, B.C.; Walbot, V.; Wang, K.; et al. An Agrobacterium-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize. *Plant. Biotechnol. J.* **2017**, *15*, 257–268. [[CrossRef](#)]
155. Zhang, S.; Zhang, R.; Song, G.; Gao, J.; Li, W.; Han, X.; Chen, M.; Li, Y.; Li, G. Targeted mutagenesis using the Agrobacterium tumefaciens-mediated CRISPR-Cas9 system in common wheat. *BMC Plant. Biol.* **2018**, *18*, 302. [[CrossRef](#)]
156. Osakabe, Y.; Liang, Z.; Ren, C.; Nishitani, C.; Osakabe, K.; Wada, M.; Komori, S.; Malnoy, M.; Velasco, R.; Poli, M.; et al. CRISPR–Cas9-mediated genome editing in apple and grapevine. *Nat. Protoc.* **2018**, *13*, 2844–2863. [[CrossRef](#)]
157. Lee, K.; Zhu, H.; Yang, B.; Wang, K. An Agrobacterium-mediated CRISPR/Cas9 platform for genome editing in maize. In *Plant Genome Editing with CRISPR Systems*; Springer: Berlin, Germany, 2019; pp. 121–143.
158. Reem, N.T.; Van Eck, J. Application of CRISPR/Cas9-mediated gene editing in tomato. In *Plant Genome Editing with CRISPR Systems*; Springer: Berlin, Germany, 2019; pp. 171–182.
159. Sun, Y.; Zhang, X.; Wu, C.; He, Y.; Ma, Y.; Hou, H.; Guo, X.; Du, W.; Zhao, Y.; Xia, L. Engineering Herbicide-Resistant Rice Plants through CRISPR/Cas9-Mediated Homologous Recombination of Acetolactate Synthase. *Mol. Plant.* **2016**, *9*, 628–631. [[CrossRef](#)]
160. Hamada, H.; Liu, Y.; Nagira, Y.; Miki, R.; Taoka, N.; Imai, R. Biolistic-delivery-based transient CRISPR/Cas9 expression enables in planta genome editing in wheat. *Sci. Rep.* **2018**, *8*, 14422. [[CrossRef](#)]
161. Ali, Z.; Abulfaraj, A.; Idris, A.; Ali, S.; Tashkandi, M.; Mahfouz, M.M. CRISPR/Cas9-mediated viral interference in plants. *Genome Biol.* **2015**, *16*, 238. [[CrossRef](#)] [[PubMed](#)]
162. Yin, K.; Han, T.; Liu, G.; Chen, T.; Wang, Y.; Yu, A.Y.L.; Liu, Y. A geminivirus-based guide RNA delivery system for CRISPR/Cas9 mediated plant genome editing. *Sci. Rep.* **2015**, *5*, 14926. [[CrossRef](#)] [[PubMed](#)]
163. Demirer, G.S.; Zhang, H.; Goh, N.S.; González-Grandío, E.; Landry, M.P. Carbon nanotube-mediated DNA delivery without transgene integration in intact plants. *Nat. Protoc.* **2019**, *14*, 2954–2971. [[CrossRef](#)] [[PubMed](#)]
164. Chandrasekaran, R.; Rajiv, P.; Abd-Elsalam, K.A. Carbon nanotubes: Plant gene delivery and genome editing. In *Carbon Nanomaterials for Agri-Food and Environmental Applications*; Elsevier BV: Amsterdam, The Netherlands, 2020; pp. 279–296.
