Review

Development of Climate Smart Fruit Plants via CRISPR/Cas Genome Editing Systems: A Spatiotemporal Review

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Abstract

Fruit production is an important part of the gross domestic product for many countries around the world especially to those who have a strong focus on agriculture. However, long-term maintenance and yield stability of fruit production may be threatened by the ongoing climate change and its consequences like extended drought periods, heavy rain events, and floodings. Genome editing, with its progressive technological developments, offers opportunities to adapt relevant fruit plant species to new climatic conditions. Among modern genome editing techniques, CRISPR/Cas, in particular, has the potential to support breeding for those fruit plant species with extended breeding cycles, e.g., perennial fruits. In this review, we discuss CRISPR/Cas and other genome editing techniques in detail and how these techniques can be applied to support the breeding of fruit plant species for adaptation to changing climates. The chronological history of CRISPR/Cas9 systems, their associated computational tools, genomic data sources, transformation methods along with their delivery

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vehicles, quality improvement, environmental-stress resiliency, limitations, and future perspectives will also be discussed with respect to securing future global fruit production.

Keywords: CRISPR/Cas; TALEN; ZFN; Fruit Plants; Genome Editing Systems

Introduction

Global climate change patterns are one of the main concerns for developing agricultural systems and the food insecurity problem. Greenhouse emissions and the subsequent accumulation of these gases in Earth's atmosphere are one of its leading causes. Even with taking proper measures to control the emissions, the damage that has already been done is going to take millennia to fully recover. All of these abrupt changes had a negative impact on the outputs of agricultural systems all around the world. As a result of droughts and floods, the global food supply is constantly declining (Nunez et al. 2019). Agriculture accounts for about 16.47% of China's total gross domestic product (GDP). Similarly, for India, it's 20.19%, for the United States 5.4%, for Turkey 5.54%, for Mexico 3.89%, and, for Brazil, it's a staggering 29%. All these countries are the top fruit producers globally and a significant portion of their GDP depends upon agriculture and agriculture-related industries. It is of foremost importance to develop fruit crops that are resilient to these environmental changes.

Our current gene editing systems can tackle these climate-related consequences by modifying the fruit plants in such a way that they adapt to the new climate by rendering their effects neutral. The gene editing method involves precise tampering with the DNA that leads to the knockdown of one or a set of genes which mostly leads to the loss of function of a trait.

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On the contrary, it can also be used to precisely insert our gene of interest that results in knock-in mutants. The well-known and researched gene editing systems include Zinc Finger Nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas (Khalil 2020). The CRISPR/Cas systems lead the packs in terms of precision, accuracy, and efficiency (Chakrabarti et al. 2019). All of these gene editing systems have paved a streamlined way to develop climate-smart or resilient plants.

Climate-smart fruits have also proven to be the great wall against food insecurity problems worldwide (Kole 2020). As an example, banana has been successfully made resistant to abiotic stress by targeting the semi-dwarfed trait. Here, CRISPR/Cas9 was used to modify five *GA20ox2* homologous genes in banana (Ma04g15900, Ma06g27710, Ma08g32850, Ma11g10500, and Ma11g17210). These gibberellin biosynthesis genes have been knocked out to establish semi-dwarfism which makes the banana plant more resistant to lodging during severe climate conditions (Shao et al. 2020). Worldwide fruit production has increased a little bit since these gene editing systems have gone mainstream development. The latest global fruit production statistics can be visualized in the graph illustrated in Figure 1.

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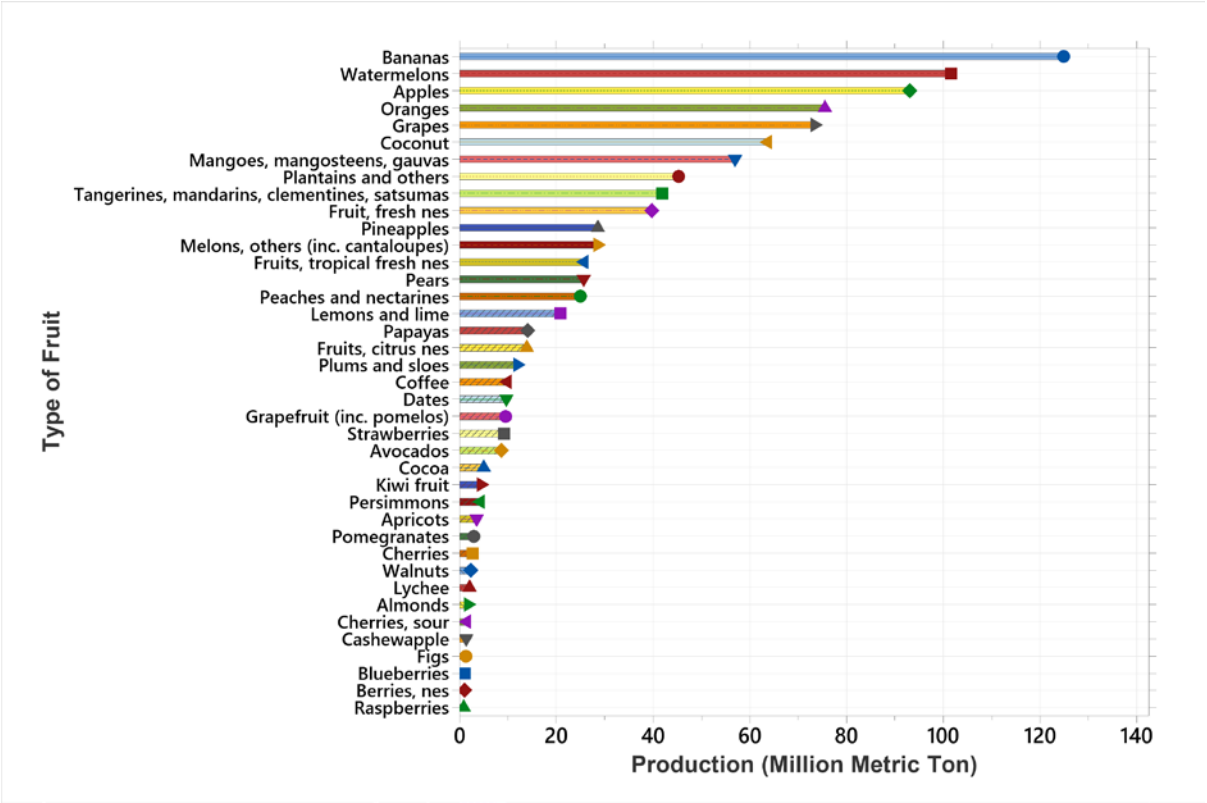

Figure 1: Descriptive statistics of global fruit production from the data taken in year 2020. The graph shows the production in million metric tons including the world's major fruits like banana, watermelon, apple, and orange, to minor contributors raspberry, fig, and cashew apple.

On the other hand, New Plant Breeding Techniques (NPBTs) are based on highly advanced molecular methods with more precision and accuracy than ever before. Both old and new plant breeding strategies improved concerning quality, quantity, and climate resilience. Similarly, to achieve the targets in fruits, different NPBTs have been utilized, e.g., genome-assisted breeding (GAB), mutation breeding, transgenic breeding, and currently, genome editing plant improvement systems (Salonia et al. 2020; Campoy et al. 2020; A. Brown, Carpentier, and Swennen 2020; Boudichevskaia et al. 2020; Basu 2020; Rugienius et al. 2020; Ramesh, Arunachalam, and Rajesh 2020; Zambounis et al. 2020; Delrot et al. 2020a; Gogorcena et al. 2020; Delrot et al. 2020b).

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This review focusses on the recent developments and efforts of these genome editing technologies, especially CRISPR/Cas systems and their applications in the development of climate-smart fruit crops, and to achieve secure future global food production goals with limitations and new opportunities that lie ahead of us.

A Brief History of Genome Editing Systems and their Mechanisms

In the past few years, several advanced genome editing tools have been developed that have paved the way to take genetic engineering to a new level of precision and specificity. These systems have different levels of priority in their utilization in different experiments due to variations and differences in their properties. The journey of genome editing begins with the development of the most notable systems including mega-nucleases and Zinc Finger Nucleases (ZFNs) (Y. G. Kim, Cha, and Chandrasegaran 1996). Then, Transcription Activator-Like Effector Nucleases (TALENs) came into action in 2009 and were identified as an efficient and highly specific genetic engineering tool, honored as “tool of the year” in 2011 (“Method of the Year 2011” 2011; Boch et al. 2009).

The CRISPR/Cas genome editing system was discovered in bacteria and utilized in plant sciences in 2013 (Shan et al. 2013; Nekrasov et al. 2013; Miao et al. 2013; W. Jiang et al. 2013; Xie and Yang 2013; Feng et al. 2013; J. F. Li et al. 2013a). Currently, several advanced versions of the CRISPR/Cas system including cytosine base editor (CBE) that allows C to T conversions, adenine base editor (ABE) that allow A to G conversions, prime editors, CRISPR-Transposases, CRISPR activation (CRISPRa), CRISPR (CRISPRi), Programmable Addition via Site-specific Targeting Elements (PASTE) (Ioannidi et al. 2021), etc., have been developed and are being used for improvement, acceleration of plant breeding

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programs, and functional characterization of newly identified genes. A general mechanism of restriction enzyme cutting in action and the timeline of all other genome editing systems preceding CRISPR/Cas systems have been given in Figures 2, 3, 4, and 5 in respective chronological order. Furthermore, a detailed and contrasting comparison of genome editing systems like Oligonucleotide-Directed Mutagenesis (ODM), ZFNs, TALENs, and CRISPR/Cas systems are provided in Table 1.

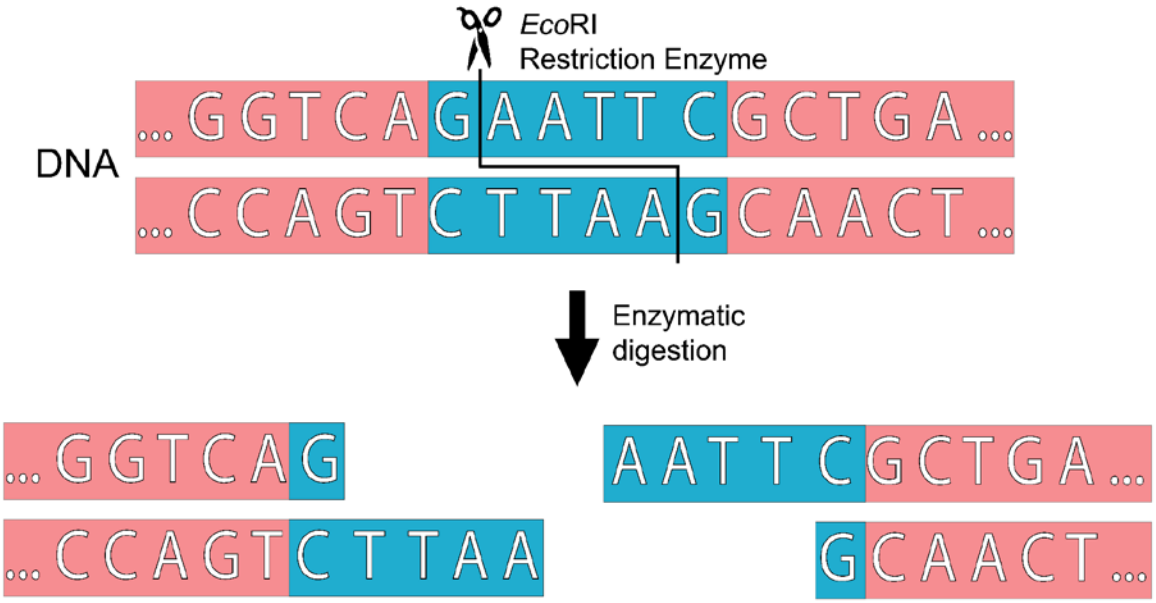

Figure 2: Restriction Enzymes (RE): the first true genome editors (1970)

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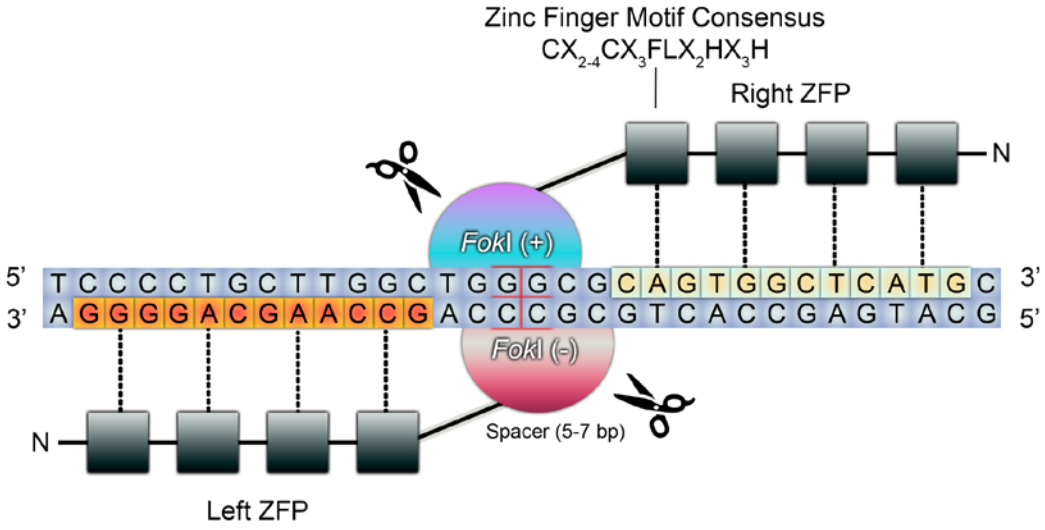

Figure 3: Zinc Finger Nucleases (ZFNs): the masters of recognition (1985)

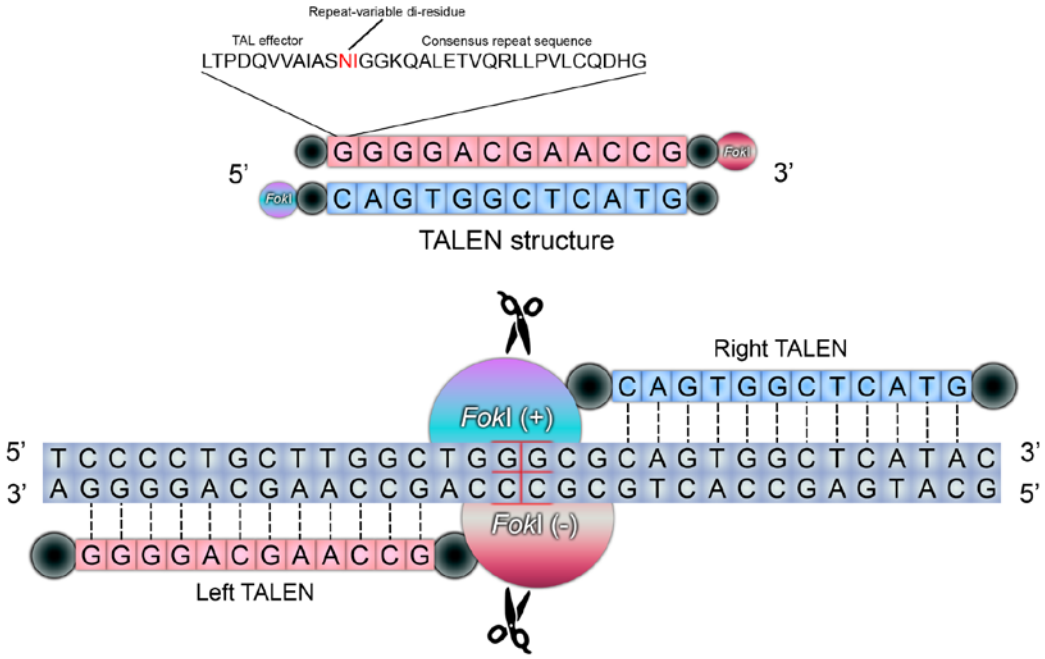

Figure 4: Transcription Activator-Like Effector Nucleases (TALENs): the experts of single nucleotide resolution (2010)

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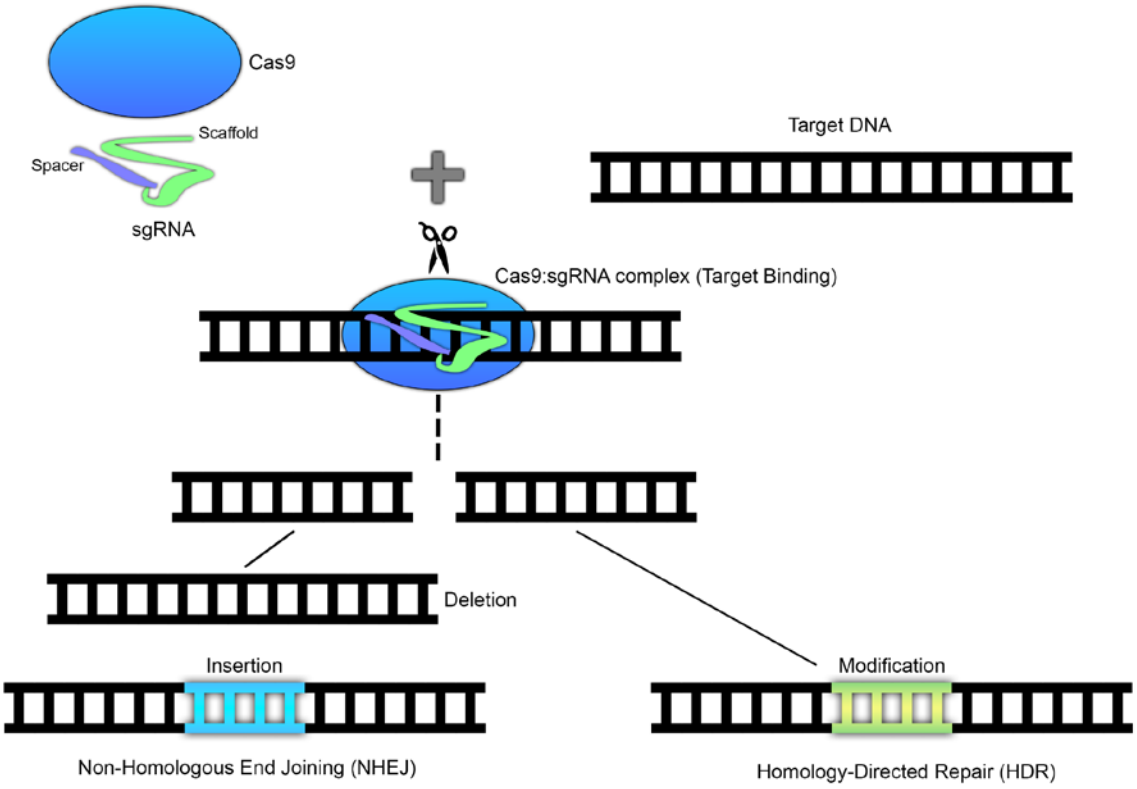

Figure 5: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR-associated protein 9 (Cas9): the revolution of precision genome editing (2012)

Table 1: Comparison of genome editing techniques in plants

Properties	***ODM***	***ZFNs***	***TALENs***	***CRISPR/Cas 9***	***Reference(s)***
Multiplexing Capability	Difficult	Difficult	Difficult	Possible	(Mao et al. 2013) (Noman, Aqeel, and He 2016)
Mutation Rate	Medium	High	Medium	Low	(Gaj, Gersbach, and Barbas 2013)

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Mode of Action	Conversion within the target region (sense strand directed)	Breaks in target DNA (Double strand)	Breaks in target DNA (Double strand)	Breaks in target DNA (Double strand)	(Mao et al. 2013)
Cloning	Not required	Necessary	Necessary	Not Required (Usually)	(Sauer et al. 2016)
Target Sequence Length [bp]	70-86	26-34	26-57	20-22	(F. Chen et al. 2017)
Targeting Efficiency	Relatively high	High	High	Very high	(Gaj, Gersbach, and Barbas 2013)
Components	Chimeraplast	Zn finger *Fok*I nuclease domain (non-specific)	TALE DNA-binding *Fok*I nuclease domain (non-specific)	Cas9 proteins, sgRNA	(V. Kumar and Jain 2015)
Structural Protein Composition	Non-protein	Proteins (dimeric)	Proteins (dimeric)	Protein (monomeric)	(J. F. Li et al. 2013b)
Catalytic Domains	Catalytic domain absent	Restriction endonuclease *Fok*I	Restriction endonuclease *Fok*I	HNH and RuvC	(Sauer et al. 2016)

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Large-Scale Libraries	Difficult	Difficult	Very Difficult	Possible	(Hsu et al. 2013)
Engineering Steps for Protein	Not required	Required	Required	Required	(Cho et al. 2013)

Key Aspects of Fruit Plant Modification through Genome Editing Systems

Generally, genome editing technology can be used mainly for any of the following two purposes. First, for plant modification or the development of their breeding material, and second is to characterize the function of genes in plants enabling also indirect plant improvement if the characterized genes are considered in conventional breeding. However, there are several key steps involved in CRISPR/Cas experiments to fulfill any of the mentioned goals. To go for either option, in fruits, the first step is the availability of the gene sequence of the target species. In this regard, genomes of various fruit species are freely available and can be used according to our needs and objectives. An exhaustive list of the genomes of major fruit species and their accompanying details have been supplied in Table S1 (Supplementary File 1) (Alioto et al. 2020; Velasco et al. 2010; F. Jiang et al. 2019; Dash and Rai 2016; Argout et al. 2010; Lantican et al. 2019; Xiao et al. 2017; Scalabrin et al. 2020; Al-Mssallem et al. 2013; Chagné et al. 2014; Mori et al. 2017; Mittal et al. 2020; Q. gang Zhu et al. 2019; Huang et al. 2013; Arumuganathan and Earle 1991; Ming et al. 2008; Verde et al. 2013; Wu et al. 2013; Ming et al. 2015; H. Xu et al. 2018; Liu et al. 2020; Luo et al. 2020; J. Wang et al. 2020; Q. Xu et al. 2012; Jaillon et al. 2007; Martínez-García et al. 2016;

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He et al. 2013). The second step involves the selection of target gene mining from the genome and then a specific region to be targeted by the CRISPR/Cas genome editing system (H. Li et al. 2020; Yi Zhang et al. 2018; Tabassum et al. 2021).

One of the ways to identify the target gene for plant improvement is a sound literature review. Different susceptibility or sensitivity-causing genes can be identified from available literature, partly transferred from other plant species, and can be targeted by a CRISPR/Cas approach in fruits for their improvement. The transferability of candidate genes for genome editing in a selected plant species appears to be particularly promising if functional domains of the related protein match in the source and target species. For this purpose, functional domains can be identified based on the gene or amino acid sequences with freely available online tools and analyzed with respect to their function such as CRISPRscan, CCTop, and Cas-OFFinder. A detailed list of modern mainstream single guide RNA (sgRNA) designing tools has been provided in Table 2. Moreover, target genes can also be identified through differential transcriptome analysis of plants under certain stress treatments. Afterward, the sgRNAs are cloned into a suitable CRISPR/Cas vector. Then, that vector is transformed into the targeted plant species through direct or indirect transformation methods. Finally, CRISPR/Cas-edited mutant plants are screened and further processed till the possible commercialization step. This whole procedure may vary from 3-5 years, depending on the species and transformation method (Gogorcena et al. 2020; Delrot et al. 2020a; Zambounis et al. 2020; Ramesh, Arunachalam, and Rajesh 2020; Rugienius et al. 2020; Basu 2020; Boudichevskaia et al. 2020; A. Brown, Carpentier, and Swennen 2020; Campoy et al. 2020; Delrot et al. 2020b).

Table 2: CRISPR/Cas sgRNA Designing Tools

Tool	*Web Address*	*Reference(s)*
CRISPRscan	http://www.crisprscan.org/	(Moreno-Mateos et al. 2015)
CHOPCHOP	https://chopchop.cbu.uib.no/	(Montague et al. 2014)
WU-CRISPR	https://crisprdb.org/wu-crispr/	(Wong, Liu, and Wang 2015)
CRISPRdirect	http://crispr.dbcls.jp/	(Naito et al. 2015)
CRISPR MultiTargeter	http://www.multicrispr.net/	(Prykhozhij et al. 2015)
E-CRISP	http://www.e-crisp.org/E-CRISP/	(Heigwer, Kerr, and Boutros 2014)
CCTop	https://cctop.cos.uni-heidelberg.de/	(Stemmer et al. 2015)
Cas-OFFinder	http://www.rgenome.net/cas-offinder/	(Bae, Park, and Kim 2014)

Delivery of CRISPR/Cas constructs in fruit plants through direct and indirect transformation methods

CRISPR/Cas systems have caused a paradigm shift from ZFNs and TALENs through their flexibility and ease of use in many complex and diverse scenarios. The dispersion of

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these site-specific nuclease systems has repurposed many biological research areas. Target discovery of disease and associated mechanisms, transcriptional trans-modulation, and the development of transgenic hybrid species of plants are the most prominent of them. One of the primary hurdles that researchers have to deal with is the delivery of the developed CRISPR/Cas constructs into the host of interest (Lino et al. 2018). Over the years, many methods have been developed and improved to deal with CRISPR/Cas payload delivery according to the varying needs of the situation and hence diverse methodologies and protocols. These advanced methods have merits and demerits in terms of efficiency and other important relevant factors.