165. Tian, S.; Jiang, L.; Gao, Q.; Zhang, J.; Zong, M.; Zhang, H.; Ren, Y.; Guo, S.; Gong, G.; Liu, F.; et al. Efficient CRISPR/Cas9-based gene knockout in watermelon. *Plant. Cell Rep.* **2016**, *36*, 399–406. [[CrossRef](#)] [[PubMed](#)]
166. Brandt, K.M.; Gunn, H.; Moretti, N.; Zemetra, R.S. A Streamlined Protocol for Wheat (*Triticum aestivum*) Protoplast Isolation and Transformation with CRISPR-Cas Ribonucleoprotein Complexes. *Front. Plant. Sci.* **2020**, *11*, 769. [[CrossRef](#)]
167. Xu, R.-F.; Li, H.; Qin, R.-Y.; Li, J.; Qiu, C.-H.; Yang, Y.-C.; Ma, H.; Li, L.; Wei, P.-C.; Yang, J.-B. Generation of inheritable and “transgene clean” targeted genome-modified rice in later generations using the CRISPR/Cas9 system. *Sci. Rep.* **2015**, *5*, 11491. [[CrossRef](#)]
168. Zhang, Y.; Liang, Z.; Zong, Y.; Wang, Y.; Liu, J.; Chen, K.; Qiu, J.-L.; Gao, C. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nat. Commun.* **2016**, *7*, 12617. [[CrossRef](#)]
169. Hua, Y.; Wang, C.; Huang, J.; Wang, K. A simple and efficient method for CRISPR/Cas9-induced mutant screening. *J. Genet. Genom.* **2017**, *44*, 207–213. [[CrossRef](#)]
170. Zhang, Z.; Ge, X.; Luo, X.; Wang, P.; Fan, Q.; Hu, G.; Xiao, J.; Li, F.; Wu, J. Simultaneous Editing of Two Copies of Gh14-3-3d Confers Enhanced Transgene-Clean Plant Defense Against *Verticillium dahliae* in Allotetraploid Upland Cotton. *Front. Plant. Sci.* **2018**, *9*, 842. [[CrossRef](#)]
171. Kim, D.; Alptekin, B.; Budak, H. CRISPR/Cas9 genome editing in wheat. *Funct. Integr. Genom.* **2017**, *18*, 31–41. [[CrossRef](#)]
172. Brinkman, E.K.; Chen, T.; Amendola, M.; Van Steensel, B. Easy quantitative assessment of genome editing by sequence trace decomposition. *Nucleic Acids Res.* **2014**, *42*, e168. [[CrossRef](#)]

173. Braatz, J.; Harloff, H.-J.; Mascher, M.; Stein, N.; Himmelbach, A.; Jung, C. CRISPR-Cas9 Targeted Mutagenesis Leads to Simultaneous Modification of Different Homoeologous Gene Copies in Polyploid Oilseed Rape (*Brassica napus*). *Plant. Physiol.* **2017**, *174*, 935–942. [[CrossRef](#)] [[PubMed](#)]
174. Feng, C.; Su, H.; Bai, H.; Wang, R.; Liu, Y.; Guo, X.; Liu, C.; Zhang, J.; Yuan, J.; Birchler, J.A.; et al. High-efficiency genome editing using a dmc1 promoter-controlled CRISPR/Cas9 system in maize. *Plant. Biotechnol. J.* **2018**, *16*, 1848–1857. [[CrossRef](#)] [[PubMed](#)]
175. Tang, X.; Liu, G.; Zhou, J.; Ren, Q.; You, Q.; Tian, L.; Xin, X.; Zhong, Z.; Liu, B.; Zheng, X.; et al. A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. *Genome Biol.* **2018**, *19*, 84. [[CrossRef](#)] [[PubMed](#)]
176. Nadakuduti, S.S.; Buell, C.R.; Voytas, D.F.; Starker, C.G.; Douches, D.S. Genome Editing for Crop Improvement—Applications in Clonally Propagated Polyploids With a Focus on Potato (*Solanum tuberosum* L.). *Front. Plant. Sci.* **2018**, *9*, 1607. [[CrossRef](#)] [[PubMed](#)]
177. Xu, R.; Yang, Y.; Qin, R.; Li, H.; Qiu, C.; Li, L.; Wei, P.; Yang, J. Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *J. Genet. Genom.* **2016**, *43*, 529–532. [[CrossRef](#)] [[PubMed](#)]