The components of CRISPR/Cas delivery systems have been categorized (Lino et al. 2018) into two groups:

1. Cargo
2. Delivery Vehicle

Cargo has been sub-divided (Lino et al. 2018) and has exploited the following three approaches:

I. DNA plasmid encoding a sgRNA and Cas protein

II. Cas translocation-mediating mRNA and a separate sgRNA

III. Ribonucleoprotein (RNP) complex (sgRNA + Cas protein)

The mediation of the above three cargoes is done by the delivery vehicles along with affecting factors like the usability of system utilizing both the *in vivo* and *in vitro* conditions. The system's usability is a very important factor to consider while dealing with a cargo-

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mediating delivery vehicle. For example, molecular compatibility and cross-checking between cargo and the delivery vehicles are very important in the case of negatively charged oligonucleotides compared to the positively charged Cas9 proteins. Cas9:sgRNA RNPs exhibit positive and negative partial charge, which may also be affected by pH. Moreover, the concentration of Cas proteins can be mediated by using DNA instead of using recombinant protein as a direct input (Sun et al. 2015). Considering all the factors, it is still impossible to predict the number of effectively interacting Cas units in a specific time frame of a given assembly system.

The properties and expansive details of all the up-to-date delivery methods along with their important respective properties have been provided in Table S2 (Supplementary File 2) (Matano et al. 2015; Ousterout et al. 2015; J 1995; Crispo et al. 2015; Raveux, Vandormael-Pournin, and Cohen-Tannoudji 2017; Dong et al. 2015; Guan et al. 2016; Maggio et al. 2016; Maddalo et al. 2014; Tabebordbar et al. 2016; Truong et al. 2015; Platt et al. 2014; Roehm et al. 2016; Koike-Yusa et al. 2013; Mout et al. 2017; Ebina et al. 2013; Kennedy et al. 2014; Schwank et al. 2013; Horii et al. 2014; Zuris et al. 2014; Sun et al. 2015; Axford, Morris, and McMurry, n.d.; D'Astolfo et al. 2015; Bates and Kostarelos 2013; Nakamura et al. 2012; Su et al. 2017; Gonzalez Porras et al. 2016; Teng et al. 2016; Brito et al. 2008). Apples and grapevines are one of those fruit crops that have been successfully edited by exploiting vector-free transformation methods (Malnoy et al. 2016). The *Agrobacterium*-mediated transformation has also been used as a delivery method in apple, banana, cacao, citrus, grapevine, kiwi, and strawberry (Nishitani et al. 2016b; Naim et al. 2018; Fister et al. 2018b; F. Zhang et al. 2017; Nakajima et al. 2017; Z. Wang et al. 2018b; Zhou, Wang, and Liu 2018).

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Exploiting the CRISPR/Cas system in fruit plants for biotic and abiotic stress management

Though the work of genome editing is challenging in fruits, there are certain applications of the CRISPR/Cas genome editing system that have been successful in modifying fruit crops for various purposes. For example, many fruits have been targeted via the CRISPR/Cas system and improved their response to various environmental stresses, yield, and quality to meet the global need for food and nutrition. The successfully modified fruit plant species include apple, banana, kiwi, fig, pear, orange, and papaya. All of these have been modified for one or more than one gene. A detailed list of CRISPR-edited fruits along with their loci, their type of modification, mode of transformation, and plant parts under modification have been provided in Table 3. Although Agrobacterium-mediated transformation and RNP transformation methods pave the way to the development of genome-edited mutants in terms of identification, these are indistinguishable from any other mutants produced by mean of other genetic modification methods like physical (UV-rays, X-rays, gamma rays, and ion beams) and chemical methods (alkylation agents, base analogs, and acridine dyes).

Table 3: CRISPR/Cas genome editing in fruits. The plants are transformed with Agrobacterium-mediated transformation protocols.

Fruits Crop	***Loci being Targeted***	***Type of Modification(s)***	***Tissue for Modification***	***Reference(s)***
Apple	*uidA*	Activity of β-glucuronidase	Leaf tissue	(Peer et al. 2015)

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	PDS	Biosynthesis of carotenoids	Leaf tissue	(Nishitani et al. 2016a)
	IdnDH	Tartaric acid biosynthesis	Leaf tissue	(Osakabe et al. 2018)
	DIPM (1, 2, and 4)	Resistance to fire blight	Protoplasm	(Malnoy et al. 2016)
Banana	*MaPDS*	Biosynthesis of carotenoids	Cell suspension (embryogenic)	(Kaur et al. 2018)
	eBSV	Viral pathogenesis control	Explant (epicotyl)	(Tripathi et al. 2019)
Kiwifruit	*acPDS*	Biosynthesis of carotenoids	Leaf tissue	(Z. Wang et al. 2018a)
Sweet Orange	*CsPDS*	Biosynthesis of carotenoids	Leaf tissue	(Jia and Nian 2014)
	DMR6	Resistance to Huanglongbing disease	Explant (epicotyl)	(“Regulation of Citrus DMR6</Em> via RNA Interference and CRISPR/Cas9-Mediated Gene Editing to Improve Huanglongbing Tolerance,” n.d.)
Fig	*uidA*	Activity of β-glucuronidase	Leaf tissue	(Peer et al. 2015)

Wanjincheng Orange	*CsLOB1* (promoter Sequence)	Resistance against citrus canker	Explant (epicotyl)	(Peng et al. 2017a)
Pear	*TFL1*	Early flowering	Cell suspension (embryogenic)	(Charrier et al. 2019)
Coffee	*CcPDS*	Biosynthesis of carotenoids	Cell suspension (meristematic)	(Breitler et al. 2018)
Cacao	*TcNPR3*	Enhanced defense response	Leaf tissue	(Fister et al. 2018a)
Grapevine	*VvPR4b*	Downy mildew resistance	Proembryogenic mass (PEM) cells	(M. Y. Li et al. 2020)
	VvMLO3	Powdery mildew	Leaf tissue	(Wan et al. 2020)
Papaya	*cp*	Resistance against Papaya ringspot virus	immature zygotic embryos	(Fitch et al. 1992)

CRISPR/Cas genome editing is also being used to produce and develop those varieties of fruits that are resistant to multiple biotic stress situations posed by the environment. For example, papaya has been made resistant to its infamous pathogen named *papaya ringspot virus* (PRSV). The virus uses insects mainly aphids as a transmission vector. The biolistic transformation approach was exploited to develop the transgenic ‘SunUp’ cultivar from the original ‘Sunset’ cultivar by utilizing genes isolated from a closely-related Hawaiian papaya strain (Fang et al. 2020). To achieve the desirable yellow flesh of papaya that the new cultivar

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was lacking, it was further crossed with a non-engineered 'Kapoho' cultivar. The resulting cultivar was also resistant to the papaya ringspot virus (Fitch et al. 1992; Conover 1964). Similarly, *Citrus* species as one of the more economically important fruit plant groups are susceptible to citrus canker. Targeted modifications at the 5'-regulatory region have been made in the effector-binding element (EBEPthA$_4$) of the *Citrus sinensis Lateral Organ Boundaries 1* (*CsLOB1*) gene that is responsible for the susceptibility. When the promoter of the gene was disrupted, it was observed that the overall resistance was improved. Complete resistance was achieved by completely deleting the EBEPthA$_4$ promoter sequence from both *CsLOB1* alleles (Jia et al. 2017a; Peng et al. 2017b).

Furthermore, through CRISPR/Cas-mediated gene disruptions , cacao has been made resistant to the fungus *Phytophthora tropicalis*, grapevine against *Botrytis cinerea*, and grapefruit against citrus canker (Jia et al. 2017b, 2016; X. Wang et al. 2018). Furthermore, Bayoud disease in date palms can also be tackled successfully using CRISPR/Cas manipulation of multiple date palm genomic loci through the universal tRNA-based approach (Sattar et al. 2017; Jaganathan et al. 2018). Plant productivity and survival are negatively impacted by abiotic stresses posed by the environment, especially and increasingly by climate change. Many genes have been identified to regulate the adaptive mechanisms related to abiotic stresses like heat, cold, salinity, and drought (Bressan, Bohnert, and Zhu 2009). For example, pear (*Pyrus communis*) has been modified to produce multiple abiotic stress tolerances at once. This has been made possible by the overexpression of the apple-derived *spermidine synthase* gene (*MdSPDS1*) modulated by the application of *Agrobacterium*-mediated transformation that alters the titers of polyamines in pear fruits (Wen et al. 2008).

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Similarly, apple has been made tolerant to cold, salt, and drought stress by overexpressing its *MdCIPK6L* gene that encodes specific CIPKs (CBL-interacting protein kinases) (Y. Wang et al. 2016; Kaur, Awasthi, and Tiwari 2020).

CRISPR/Cas genome editing has been used to control the browning in apple fruits. The browning of the apple's flesh happens when the underlying phenolic compounds are oxidized by polyphenol oxidases (PPOs). An approach was successful to develop multiple varieties of apples by using a CRISPR/Cas system to silence the genes mediating the activity of PPOs (Butiuc-Keul et al. 2022). Similarly, as a proof of concept of the great potential of the CRISPR/Cas system, Pinkglow™ pineapple was developed by modifying the carotenoid synthesis pathway through the expression of the tangerine (*Citrus reticulata*) *PSY* gene. The pink color is due to the subsequence accumulation of lycopene that is formed as an intermediate during general carotenoid synthesis. In tomatoes, enhancements have been made to their floral architecture and fruit size through mobile CLV3 peptide (regulates floral stem cells) promoter edited by CRISPR/Cas (Rodríguez-Leal et al. 2017).

Limitations and their possible solutions in engineering climate-smart fruit plants

One of the major concerns that limit the efficacy of CRISPR/Cas systems are undesirable off-target effects, meaning unintended editing events. They can be caused by PAM (protospacer adjacent motif) sequences and sgRNA binding sites besides the intended target when implemented *in vivo*. *In silico* techniques are readily utilized to precisely predict these off-target cleavages but are limited by the exceptionally complex interaction of epigenetic modifications that are almost impossible to predict with the current technological standards.

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Moreover, these programs are limited to the examination of only homologous genes (Yee and Yee 2016; H. C. Yang and Chen 2018; Suleiman, Saedi, and Muhaidi 2021; Zischewski, Fischer, and Bortesi 2017). High-throughput NGS or genome-wide next-generation sequencing can contribute to reducing off-target effects by designing extremely target-specific sgRNAs using existing gene sequences. If off-targets cannot be completely excluded, gene sequences can at least be used to predict possible off-targets, which can then be checked for unintended editing via DNA sequencing. One of the strategies that can be applied directly to a CRISPR/Cas system is reducing the functional time frame of activity as well as target locus alterations and enhancing the specificity of nuclease cleavages. The newly engineered CRISPR-associated proteins like Sniper-Cas9, HF-Cas9, eSpCas9, and HypaCas9 exhibit on-target specificity with great efficiency reducing the off-target effects (Hu et al. 2018; J. K. Lee et al. 2018; J. S. Chen et al. 2017; J. Lee et al. 2019; Davis et al. 2015). Online tools such as Cas-OFFinder and CCTop can be utilized to predict potential off-targets involved in the intended CRISPR/Cas approach (Bae, Park, and Kim 2014; Stemmer et al. 2015).

Furthermore, the availability of PAM sequences at the locus of interest is limited. This restriction can be circumvented by using variants of CRISPR-associated proteins such as Cas12a and SpCas9 or alternative PAMs with lower efficiency. The indel target specificity can be further enhanced by exploiting artificial intelligence-mediated predictions and analysis (H. K. Kim et al. 2018; Kleinstiver et al. 2015; Gao et al. 2017). To obtain single point mutations without the induction of DNA double strand breaks, base editors are exploited because of their unmatched efficiency. These include cytosine and adenine base editors that convert C-G base pairs to T-A pairs and A-T pairs to G-C pairs, respectively

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(Gaudelli et al. 2017; Komor et al. 2016; Rees and Liu 2018; Y. Yang et al. 2021). Other prominent limitations hindering the efficacy of CRISPR/Cas systems are mosaicism, RNA instability, and Cas-associated immunogenicity (P. Kumar et al. 2020). The mosaicism problem can be tackled through the proper optimization of the transformation pathway being used for the induction of faster editing. RNA instability can be corrected by avoiding RNase contaminations in the process. Furthermore, it has been shown that the Cas proteins-associated immunogenic response can be reduced by the induction of two consecutive mutations in the epitope anchor residues (Ferdosi et al. 2019).