178. Shi, J.; Gao, H.; Wang, H.; Lafitte, H.R.; Archibald, R.L.; Yang, M.; Hakimi, S.M.; Mo, H.; Habben, J.E. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant. Biotechnol. J.* **2016**, *15*, 207–216. [[CrossRef](#)]
179. Nieves-Cordones, M.; Mohamed, S.; Tanoi, K.; Kobayashi, N.I.; Takagi, K.; Vernet, A.; Guiderdoni, E.; Périn, C.; Sentenac, H.; Véry, A.-A. Production of low-Cs⁺ rice plants by inactivation of the K⁺ transporter Os HAK 1 with the CRISPR -Cas system. *Plant. J.* **2017**, *92*, 43–56. [[CrossRef](#)] [[PubMed](#)]
180. Haque, E.; Taniguchi, H.; Hassan, M.; Bhowmik, P.; Karim, M.R.; Śmiech, M.; Zhao, K.; Rahman, M.; Islam, T. Application of CRISPR/Cas9 Genome Editing Technology for the Improvement of Crops Cultivated in Tropical Climates: Recent Progress, Prospects, and Challenges. *Front. Plant. Sci.* **2018**, *9*, 617. [[CrossRef](#)]
181. Woo, J.W.; Kim, J.; Kwon, S.I.; Corvalán, C.; Cho, S.W.; Kim, H.; Kim, S.-G.; Kim, S.-T.; Choe, S.; Kim, J.-S. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nat. Biotechnol.* **2015**, *33*, 1162–1164. [[CrossRef](#)]
182. Kim, J.; Kim, J.-S. Bypassing GMO regulations with CRISPR gene editing. *Nat. Biotechnol.* **2016**, *34*, 1014–1015. [[CrossRef](#)]
183. Hilscher, J.; Bürstmayr, H.; Stoger, E. Targeted modification of plant genomes for precision crop breeding. *Biotechnol. J.* **2016**, *12*, 1600173. [[CrossRef](#)] [[PubMed](#)]
184. Ishii, T.; Araki, M. A future scenario of the global regulatory landscape regarding genome-edited crops. *GM Crop. Food* **2017**, *8*, 44–56. [[CrossRef](#)] [[PubMed](#)]
185. Waltz, E. *CRISPR-Edited Crops Free to Enter. Market., Skip Regulation*; Nature Publishing Group: Berlin, Germany, 2016.
186. Sander, J.; Jeschke, M. CRISPR-Cas Advanced Plant Breeding. *Crop Insights* **2016**, *26*, 18. Available online: https://crisprcas.pioneer.com/wp-content/uploads/2017/01/CRISPR-Cas_Advanced_Plant_Breeding_CII161215.pdf (accessed on 1 September 2020).
187. Wiczorek, A.; Wright, M.G. History of agricultural biotechnology: How crop development has evolved. *Nat. Educ. Knowl.* **2012**, *3*, 9.
188. Kim, H.; Kim, J.-S. A guide to genome engineering with programmable nucleases. *Nat. Rev. Genet.* **2014**, *15*, 321–334. [[CrossRef](#)]
189. Ishii, T.; Araki, M. Consumer acceptance of food crops developed by genome editing. *Plant. Cell Rep.* **2016**, *35*, 1507–1518. [[CrossRef](#)]
190. Globus, R.; Qimron, U. A technological and regulatory outlook on CRISPR crop editing. *J. Cell. Biochem.* **2017**, *119*, 1291–1298. [[CrossRef](#)]
191. Liu, X.; Xie, C.; Si, H.; Yang, J. CRISPR/Cas9-mediated genome editing in plants. *Methods* **2017**, *121*, 94–102. [[CrossRef](#)]
192. USDA Re: Confirmation of Regulatory Status of Waxy Com Developed by CRISPR-Cas Technology. Available online: https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-352-01_air_response_signed.pdf (accessed on 2 October 2020).

193. USDA Re: Request for Confirmation that Transgene-Free, CRISPR-Edited Mushroom Is Not a Regulated Article. Available online: https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-321-01_air_response_signed.pdf (accessed on 2 October 2020).
194. Songstad, D.D.; Petolino, J.F.; Voytas, D.F.; Reichert, N.A. Genome Editing of Plants. *Crit. Rev. Plant. Sci.* **2017**, *36*, 1–23. [CrossRef]
195. USDA Secretary Perdue Issues USDA Statement on Plant Breeding Innovation. Available online: <https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation> (accessed on 2 October 2020).
196. USDA-APHIS Regulated Article Letters of Inquiry. Available online: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/Regulated_Article_Letters_of_Inquiry (accessed on 2 October 2020).
197. Barrangou, R. *Finding SECURE Ground: USDA Edits the Biotechnology Regulatory Framework*; Mary Ann Liebert, Inc.: New Rochelle, NY, USA, 2020.
198. USDA-APHIS about the SECURE Rule. Available online: <https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/biotech-rule-revision> (accessed on 2 October 2020).
199. Agency, C.F.I. Regulatory Oversight of Plant Products Developed using Biotechnology. Available online: <https://www.inspection.gc.ca/plant-varieties/plants-with-novel-traits/gene-editing-techniques/eng/1541800629219/1541800629556> (accessed on 2 October 2020).
200. Wight, A.J. Strict EU ruling on gene-edited crops squeezes science. *Nature* **2018**, *563*, 15–16. [CrossRef]
201. Callaway, E. CRISPR plants now subject to tough GM laws in European Union. *Nature* **2018**, *560*, 16. [CrossRef] [PubMed]
202. Mallapaty, S. Australian gene-editing rules adopt ‘middle ground’. *Nature* **2019**, *10*. [CrossRef] [PubMed]
203. Metje-Sprink, J.; Sprink, T.; Hartung, F. Genome-edited plants in the field. *Curr. Opin. Biotechnol.* **2020**, *61*, 1–6. [CrossRef] [PubMed]
204. MOEF Rules for the Manufacture, Use/Import/Export and Storage of Hazardous Micro Organisms/ genetically Engineered Organisms or Cells. Available online: <https://geacindia.gov.in/resource-documents/biosafety-regulations/acts-and-rules/Rules-for-the-manufacture-use-import-export-and-storage-1989.pdf> (accessed on 2 October 2020).
205. Pillay, S.; Thaldar, D. CRISPR: Challenges to South African biotechnology law. *S. Afr. J. Bioeth. Law* **2018**, *11*, 89–92. [CrossRef]
206. MALF Resolution 173/2015. Available online: <http://servicios.infoleg.gob.ar/infolegInternet/anexos/245000-249999/246978/norma.htm> (accessed on 2 October 2020).
207. Synthego CRISPR in Agriculture: An Era of Food Evolution. Available online: <https://www.synthego.com/blog/crispr-agriculture-foods#crops> (accessed on 2 October 2020).
208. Calyxt, I. First Commercial Sale of Calyxt High Oleic Soybean Oil on the U.S. Market. Available online: <https://calyxt.com/first-commercial-sale-of-calyxt-high-oleic-soybean-oil-on-the-u-s-market/> (accessed on 2 October 2020).
209. Bartkowski, B.; Theesfeld, I.; Pirscher, F.; Timaeus, J. Snipping around for food: Economic, ethical and policy implications of CRISPR/Cas genome editing. *Geoforum* **2018**, *96*, 172–180. [CrossRef]
210. Wang, T.; Zhang, H.; Zhu, H. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. *Hortic. Res.* **2019**, *6*, 77. [CrossRef]
211. Bain, C.; Lindberg, S.; Selfa, T. Emerging sociotechnical imaginaries for gene edited crops for foods in the United States: Implications for governance. *Agric. Hum. Values* **2019**, *37*, 265–279. [CrossRef]

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).