Conclusions and Future Perspectives

The original prokaryotic defense system against bacteriophages, i.e., modern CRISPR/Cas technology, holds virtually infinite potential and clings tightly to an immensely positive future outlook. It has already affected and turned the face of global food insecurity by a mile due to its easiness of use in developing multiple climate-smart fruit crops. Through the precision and accuracy of CRISPR/Cas technology, researchers can implement the features of C_4-plants in C_3-plants to cope with the yield losses due to the deficiency in the overall photosynthesis rate, especially in warmer climates (Cui 2021; N. J. Brown et al. 2011). Although, the underlying mechanisms of C_4-plants are highly complex (Sedelnikova, Hughes, and Langdale 2018; Furbank 2016; H. Zhu, Li, and Gao 2020; J. H. Lee et al. 2020; Yingxiao Zhang et al. 2019), recent important developments have paved to the way for the new C_4-plants to emerge (Lundgren et al. 2016; Newell et al. 2010; Brutnell et al. 2010). Moreover, CRISPR biosynthetic pathway modification is already being applied to fruits like apples, bananas, citrus, pineapple, pear, fig, and kiwi to introduce novel mechanisms like

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carotenoid biosynthesis as a proof-of-concept and regulation in the activities of different enzymes. These modifications lead the way in achieving resistance against climate change by enhancing the biotic and abiotic stress tolerance profile in fruit crops (South et al. 2019; Narayanan et al. 2019; Taylor et al. 2019). The recent advances in base and prime editing have demonstrated that the capacity and scope of CRISPR/Cas technology are still expanding (Grünewald et al. 2020; Lin et al. 2020; Anzalone et al. 2019).

Furthermore, a lot of potential of CRISPR/Cas systems has been exploited to make fruits resistant to biotic and abiotic stresses of the environment along with enhancing their overall nutritional value. All of these developments are ultimately necessary especially when the world population is rising at an alarming rate along with climate change. To get the most out of these revolutionary genome editing technologies, the general population also needs to be guided about the long-term benefits and perks of using climate-smart fruits generated through CRISPR/Cas and other systems provided that their concerns are justified. Maybe in the distant future, this technology morphs into a new form with its current precise, accurate, and efficient editing and without its limitations, which will surely help to use the uncharted and gigantic genetic resources of plants.

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Authors' Contribution

M.S.,[1st] T.B., and M.S.[3rd] retrieved the data, made an outline, and discussed literature. M.S.[1st] compiled the data, wrote the original draft, and created the figures and tables. S.H.K., M.T.A., and R.M.A. helped in literature curation and setting up outline; M.F. and I.A.R. conceptualized the idea, supervised, proofread and thoroughly reviewed the manuscript.

Supplementary File(s)

Supplementary File 1: Major Fruits and their Genome Sequence Information

Supplementary File 2: Common Features of Delivery Vehicles for CRISPR/Cas system

Conflict of interests

The authors declare that they have no conflict of interest.

Data Availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request

References

Al-Mssallem, Ibrahim S., Songnian Hu, Xiaowei Zhang, Qiang Lin, Wanfei Liu, Jun Tan, Xiaoguang Yu, et al. 2013. “Genome Sequence of the Date Palm Phoenix Dactylifera L.” *Nature Communications 2013 4:1* 4 (1): 1–9. https://doi.org/10.1038/ncomms3274.

Alioto, Tyler, Konstantinos G. Alexiou, Amélie Bardil, Fabio Barteri, Raúl Castanera, Fernando Cruz, Amit Dhingra, et al. 2020. “Transposons Played a Major Role in the Diversification between the Closely Related Almond and Peach Genomes: Results from the Almond Genome Sequence.” *The Plant Journal : For Cell and Molecular Biology* 101 (2): 455–72. https://doi.org/10.1111/TPJ.14538.

Anzalone, Andrew V, Peyton B Randolph, Jessie R Davis, Alexander A Sousa, Luke W Koblan, Jonathan M Levy, Peter J Chen, et al. 2019. “Search-and-Replace Genome Editing without Double-Strand Breaks or Donor DNA.” *Nature* 576 (7785): 149–57. https://doi.org/10.1038/s41586-019-1711-4.

Argout, Xavier, Jerome Salse, Jean Marc Aury, Mark J. Guiltinan, Gaetan Droc, Jerome Gouzy, Mathilde Allegre, et al. 2010. “The Genome of Theobroma Cacao.” *Nature Genetics 2010 43:2* 43 (2): 101–8. https://doi.org/10.1038/ng.736.

Arumuganathan, K., and E. D. Earle. 1991. “Nuclear DNA Content of Some Important Plant Species.” *Plant Molecular Biology Reporter 1991 9:3* 9 (3): 208–18. https://doi.org/10.1007/BF02672069.

Axford, David S., Daniel P. Morris, and Jonathan L McMurry. n.d. “Cell Penetrating Peptide-Mediated Nuclear Delivery of Cas9 to Enhance the Utility of CRISPR/Cas Genome Editing.” *The FASEB Journal* 31: 909.4-909.4. https://doi.org/10.1096/FASEBJ.31.1_SUPPLEMENT.909.4.

Bae, Sangsu, Jeongbin Park, and Jin Soo Kim. 2014. “Cas-OFFinder: A Fast and Versatile Algorithm That Searches for Potential off-Target Sites of Cas9 RNA-Guided Endonucleases.” *Bioinformatics (Oxford, England)* 30 (10): 1473–75. https://doi.org/10.1093/BIOINFORMATICS/BTU048.

Basu, Supratim. 2020. “Toward Development of Climate-Resilient Citrus.” *Genomic Designing of Climate-Smart Fruit Crops*, 117–34. https://doi.org/10.1007/978-3-319-97946-5_5.

Bates, Katie, and Kostas Kostarelos. 2013. “Carbon Nanotubes as Vectors for Gene Therapy: Past Achievements, Present Challenges and Future Goals.” *Advanced Drug Delivery Reviews* 65 (15): 2023–33. https://doi.org/10.1016/J.ADDR.2013.10.003.

Boch, Jens, Heidi Scholze, Sebastian Schornack, Angelika Landgraf, Simone Hahn, Sabine Kay, Thomas Lahaye, Anja Nickstadt, and Ulla Bonas. 2009. “Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors.” *Science (New York, N.Y.)* 326 (5959): 1509–12. https://doi.org/10.1126/SCIENCE.1178811.

Boudichevskaia, Anastassia, Gulshan Kumar, Yogesh Sharma, Ritu Kapoor, and Anil Kumar Singh. 2020. “Challenges and Strategies for Developing Climate-Smart Apple Varieties Through Genomic Approaches.” *Genomic Designing of Climate-Smart Fruit Crops*, 23–71. https://doi.org/10.1007/978-3-319-97946-5_2.

Breitler, Jean Christophe, Eveline Dechamp, Claudine Campa, Leonardo Augusto Zebral Rodrigues, Romain Guyot, Pierre Marraccini, and Hervé Etienne. 2018. “CRISPR/Cas9-Mediated Efficient Targeted Mutagenesis Has the Potential to Accelerate the Domestication of Coffea Canephora.” *Plant Cell, Tissue and Organ Culture* 134 (3): 383–94. https://doi.org/10.1007/S11240-018-1429-2/FIGURES/3.

Bressan, Ray, Hans Bohnert, and Jian Kang Zhu. 2009. “Abiotic Stress Tolerance: From Gene Discovery in Model Organisms to Crop Improvement.” *Molecular Plant* 2 (1): 1–2. https://doi.org/10.1093/MP/SSN097.

Brito, Jose L.R., Faith E. Davies, David Gonzalez, and Gareth J. Morgan. 2008. “Streptolysin-O Reversible Permeabilisation Is an Effective Method to Transfect SiRNAs into Myeloma Cells.” *Journal of Immunological Methods* 333 (1–2): 147–55. https://doi.org/10.1016/J.JIM.2008.01.009.

Brown, Allan, Sebastien C. Carpentier, and Rony Swennen. 2020. “Breeding Climate-Resilient Bananas.” *Genomic Designing of Climate-Smart Fruit Crops*, 91–115. https://doi.org/10.1007/978-3-319-97946-5_4.

Brown, Naomi J, Christine A Newell, Susan Stanley, Jit E Chen, Abigail J Perrin, Kaisa Kajala, and Julian M Hibberd. 2011. “Independent and Parallel Recruitment of Preexisting Mechanisms Underlying C_4 Photosynthesis.” *Science (New York, N.Y.)*

331 (6023): 1436–39. https://doi.org/10.1126/science.1201248.

Brutnell, Thomas P, Lin Wang, Kerry Swartwood, Alexander Goldschmidt, David Jackson, Xin-Guang Zhu, Elizabeth Kellogg, and Joyce Van Eck. 2010. "Setaria Viridis: A Model for C4 Photosynthesis." *The Plant Cell* 22 (8): 2537–44.

Butiuc-Keul, Anca, Anca Farkas, Rahela Carpa, Cristina Dobrota, and Dumitrana Iordache. 2022. "Development of Smart Fruit Crops by Genome Editing." *Turkish Journal of Agriculture and Forestry* 46 (2): 129–40. https://doi.org/10.3906/tar-2012-104.

Campoy, Jose A., Jean M. Audergon, D. Ruiz, and Pedro Martínez-Gómez. 2020. "Genomic Designing for New Climate-Resilient Apricot Varieties in a Warming Context." *Genomic Designing of Climate-Smart Fruit Crops*, 73–89. https://doi.org/10.1007/978-3-319-97946-5_3.

Chagné, David, Ross N. Crowhurst, Massimo Pindo, Amali Thrimawithana, Cecilia Deng, Hilary Ireland, Mark Fiers, et al. 2014. "The Draft Genome Sequence of European Pear (Pyrus Communis L. 'Bartlett')." *PloS One* 9 (4). https://doi.org/10.1371/JOURNAL.PONE.0092644.

Chakrabarti, Anob M, Tristan Henser-Brownhill, Josep Monserrat, Anna R Poetsch, Nicholas M Luscombe, and Paola Scaffidi. 2019. "Target-Specific Precision of CRISPR-Mediated Genome Editing." *Molecular Cell* 73 (4): 699-713.e6. https://doi.org/10.1016/j.molcel.2018.11.031.

Charrier, Aurélie, Emilie Vergne, Nicolas Dousset, Andréa Richer, Aurélien Petiteau, and Elisabeth Chevreau. 2019. "Efficient Targeted Mutagenesis in Apple and First Time Edition of Pear Using the CRISPR-Cas9 System." *Frontiers in Plant Science* 10 (February): 40. https://doi.org/10.3389/FPLS.2019.00040/BIBTEX.

Chen, Fuqiang, Xiao Ding, Yongmei Feng, Timothy Seebeck, Yanfang Jiang, and Gregory D Davis. 2017. "Targeted Activation of Diverse CRISPR-Cas Systems for Mammalian Genome Editing via Proximal CRISPR Targeting." *Nature Communications* 8 (1): 14958. https://doi.org/10.1038/ncomms14958.

Chen, Janice S., Yavuz S. Dagdas, Benjamin P. Kleinstiver, Moira M. Welch, Alexander A. Sousa, Lucas B. Harrington, Samuel H. Sternberg, J. Keith Joung, Ahmet Yildiz, and Jennifer A. Doudna. 2017. "Enhanced Proofreading Governs CRISPR–Cas9 Targeting Accuracy." *Nature 2017 550:7676* 550 (7676): 407–10. https://doi.org/10.1038/nature24268.

Cho, Seung Woo, Sojung Kim, Jong Min Kim, and Jin Soo Kim. 2013. "Targeted Genome Engineering in Human Cells with the Cas9 RNA-Guided Endonuclease." *Nature Biotechnology 2013 31:3* 31 (3): 230–32. https://doi.org/10.1038/nbt.2507.

Conover, Robert A. 1964. "Distortion Ringspot, a Severe Virus Disease of Papaya in Florida." *Proceedings of Florida State Horticultural Society* 77: 440–44.

Crispo, M., A. P. Mulet, L. Tesson, N. Barrera, F. Cuadro, P. C. Dos Santos-Neto, T. H. Nguyen, et al. 2015. "Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes." *PloS One* 10 (8). https://doi.org/10.1371/JOURNAL.PONE.0136690.

Cui, Hongchang. 2021. "Challenges and Approaches to Crop Improvement Through C3-to-C4 Engineering ." *Frontiers in Plant Science* .

https://www.frontiersin.org/articles/10.3389/fpls.2021.715391.

D'Astolfo, Diego S., Romina J. Pagliero, Anita Pras, Wouter R. Karthaus, Hans Clevers, Vikram Prasad, Robert Jan Lebbink, Holger Rehmann, and Niels Geijsen. 2015. "Efficient Intracellular Delivery of Native Proteins." *Cell* 161 (3): 674–90. https://doi.org/10.1016/J.CELL.2015.03.028.

Dash, Prasanta K., and Rhitu Rai. 2016. "Translating the 'Banana Genome' to Delineate Stress Resistance, Dwarfing, Parthenocarpy and Mechanisms of Fruit Ripening." *Frontiers in Plant Science* 7 (OCTOBER2016): 1543. https://doi.org/10.3389/FPLS.2016.01543/BIBTEX.

Davis, Kevin M., Vikram Pattanayak, David B. Thompson, John A. Zuris, and David R. Liu. 2015. "Small Molecule–Triggered Cas9 Protein with Improved Genome-Editing Specificity." *Nature Chemical Biology 2015 11:5* 11 (5): 316–18. https://doi.org/10.1038/nchembio.1793.

Delrot, Serge, Jérôme Grimplet, Pablo Carbonell-Bejerano, Anna Schwandner, Pierre-François Bert, Luigi Bavaresco, Lorenza Dalla Costa, et al. 2020a. "Genetic and Genomic Approaches for Adaptation of Grapevine to Climate Change." *Genomic Designing of Climate-Smart Fruit Crops*, 157–270. https://doi.org/10.1007/978-3-319-97946-5_7.

———. 2020b. "Genetic and Genomic Approaches for Adaptation of Grapevine to Climate Change BT - Genomic Designing of Climate-Smart Fruit Crops." Edited by Chittaranjan Kole, 157–270. https://doi.org/10.1007/978-3-319-97946-5_7.

Dong, Chunsheng, Liang Qu, Haoyi Wang, Lin Wei, Yuansu Dong, and Sidong Xiong. 2015. "Targeting Hepatitis B Virus CccDNA by CRISPR/Cas9 Nuclease Efficiently Inhibits Viral Replication." *Antiviral Research* 118 (June): 110–17. https://doi.org/10.1016/J.ANTIVIRAL.2015.03.015.

Ebina, Hirotaka, Naoko Misawa, Yuka Kanemura, and Yoshio Koyanagi. 2013. "Harnessing the CRISPR/Cas9 System to Disrupt Latent HIV-1 Provirus." *Scientific Reports* 3. https://doi.org/10.1038/SREP02510.

Fang, Jingping, Jingping Fang, Jingping Fang, Jingping Fang, Andrew Michael Wood, Youqiang Chen, Youqiang Chen, Jingjing Yue, Ray Ming, and Ray Ming. 2020. "Genomic Variation between PRSV Resistant Transgenic SunUp and Its Progenitor Cultivar Sunset." *BMC Genomics* 21 (1): 1–21. https://doi.org/10.1186/S12864-020-06804-7/TABLES/8.

Feng, Zhengyan, Botao Zhang, Wona Ding, Xiaodong Liu, Dong Lei Yang, Pengliang Wei, Fengqiu Cao, et al. 2013. "Efficient Genome Editing in Plants Using a CRISPR/Cas System." *Cell Research* 23 (10): 1229–32. https://doi.org/10.1038/CR.2013.114.

Ferdosi, Shayesteh R, Radwa Ewaisha, Farzaneh Moghadam, Sri Krishna, Jin G Park, Mo R Ebrahimkhani, Samira Kiani, and Karen S Anderson. 2019. "Multifunctional CRISPR-Cas9 with Engineered Immunosilenced Human T Cell Epitopes." *Nature Communications* 10 (1): 1842. https://doi.org/10.1038/s41467-019-09693-x.

Fister, Andrew S., Lena Landherr, Siela N. Maximova, and Mark J. Guiltinan. 2018a. "Transient Expression of CRISPR/Cas9 Machinery Targeting TcNPR3 Enhances Defense Response in Theobroma Cacao." *Frontiers in Plant Science* 9 (March): 268.

https://doi.org/10.3389/FPLS.2018.00268/BIBTEX.

———. 2018b. “Transient Expression of CRISPR/Cas9 Machinery Targeting TcNPR3 Enhances Defense Response in Theobroma Cacao.” *Frontiers in Plant Science* 9 (March). https://doi.org/10.3389/FPLS.2018.00268.

Fitch, Maureen M.M., Richard M. Manshardt, Dennis Gonsalves, Jerry L. Slightom, and John C. Sanford. 1992. “Virus Resistant Papaya Plants Derived from Tissues Bombarded with the Coat Protein Gene of Papaya Ringspot Virus.” *Bio/Technology 1992 10:11* 10 (11): 1466–72. https://doi.org/10.1038/nbt1192-1466.

Furbank, Robert T. 2016. “Walking the C4 Pathway: Past, Present, and Future.” *Journal of Experimental Botany* 67 (14): 4057–66.

Gaj, Thomas, Charles A. Gersbach, and Carlos F. Barbas. 2013. “ZFN, TALEN, and CRISPR/Cas-Based Methods for Genome Engineering.” *Trends in Biotechnology* 31 (7): 397–405. https://doi.org/10.1016/J.TIBTECH.2013.04.004.

Gao, Linyi, David B.T. Cox, Winston X. Yan, John C. Manteiga, Martin W. Schneider, Takashi Yamano, Hiroshi Nishimasu, Osamu Nureki, Nicola Crosetto, and Feng Zhang. 2017. “Engineered Cpf1 Variants with Altered PAM Specificities.” *Nature Biotechnology 2017 35:8* 35 (8): 789–92. https://doi.org/10.1038/nbt.3900.

Gaudelli, Nicole M., Alexis C. Komor, Holly A. Rees, Michael S. Packer, Ahmed H. Badran, David I. Bryson, and David R. Liu. 2017. “Programmable Base Editing of A•T to G•C in Genomic DNA without DNA Cleavage.” *Nature 2017 551:7681* 551 (7681): 464–71. https://doi.org/10.1038/nature24644.

Gogorcena, Yolanda, Gerardo Sánchez, Santiago Moreno-Vázquez, Salvador Pérez, and Najla Ksouri. 2020. “Genomic-Based Breeding for Climate-Smart Peach Varieties.” *Genomic Designing of Climate-Smart Fruit Crops*, 271–331. https://doi.org/10.1007/978-3-319-97946-5_8.

Gonzalez Porras, Maria A., Paul N. Durfee, Ashley M. Gregory, Gary C. Sieck, C. Jeffrey Brinker, and Carlos B. Mantilla. 2016. “A Novel Approach for Targeted Delivery to Motoneurons Using Cholera Toxin-B Modified Protocells.” *Journal of Neuroscience Methods* 273 (November): 160–74. https://doi.org/10.1016/J.JNEUMETH.2016.09.003.

Grünewald, Julian, Ronghao Zhou, Caleb A Lareau, Sara P Garcia, Sowmya Iyer, Bret R Miller, Lukas M Langner, Jonathan Y Hsu, Martin J Aryee, and J Keith Joung. 2020. “A Dual-Deaminase CRISPR Base Editor Enables Concurrent Adenine and Cytosine Editing.” *Nature Biotechnology* 38 (7): 861–64. https://doi.org/10.1038/s41587-020-0535-y.

Guan, Yuting, Yanlin Ma, Qi Li, Zhenliang Sun, Lie Ma, Lijuan Wu, Liren Wang, et al. 2016. “CRISPR/Cas9-Mediated Somatic Correction of a Novel Coagulator Factor IX Gene Mutation Ameliorates Hemophilia in Mouse.” *EMBO Molecular Medicine* 8 (5): 477–88. https://doi.org/10.15252/EMMM.201506039.

He, Ningjia, Chi Zhang, Xiwu Qi, Shancen Zhao, Yong Tao, Guojun Yang, Tae Ho Lee, et al. 2013. “Draft Genome Sequence of the Mulberry Tree Morus Notabilis.” *Nature Communications 2013 4:1* 4 (1): 1–9. https://doi.org/10.1038/ncomms3445.

Heigwer, Florian, Grainne Kerr, and Michael Boutros. 2014. “E-CRISP: Fast CRISPR Target

Site Identification." *Nature Methods 2014 11:2* 11 (2): 122–23. https://doi.org/10.1038/nmeth.2812.

Horii, Takuro, Yuji Arai, Miho Yamazaki, Sumiyo Morita, Mika Kimura, Masahiro Itoh, Yumiko Abe, and Izuho Hatada. 2014. "Validation of Microinjection Methods for Generating Knockout Mice by CRISPR/Cas-Mediated Genome Engineering." *Scientific Reports* 4 (March). https://doi.org/10.1038/SREP04513.

Hsu, Patrick D., David A. Scott, Joshua A. Weinstein, F. Ann Ran, Silvana Konermann, Vineeta Agarwala, Yinqing Li, et al. 2013. "DNA Targeting Specificity of RNA-Guided Cas9 Nucleases." *Nature Biotechnology 2013 31:9* 31 (9): 827–32. https://doi.org/10.1038/nbt.2647.

Hu, Johnny H., Shannon M. Miller, Maarten H. Geurts, Weixin Tang, Liwei Chen, Ning Sun, Christina M. Zeina, et al. 2018. "Evolved Cas9 Variants with Broad PAM Compatibility and High DNA Specificity." *Nature 2018 556:7699* 556 (7699): 57–63. https://doi.org/10.1038/nature26155.

Huang, Shengxiong, Jian Ding, Dejing Deng, Wei Tang, Honghe Sun, Dongyuan Liu, Lei Zhang, et al. 2013. "Draft Genome of the Kiwifruit Actinidia Chinensis." *Nature Communications* 4. https://doi.org/10.1038/NCOMMS3640.

Ioannidi, Eleonora I., Matthew T. N. Yarnall, Cian Schmitt-Ulms, Rohan N. Krajeski, Justin Lim, Lukas Villiger, Wenyuan Zhou, et al. 2021. "Drag-and-Drop Genome Insertion without DNA Cleavage with CRISPR-Directed Integrases." *BioRxiv*, November, 2021.11.01.466786. https://doi.org/10.1101/2021.11.01.466786.

J, Ross. 1995. "MRNA Stability in Mammalian Cells." *Microbiological Reviews* 59 (3): 423–50. https://doi.org/10.1128/MR.59.3.423-450.1995.

Jaganathan, Deepa, Karthikeyan Ramasamy, Gothandapani Sellamuthu, Shilpha Jayabalan, and Gayatri Venkataraman. 2018. "CRISPR for Crop Improvement: An Update Review." *Frontiers in Plant Science* 9 (July): 985. https://doi.org/10.3389/FPLS.2018.00985/BIBTEX.

Jaillon, Olivier, Jean Marc Aury, Benjamin Noel, Alberto Policriti, Christian Clepet, Alberto Casagrande, Nathalie Choisne, et al. 2007. "The Grapevine Genome Sequence Suggests Ancestral Hexaploidization in Major Angiosperm Phyla." *Nature 2007 449:7161* 449 (7161): 463–67. https://doi.org/10.1038/nature06148.

Jia, Hongge, and Wang Nian. 2014. "Targeted Genome Editing of Sweet Orange Using Cas9/SgRNA." *PloS One* 9 (4). https://doi.org/10.1371/JOURNAL.PONE.0093806.

Jia, Hongge, Vladimir Orbovic, Jeffrey B. Jones, and Nian Wang. 2016. "Modification of the PthA4 Effector Binding Elements in Type I CsLOB1 Promoter Using Cas9/SgRNA to Produce Transgenic Duncan Grapefruit Alleviating XccΔpthA4:DCsLOB1.3 Infection." *Plant Biotechnology Journal* 14 (5): 1291–1301. https://doi.org/10.1111/PBI.12495.

Jia, Hongge, Yunzeng Zhang, Vladimir Orbović, Jin Xu, Frank F. White, Jeffrey B. Jones, and Nian Wang. 2017a. "Genome Editing of the Disease Susceptibility Gene CsLOB1 in Citrus Confers Resistance to Citrus Canker." *Plant Biotechnology Journal* 15 (7): 817–23. https://doi.org/10.1111/PBI.12677.

———. 2017b. "Genome Editing of the Disease Susceptibility Gene CsLOB1 in Citrus

Confers Resistance to Citrus Canker." *Plant Biotechnology Journal* 15 (7): 817–23. https://doi.org/10.1111/PBI.12677.

Jiang, Fengchao, Junhuan Zhang, Sen Wang, Li Yang, Yingfeng Luo, Shenghan Gao, Meiling Zhang, et al. 2019. "The Apricot (Prunus Armeniaca L.) Genome Elucidates Rosaceae Evolution and Beta-Carotenoid Synthesis." *Horticulture Research 2019 6:1* 6 (1): 1–12. https://doi.org/10.1038/s41438-019-0215-6.

Jiang, Wenzhi, Huanbin Zhou, Honghao Bi, Michael Fromm, Bing Yang, and Donald P. Weeks. 2013. "Demonstration of CRISPR/Cas9/SgRNA-Mediated Targeted Gene Modification in Arabidopsis, Tobacco, Sorghum and Rice." *Nucleic Acids Research* 41 (20). https://doi.org/10.1093/NAR/GKT780.

Kaur, Navneet, Anshu Alok, Shivani, Navjot Kaur, Pankaj Pandey, Praveen Awasthi, and Siddharth Tiwari. 2018. "CRISPR/Cas9-Mediated Efficient Editing in Phytoene Desaturase (PDS) Demonstrates Precise Manipulation in Banana Cv. Rasthali Genome." *Functional & Integrative Genomics* 18 (1): 89–99. https://doi.org/10.1007/S10142-017-0577-5.

Kaur, Navneet, Praveen Awasthi, and Siddharth Tiwari. 2020. *Fruit Crops Improvement Using CRISPR/Cas9 System*. *Genome Engineering via CRISPR-Cas9 System*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-818140-9.00012-x.

Kennedy, Edward M., Anand V. R. Kornepati, Michael Goldstein, Hal P. Bogerd, Brigid C. Poling, Adam W. Whisnant, Michael B. Kastan, and Bryan R. Cullen. 2014. "Inactivation of the Human Papillomavirus E6 or E7 Gene in Cervical Carcinoma Cells by Using a Bacterial CRISPR/Cas RNA-Guided Endonuclease." *Journal of Virology* 88 (20): 11965–72. https://doi.org/10.1128/JVI.01879-14.

Khalil, Ahmad M. 2020. "The Genome Editing Revolution: Review." *Journal, Genetic Engineering & Biotechnology* 18 (1): 68. https://doi.org/10.1186/s43141-020-00078-y.

Kim, Hui Kwon, Seonwoo Min, Myungjae Song, Soobin Jung, Jae Woo Choi, Younggwang Kim, Sangeun Lee, Sungroh Yoon, and Hyongbum Kim. 2018. "Deep Learning Improves Prediction of CRISPR–Cpf1 Guide RNA Activity." *Nature Biotechnology 2018 36:3* 36 (3): 239–41. https://doi.org/10.1038/nbt.4061.

Kim, Yang Gyun, Jooyeun Cha, and Srinivasan Chandrasegaran. 1996. "Hybrid Restriction Enzymes: Zinc Finger Fusions to Fok I Cleavage Domain." *Proceedings of the National Academy of Sciences of the United States of America* 93 (3): 1156. https://doi.org/10.1073/PNAS.93.3.1156.

Kleinstiver, Benjamin P., Michelle S. Prew, Shengdar Q. Tsai, Ved V. Topkar, Nhu T. Nguyen, Zongli Zheng, Andrew P.W. Gonzales, et al. 2015. "Engineered CRISPR-Cas9 Nucleases with Altered PAM Specificities." *Nature 2015 523:7561* 523 (7561): 481–85. https://doi.org/10.1038/nature14592.

Koike-Yusa, Hiroko, Yilong Li, E. Pien Tan, Martin Del Castillo Velasco-Herrera, and Kosuke Yusa. 2013. "Genome-Wide Recessive Genetic Screening in Mammalian Cells with a Lentiviral CRISPR-Guide RNA Library." *Nature Biotechnology 2013 32:3* 32 (3): 267–73. https://doi.org/10.1038/nbt.2800.

Kole, Chittaranjan. 2020. "Genomic Designing of Climate-Smart Fruit Crops." *Genomic Designing of Climate-Smart Fruit Crops*, January, 1–404. https://doi.org/10.1007/978-

3-319-97946-5/COVER.

Komor, Alexis C., Yongjoo B. Kim, Michael S. Packer, John A. Zuris, and David R. Liu. 2016. “Programmable Editing of a Target Base in Genomic DNA without Double-Stranded DNA Cleavage.” *Nature 2015 533:7603* 533 (7603): 420–24. https://doi.org/10.1038/nature17946.

Kumar, Prashant, Yashpal Singh Malik, Balasubramanian Ganesh, Somnath Rahangdale, Sharad Saurabh, Senthilkumar Natesan, Ashish Srivastava, et al. 2020. “CRISPR-Cas System: An Approach With Potentials for COVID-19 Diagnosis and Therapeutics.” *Frontiers in Cellular and Infection Microbiology* 10 (November): 639. https://doi.org/10.3389/FCIMB.2020.576875/BIBTEX.

Kumar, Vinay, and Mukesh Jain. 2015. “The CRISPR–Cas System for Plant Genome Editing: Advances and Opportunities.” *Journal of Experimental Botany* 66 (1): 47–57. https://doi.org/10.1093/JXB/ERU429.

Lantican, Darlon V., Susan R. Strickler, Alma O. Canama, Roanne R. Gardoce, Lukas A. Mueller, and Hayde F. Galvez. 2019. “De Novo Genome Sequence Assembly of Dwarf Coconut (Cocos Nucifera L. ‘Catigan Green Dwarf’) Provides Insights into Genomic Variation Between Coconut Types and Related Palm Species.” *G3 Genes|Genomes|Genetics* 9 (8): 2377–93. https://doi.org/10.1534/G3.119.400215.

Lee, Jae Hoon, Hyo Jun Won, Eun-Seok Oh, Man-Ho Oh, and Je Hyeong Jung. 2020. “Golden Gate Cloning-Compatible DNA Replicon/2A-Mediated Polycistronic Vectors for Plants.” *Frontiers in Plant Science* 11: 559365.

Lee, Joonsun, Min Hee Jung, Euihwan Jeong, and Jungjoon K. Lee. 2019. “Using Sniper-Cas9 to Minimize Off-Target Effects of CRISPR-Cas9 Without the Loss of On-Target Activity Via Directed Evolution.” *JoVE (Journal of Visualized Experiments)* 2019 (144): e59202. https://doi.org/10.3791/59202.

Lee, Jungjoon K., Euihwan Jeong, Joonsun Lee, Minhee Jung, Eunji Shin, Young hoon Kim, Kangin Lee, et al. 2018. “Directed Evolution of CRISPR-Cas9 to Increase Its Specificity.” *Nature Communications 2018 9:1* 9 (1): 1–10. https://doi.org/10.1038/s41467-018-05477-x.

Li, Hongyi, Yang Yang, Weiqi Hong, Mengyuan Huang, Min Wu, and Xia Zhao. 2020. “Applications of Genome Editing Technology in the Targeted Therapy of Human Diseases: Mechanisms, Advances and Prospects.” *Signal Transduction and Targeted Therapy 2020 5:1* 5 (1): 1–23. https://doi.org/10.1038/s41392-019-0089-y.

Li, Jian Feng, Julie E. Norville, John Aach, Matthew McCormack, Dandan Zhang, Jenifer Bush, George M. Church, and Jen Sheen. 2013a. “Multiplex and Homologous Recombination-Mediated Genome Editing in Arabidopsis and Nicotiana Benthamiana Using Guide RNA and Cas9.” *Nature Biotechnology* 31 (8): 688–91. https://doi.org/10.1038/NBT.2654.

———. 2013b. “Multiplex and Homologous Recombination–Mediated Genome Editing in Arabidopsis and Nicotiana Benthamiana Using Guide RNA and Cas9.” *Nature Biotechnology 2013 31:8* 31 (8): 688–91. https://doi.org/10.1038/nbt.2654.

Li, Meng Yuan, Yun Tong Jiao, Yu Ting Wang, Na Zhang, Bian Bian Wang, Rui Qi Liu, Xiao Yin, Yan Xu, and Guo Tian Liu. 2020. “CRISPR/Cas9-Mediated VvPR4b Editing

Decreases Downy Mildew Resistance in Grapevine (Vitis Vinifera L.).” *Horticulture Research 2020 7:1* 7 (1): 1–11. https://doi.org/10.1038/s41438-020-00371-4.

Lin, Qiupeng, Yuan Zong, Chenxiao Xue, Shengxing Wang, Shuai Jin, Zixu Zhu, Yanpeng Wang, et al. 2020. “Prime Genome Editing in Rice and Wheat.” *Nature Biotechnology* 38 (5): 582–85. https://doi.org/10.1038/s41587-020-0455-x.

Lino, Christopher A., Jason C. Harper, James P. Carney, and Jerilyn A. Timlin. 2018. “Delivering Crispr: A Review of the Challenges and Approaches.” *Drug Delivery* 25 (1): 1234–57. https://doi.org/10.1080/10717544.2018.1474964.

Liu, Chaoyang, Chao Feng, Weizhuo Peng, Jingjing Hao, Juntao Wang, Jianjun Pan, and Yehua He. 2020. “Chromosome-Level Draft Genome of a Diploid Plum (Prunus Salicina).” *GigaScience* 9 (12). https://doi.org/10.1093/GIGASCIENCE/GIAA130.

Lundgren, Marjorie R, Pascal-Antoine Christin, Emmanuel Gonzalez Escobar, Brad S Ripley, Guillaume Besnard, Christine M Long, Paul W Hattersley, Roger P Ellis, Richard C Leegood, and Colin P Osborne. 2016. “Evolutionary Implications of C3–C4 Intermediates in the Grass Alloteropsis Semialata.” *Plant, Cell & Environment* 39 (9): 1874–85.

Luo, Xiang, Haoxian Li, Zhikun Wu, Wen Yao, Peng Zhao, Da Cao, Haiyan Yu, et al. 2020. “The Pomegranate (Punica Granatum L.) Draft Genome Dissects Genetic Divergence between Soft- and Hard-seeded Cultivars.” *Plant Biotechnology Journal* 18 (4): 955. https://doi.org/10.1111/PBI.13260.

Maddalo, Danilo, Eusebio Manchado, Carla P. Concepcion, Ciro Bonetti, Joana A. Vidigal, Yoon Chi Han, Paul Ogrodowski, et al. 2014. “In Vivo Engineering of Oncogenic Chromosomal Rearrangements with the CRISPR/Cas9 System.” *Nature* 516 (7531): 423–28. https://doi.org/10.1038/NATURE13902.

Maggio, Ignazio, Luca Stefanucci, Josephine M. Janssen, Jin Liu, Xiaoyu Chen, Vincent Mouly, and Manuel A.F.V. Gonçalves. 2016. “Selection-Free Gene Repair after Adenoviral Vector Transduction of Designer Nucleases: Rescue of Dystrophin Synthesis in DMD Muscle Cell Populations.” *Nucleic Acids Research* 44 (3): 1449–70. https://doi.org/10.1093/NAR/GKV1540.

Malnoy, Mickael, Roberto Viola, Min Hee Jung, Ok Jae Koo, Seokjoong Kim, Jin Soo Kim, Riccardo Velasco, and Chidananda Nagamangala Kanchiswamy. 2016. “DNA-Free Genetically Edited Grapevine and Apple Protoplast Using CRISPR/Cas9 Ribonucleoproteins.” *Frontiers in Plant Science* 7 (DECEMBER2016): 1904. https://doi.org/10.3389/FPLS.2016.01904/BIBTEX.

Mao, Yanfei, Hui Zhang, Nanfei Xu, Botao Zhang, Feng Gou, and Jian Kang Zhu. 2013. “Application of the CRISPR–Cas System for Efficient Genome Engineering in Plants.” *Molecular Plant* 6 (6): 2008–11. https://doi.org/10.1093/MP/SST121.

Martínez-García, Pedro J., Marc W. Crepeau, Daniela Puiu, Daniel Gonzalez-Ibeas, Jeanne Whalen, Kristian A. Stevens, Robin Paul, et al. 2016. “The Walnut (Juglans Regia) Genome Sequence Reveals Diversity in Genes Coding for the Biosynthesis of Non-Structural Polyphenols.” *The Plant Journal : For Cell and Molecular Biology* 87 (5): 507–32. https://doi.org/10.1111/TPJ.13207.

Matano, Mami, Shoichi Date, Mariko Shimokawa, Ai Takano, Masayuki Fujii, Yuki Ohta,

Toshiaki Watanabe, Takanori Kanai, and Toshiro Sato. 2015. “Modeling Colorectal Cancer Using CRISPR-Cas9-Mediated Engineering of Human Intestinal Organoids.” *Nature Medicine* 21 (3): 256–62. https://doi.org/10.1038/NM.3802.

“Method of the Year 2011.” 2011. *Nature Methods 2012 9:1* 9 (1): 1–1. https://doi.org/10.1038/nmeth.1852.

Miao, Jin, Dongshu Guo, Jinzhe Zhang, Qingpei Huang, Genji Qin, Xin Zhang, Jianmin Wan, Hongya Gu, and Li Jia Qu. 2013. “Targeted Mutagenesis in Rice Using CRISPR-Cas System.” *Cell Research 2013 23:10* 23 (10): 1233–36. https://doi.org/10.1038/cr.2013.123.

Ming, Ray, Shaobin Hou, Yun Feng, Qingyi Yu, Alexandre Dionne-Laporte, Jimmy H. Saw, Pavel Senin, et al. 2008. “The Draft Genome of the Transgenic Tropical Fruit Tree Papaya (Carica Papaya Linnaeus).” *Nature* 452 (7190): 991–96. https://doi.org/10.1038/NATURE06856.

Ming, Ray, Robert VanBuren, Ching Man Wai, Haibao Tang, Michael C. Schatz, John E. Bowers, Eric Lyons, et al. 2015. “The Pineapple Genome and the Evolution of CAM Photosynthesis.” *Nature Genetics* 47 (12): 1435–42. https://doi.org/10.1038/NG.3435.

Mittal, Amandeep, Inderjit Singh Yadav, Naresh Kumar Arora, Rajbir Singh Boora, Meenakshi Mittal, Parwinder Kaur, William Erskine, Parveen Chhuneja, Manav Indra Singh Gill, and Kuldeep Singh. 2020. “RNA-Sequencing Based Gene Expression Landscape of Guava Cv. Allahabad Safeda and Comparative Analysis to Colored Cultivars.” *BMC Genomics* 21 (1): 1–19. https://doi.org/10.1186/S12864-020-06883-6/FIGURES/9.

Montague, Tessa G., José M. Cruz, James A. Gagnon, George M. Church, and Eivind Valen. 2014. “CHOPCHOP: A CRISPR/Cas9 and TALEN Web Tool for Genome Editing.” *Nucleic Acids Research* 42 (W1): W401–7. https://doi.org/10.1093/NAR/GKU410.

Moreno-Mateos, Miguel A., Charles E. Vejnar, Jean Denis Beaudoin, Juan P. Fernandez, Emily K. Mis, Mustafa K. Khokha, and Antonio J. Giraldez. 2015. “CRISPRscan: Designing Highly Efficient SgRNAs for CRISPR-Cas9 Targeting in Vivo.” *Nature Methods 2015 12:10* 12 (10): 982–88. https://doi.org/10.1038/nmeth.3543.

Mori, Kazuki, Kenta Shirasawa, Hitoshi Nogata, Chiharu Hirata, Kosuke Tashiro, Tsuyoshi Habu, Sangwan Kim, Shuichi Himeno, Satoru Kuhara, and Hidetoshi Ikegami. 2017. “Identification of RAN1 Orthologue Associated with Sex Determination through Whole Genome Sequencing Analysis in Fig (Ficus Carica L.).” *Scientific Reports 2017 7:1* 7 (1): 1–15. https://doi.org/10.1038/srep41124.

Mout, Rubul, Moumita Ray, Gulen Yesilbag Tonga, Yi Wei Lee, Tristan Tay, Kanae Sasaki, and Vincent M. Rotello. 2017. “Direct Cytosolic Delivery of CRISPR/Cas9-Ribonucleoprotein for Efficient Gene Editing.” *ACS Nano* 11 (3): 2452–58. https://doi.org/10.1021/ACSNANO.6B07600/SUPPL_FILE/NN6B07600_SI_004.AVI.

Naim, Fatima, Benjamin Dugdale, Jennifer Kleidon, Anthony Brinin, Kylie Shand, Peter Waterhouse, and James Dale. 2018. “Gene Editing the Phytoene Desaturase Alleles of Cavendish Banana Using CRISPR/Cas9.” *Transgenic Research* 27 (5): 451–60. https://doi.org/10.1007/S11248-018-0083-0.

Naito, Yuki, Kimihiro Hino, Hidemasa Bono, and Kumiko Ui-Tei. 2015. “CRISPRdirect: Software for Designing CRISPR/Cas Guide RNA with Reduced off-Target Sites.” *Bioinformatics* 31 (7): 1120–23. https://doi.org/10.1093/BIOINFORMATICS/BTU743.

Nakajima, Ikuko, Yusuke Ban, Akifumi Azuma, Noriyuki Onoue, Takaya Moriguchi, Toshiya Yamamoto, Seiichi Toki, and Masaki Endo. 2017. “CRISPR/Cas9-Mediated Targeted Mutagenesis in Grape.” *PloS One* 12 (5). https://doi.org/10.1371/JOURNAL.PONE.0177966.

Nakamura, T., H. Akita, Y. Yamada, H. Hatakeyama, and H. Harashima. 2012. “A Multifunctional Envelope-Type Nanodevice for Use in Nanomedicine: Concept and Applications.” *Accounts of Chemical Research* 45 (7): 1113–21. https://doi.org/10.1021/AR200254S/ASSET/IMAGES/MEDIUM/AR-2011-00254S_0006.GIF.

Narayanan, Narayanan, Getu Beyene, Raj Deepika Chauhan, Eliana Gaitán-Solís, Jackson Gehan, Paula Butts, Dimuth Siritunga, et al. 2019. “Biofortification of Field-Grown Cassava by Engineering Expression of an Iron Transporter and Ferritin.” *Nature Biotechnology 2019 37:2* 37 (2): 144–51. https://doi.org/10.1038/s41587-018-0002-1.

Nekrasov, Vladimir, Brian Staskawicz, Detlef Weigel, Jonathan D.G. Jones, and Sophien Kamoun. 2013. “Targeted Mutagenesis in the Model Plant Nicotiana Benthamiana Using Cas9 RNA-Guided Endonuclease.” *Nature Biotechnology* 31 (8): 691–93. https://doi.org/10.1038/NBT.2655.

Newell, Christine A, Naomi J Brown, Zheng Liu, Alexander Pflug, Udo Gowik, Peter Westhoff, and Julian M Hibberd. 2010. “Agrobacterium Tumefaciens-Mediated Transformation of Cleome Gynandra L., a C4 Dicotyledon That Is Closely Related to Arabidopsis Thaliana.” *Journal of Experimental Botany* 61 (5): 1311–19.

Nishitani, Chikako, Narumi Hirai, Sadao Komori, Masato Wada, Kazuma Okada, Keishi Osakabe, Toshiya Yamamoto, and Yuriko Osakabe. 2016a. “Efficient Genome Editing in Apple Using a CRISPR/Cas9 System.” *Scientific Reports 2016 6:1* 6 (1): 1–8. https://doi.org/10.1038/srep31481.

———. 2016b. “Efficient Genome Editing in Apple Using a CRISPR/Cas9 System.” *Scientific Reports* 6 (August). https://doi.org/10.1038/SREP31481.

Noman, Ali, Muhammad Aqeel, and Shuilin He. 2016. “CRISPR-Cas9: Tool for Qualitative and Quantitative Plant Genome Editing.” *Frontiers in Plant Science* 7 (NOVEMBER2016): 1740. https://doi.org/10.3389/FPLS.2016.01740/BIBTEX.

Nunez, Sarahi, Eric Arets, Rob Alkemade, Caspar Verwer, and Rik Leemans. 2019. “Assessing the Impacts of Climate Change on Biodiversity: Is below 2° C Enough?” *Climatic Change* 154: 351–65.

Osakabe, Yuriko, Zhenchang Liang, Chong Ren, Chikako Nishitani, Keishi Osakabe, Masato Wada, Sadao Komori, et al. 2018. “CRISPR–Cas9-Mediated Genome Editing in Apple and Grapevine.” *Nature Protocols 2018 13:12* 13 (12): 2844–63. https://doi.org/10.1038/s41596-018-0067-9.

Ousterout, David G., Ami M. Kabadi, Pratiksha I. Thakore, William H. Majoros, Timothy E. Reddy, and Charles A. Gersbach. 2015. “Multiplex CRISPR/Cas9-Based Genome

Editing for Correction of Dystrophin Mutations That Cause Duchenne Muscular Dystrophy." *Nature Communications 2015 6:1* 6 (1): 1–13. https://doi.org/10.1038/ncomms7244.

Peer, Reut, Gil Rivlin, Sara Golobovitch, Moshe Lapidot, Amit Gal-On, Alexander Vainstein, Tzvi Tzfira, and Moshe A. Flaishman. 2015. "Targeted Mutagenesis Using Zinc-Finger Nucleases in Perennial Fruit Trees." *Planta* 241 (4): 941–51. https://doi.org/10.1007/S00425-014-2224-X.

Peng, Aihong, Shanchun Chen, Tiangang Lei, Lanzhen Xu, Yongrui He, Liu Wu, Lixiao Yao, and Xiuping Zou. 2017a. "Engineering Canker-Resistant Plants through CRISPR/Cas9-Targeted Editing of the Susceptibility Gene CsLOB1 Promoter in Citrus." *Plant Biotechnology Journal* 15 (12): 1509–19. https://doi.org/10.1111/PBI.12733.

———. 2017b. "Engineering Canker-Resistant Plants through CRISPR/Cas9-Targeted Editing of the Susceptibility Gene CsLOB1 Promoter in Citrus." *Plant Biotechnology Journal* 15 (12): 1509–19. https://doi.org/10.1111/PBI.12733.

Platt, Randall J., Sidi Chen, Yang Zhou, Michael J. Yim, Lukasz Swiech, Hannah R. Kempton, James E. Dahlman, et al. 2014. "CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling." *Cell* 159 (2): 440–55. https://doi.org/10.1016/J.CELL.2014.09.014.

Prykhozhij, Sergey V., Vinothkumar Rajan, Daniel Gaston, and Jason N. Berman. 2015. "CRISPR MultiTargeter: A Web Tool to Find Common and Unique CRISPR Single Guide RNA Targets in a Set of Similar Sequences." *PLOS ONE* 10 (3): e0119372. https://doi.org/10.1371/JOURNAL.PONE.0119372.

Ramesh, S. V., V. Arunachalam, and M. K. Rajesh. 2020. "Genomic Designing of Climate-Smart Coconut." *Genomic Designing of Climate-Smart Fruit Crops*, 135–56. https://doi.org/10.1007/978-3-319-97946-5_6.

Raveux, Aurélien, Sandrine Vandormael-Pournin, and Michel Cohen-Tannoudji. 2017. "Optimization of the Production of Knock-in Alleles by CRISPR/Cas9 Microinjection into the Mouse Zygote." *Scientific Reports* 7 (February). https://doi.org/10.1038/SREP42661.

Rees, Holly A., and David R. Liu. 2018. "Base Editing: Precision Chemistry on the Genome and Transcriptome of Living Cells." *Nature Reviews Genetics 2018 19:12* 19 (12): 770–88. https://doi.org/10.1038/s41576-018-0059-1.

"Regulation of Citrus DMR6</Em> via RNA Interference and CRISPR/Cas9-Mediated Gene Editing to Improve Huanglongbing Tolerance." n.d.

Rodríguez-Leal, Daniel, Zachary H Lemmon, Jarrett Man, Madelaine E Bartlett, and Zachary B Lippman. 2017. "Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing." *Cell* 171 (2): 470-480.e8. https://doi.org/10.1016/j.cell.2017.08.030.

Roehm, Pamela C., Masoud Shekarabi, Hassen S. Wollebo, Anna Bellizzi, Lifan He, Julian Salkind, and Kamel Khalili. 2016. "Inhibition of HSV-1 Replication by Gene Editing Strategy." *Scientific Reports 2016 6:1* 6 (1): 1–11. https://doi.org/10.1038/srep23146.

Rugienius, Rytis, Birutė Frercks, Ingrida Mažeikienė, Neringa Rasiukevičiūtė, Danas Baniulis, and Vidmantas Stanys. 2020. "Development of Climate-Resilient Varieties in

Rosaceous Berries." *Genomic Designing of Climate-Smart Fruit Crops*, 333–84. https://doi.org/10.1007/978-3-319-97946-5_9.

Salonia, Fabrizio, Angelo Ciacciulli, Lara Poles, Helena Domenica Pappalardo, Stefano La Malfa, and Concetta Licciardello. 2020. "New Plant Breeding Techniques in Citrus for the Improvement of Important Agronomic Traits. A Review." *Frontiers in Plant Science* 11. https://doi.org/10.3389/fpls.2020.01234.

Sattar, Muhammad N., Zafar Iqbal, Muhammad N. Tahir, Muhammad S. Shahid, Muhammad Khurshid, Abdullatif A. Al-Khateeb, and Suliman A. Al-Khateeb. 2017. "CRISPR/Cas9: A Practical Approach in Date Palm Genome Editing." *Frontiers in Plant Science* 8 (August). https://doi.org/10.3389/FPLS.2017.01469/FULL.

Sauer, Noel J., Jerry Mozoruk, Ryan B. Miller, Zachary J. Warburg, Keith A. Walker, Peter R. Beetham, Christian R. Schöpke, and Greg F.W. Gocal. 2016. "Oligonucleotide-Directed Mutagenesis for Precision Gene Editing." *Plant Biotechnology Journal* 14 (2): 496–502. https://doi.org/10.1111/PBI.12496.

Scalabrin, Simone, Lucile Toniutti, Gabriele Di Gaspero, Davide Scaglione, Gabriele Magris, Michele Vidotto, Sara Pinosio, et al. 2020. "A Single Polyploidization Event at the Origin of the Tetraploid Genome of Coffea Arabica Is Responsible for the Extremely Low Genetic Variation in Wild and Cultivated Germplasm." *Scientific Reports 2020 10:1* 10 (1): 1–13. https://doi.org/10.1038/s41598-020-61216-7.

Schwank, Gerald, Bon Kyoung Koo, Valentina Sasselli, Johanna F. Dekkers, Inha Heo, Turan Demircan, Nobuo Sasaki, et al. 2013. "Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients." *Cell Stem Cell* 13 (6): 653–58. https://doi.org/10.1016/J.STEM.2013.11.002.

Sedelnikova, Olga V, Thomas E Hughes, and Jane A Langdale. 2018. "Understanding the Genetic Basis of C4 Kranz Anatomy with a View to Engineering C3 Crops." *Annual Review of Genetics* 52: 249–70.

Shan, Qiwei, Yanpeng Wang, Jun Li, Yi Zhang, Kunling Chen, Zhen Liang, Kang Zhang, et al. 2013. "Targeted Genome Modification of Crop Plants Using a CRISPR-Cas System." *Nature Biotechnology* 31 (8): 686–88. https://doi.org/10.1038/NBT.2650.

Shao, Xiuhong, Shaoping Wu, Tongxin Dou, Haocheng Zhu, Chunhua Hu, Heqiang Huo, Weidi He, et al. 2020. "Using CRISPR/Cas9 Genome Editing System to Create MaGA20ox2 Gene-Modified Semi-Dwarf Banana." *Plant Biotechnology Journal* 18 (1): 17–19. https://doi.org/10.1111/pbi.13216.

South, Paul F., Amanda P. Cavanagh, Helen W. Liu, and Donald R. Ort. 2019. "Synthetic Glycolate Metabolism Pathways Stimulate Crop Growth and Productivity in the Field." *Science* 363 (6422). https://doi.org/10.1126/SCIENCE.AAT9077/SUPPL_FILE/DATASETS_S1-S11_S16-S20.PDF.

Stemmer, Manuel, Thomas Thumberger, Maria Del Sol Keyer, Joachim Wittbrodt, and Juan L. Mateo. 2015. "CCTop: An Intuitive, Flexible and Reliable CRISPR/Cas9 Target Prediction Tool." *PLOS ONE* 10 (4): e0124633. https://doi.org/10.1371/JOURNAL.PONE.0124633.

Su, Jinghan, Huiping Sun, Qingshuo Meng, Pengcheng Zhang, Qi Yin, and Yaping Li. 2017.

"Enhanced Blood Suspensibility and Laser-Activated Tumor-Specific Drug Release of Theranostic Mesoporous Silica Nanoparticles by Functionalizing with Erythrocyte Membranes." *Theranostics* 7 (3): 523–37. https://doi.org/10.7150/THNO.17259.

Suleiman, Ahmed Abdul Jabbar, Walaa Yahya Saedi, and Mohammed Jobair Muhaidi. 2021. "Widely Used Gene Editing Strategies in Cancer Treatment a Systematic Review." *Gene Reports* 22 (March): 100983. https://doi.org/10.1016/J.GENREP.2020.100983.

Sun, Wujin, Wenyan Ji, Jordan M. Hall, Quanyin Hu, Chao Wang, Chase L. Beisel, and Zhen Gu. 2015. "Self-Assembled DNA Nanoclews for the Efficient Delivery of CRISPR-Cas9 for Genome Editing." *Angewandte Chemie (International Ed. in English)* 54 (41): 12029–33. https://doi.org/10.1002/ANIE.201506030.

Tabassum, Javaria, Shakeel Ahmad, Babar Hussain, Amos Musyoki Mawia, Aqib Zeb, and Luo Ju. 2021. "Applications and Potential of Genome-Editing Systems in Rice Improvement: Current and Future Perspectives." *Agronomy 2021, Vol. 11, Page 1359* 11 (7): 1359. https://doi.org/10.3390/AGRONOMY11071359.

Tabebordbar, Mohammadsharif, Kexian Zhu, Jason K.W. Cheng, Wei Leong Chew, Jeffrey J. Widrick, Winston X. Yan, Claire Maesner, et al. 2016. "In Vivo Gene Editing in Dystrophic Mouse Muscle and Muscle Stem Cells." *Science (New York, N.Y.)* 351 (6271): 407–11. https://doi.org/10.1126/SCIENCE.AAD5177.

Taylor, Rachel A., Sadie J. Ryan, Catherine A. Lippi, David G. Hall, Hossein A. Narouei-Khandan, Jason R. Rohr, and Leah R. Johnson. 2019. "Predicting the Fundamental Thermal Niche of Crop Pests and Diseases in a Changing World: A Case Study on Citrus Greening." *Journal of Applied Ecology* 56 (8): 2057–68. https://doi.org/10.1111/1365-2664.13455.

Teng, Kai Wen, Yuji Ishitsuka, Pin Ren, Yeoan Youn, Xiang Deng, Pinghua Ge, Andrew S. Belmont, and Paul R. Selvin. 2016. "Labeling Proteins inside Living Cells Using External Fluorophores for Microscopy." *ELife* 5 (DECEMBER2016). https://doi.org/10.7554/ELIFE.20378.

Tripathi, Jaindra N., Valentine O. Ntui, Mily Ron, Samwel K. Muiruri, Anne Britt, and Leena Tripathi. 2019. "CRISPR/Cas9 Editing of Endogenous Banana Streak Virus in the B Genome of Musa Spp. Overcomes a Major Challenge in Banana Breeding." *Communications Biology 2019 2:1* 2 (1): 1–11. https://doi.org/10.1038/s42003-019-0288-7.

Truong, Dong Jiunn Jeffery, Karin Kühner, Ralf Kühn, Stanislas Werfel, Stefan Engelhardt, Wolfgang Wurst, and Oskar Ortiz. 2015. "Development of an Intein-Mediated Split-Cas9 System for Gene Therapy." *Nucleic Acids Research* 43 (13): 6450–58. https://doi.org/10.1093/NAR/GKV601.

Velasco, Riccardo, Andrey Zharkikh, Jason Affourtit, Amit Dhingra, Alessandro Cestaro, Ananth Kalyanaraman, Paolo Fontana, et al. 2010. "The Genome of the Domesticated Apple (Malus × Domestica Borkh.)." *Nature Genetics* 42 (10): 833–39. https://doi.org/10.1038/NG.654.

Verde, Ignazio, Albert G. Abbott, Simone Scalabrin, Sook Jung, Shengqiang Shu, Fabio Marroni, Tatyana Zhebentyayeva, et al. 2013. "The High-Quality Draft Genome of Peach (Prunus Persica) Identifies Unique Patterns of Genetic Diversity, Domestication

and Genome Evolution." *Nature Genetics 2013 45:5* 45 (5): 487–94. https://doi.org/10.1038/ng.2586.

Wan, Dong-Yan, Ye Guo, Yuan Cheng, Yang Hu, Shunyuan Xiao, Yuejin Wang, and Ying-Qiang Wen. 2020. "CRISPR/Cas9-Mediated Mutagenesis of VvMLO3 Results in Enhanced Resistance to Powdery Mildew in Grapevine (Vitis Vinifera)." *Horticulture Research* 7 (January): 116. https://doi.org/10.1038/s41438-020-0339-8.

Wang, Jiawei, Weizhen Liu, Dongzi Zhu, Po Hong, Shizhong Zhang, Shijun Xiao, Yue Tan, et al. 2020. "Chromosome-Scale Genome Assembly of Sweet Cherry (Prunus Avium L.) Cv. Tieton Obtained Using Long-Read and Hi-C Sequencing." *Horticulture Research 2020 7:1* 7 (1): 1–11. https://doi.org/10.1038/s41438-020-00343-8.

Wang, Xianhang, Mingxing Tu, Dejun Wang, Jianwei Liu, Yajuan Li, Zhi Li, Yuejin Wang, and Xiping Wang. 2018. "CRISPR/Cas9-Mediated Efficient Targeted Mutagenesis in Grape in the First Generation." *Plant Biotechnology Journal* 16 (4): 844–55. https://doi.org/10.1111/PBI.12832.

Wang, Yan, Tao Sun, Tingting Li, Meng Wang, Guangxiao Yang, and Guangyuan He. 2016. "A CBL-Interacting Protein Kinase TaCIPK2 Confers Drought Tolerance in Transgenic Tobacco Plants through Regulating the Stomatal Movement." *PLOS ONE* 11 (12): e0167962. https://doi.org/10.1371/JOURNAL.PONE.0167962.

Wang, Zupeng, Shuaibin Wang, Dawei Li, Qiong Zhang, Li Li, Caihong Zhong, Yifei Liu, and Hongwen Huang. 2018a. "Optimized Paired-SgRNA/Cas9 Cloning and Expression Cassette Triggers High-Efficiency Multiplex Genome Editing in Kiwifruit." *Plant Biotechnology Journal* 16 (8): 1424–33. https://doi.org/10.1111/PBI.12884.

———. 2018b. "Optimized Paired-SgRNA/Cas9 Cloning and Expression Cassette Triggers High-Efficiency Multiplex Genome Editing in Kiwifruit." *Plant Biotechnology Journal* 16 (8): 1424–33. https://doi.org/10.1111/PBI.12884.

Wen, Xiao Peng, Xiao Ming Pang, Narumi Matsuda, Masayuki Kita, Hiromichi Inoue, Yu Jin Hao, Chikako Honda, and Takaya Moriguchi. 2008. "Over-Expression of the Apple Spermidine Synthase Gene in Pear Confers Multiple Abiotic Stress Tolerance by Altering Polyamine Titers." *Transgenic Research* 17 (2): 251–63. https://doi.org/10.1007/S11248-007-9098-7.

Wong, Nathan, Weijun Liu, and Xiaowei Wang. 2015. "WU-CRISPR: Characteristics of Functional Guide RNAs for the CRISPR/Cas9 System." *Genome Biology* 16 (1): 1–8. https://doi.org/10.1186/S13059-015-0784-0/FIGURES/4.

Wu, Jun, Zhiwen Wang, Zebin Shi, Shu Zhang, Ray Ming, Shilin Zhu, M. Awais Khan, et al. 2013. "The Genome of the Pear (Pyrus Bretschneideri Rehd.)." *Genome Research* 23 (2): 396–408. https://doi.org/10.1101/GR.144311.112.

Xiao, Yong, Pengwei Xu, Haikuo Fan, Luc Baudouin, Wei Xia, Stéphanie Bocs, Junyang Xu, et al. 2017. "The Genome Draft of Coconut (Cocos Nucifera)." *GigaScience* 6 (11): 1. https://doi.org/10.1093/GIGASCIENCE/GIX095.

Xie, Kabin, and Yinong Yang. 2013. "RNA-Guided Genome Editing in Plants Using a CRISPR-Cas System." *Molecular Plant* 6 (6): 1975–83. https://doi.org/10.1093/MP/SST119.

Xu, Huimin, Qingyi Yu, Yan Shi, Xiuting Hua, Haibao Tang, Long Yang, Ray Ming, and

Jisen Zhang. 2018. “PGD: Pineapple Genomics Database.” *Horticulture Research 2018 5:1* 5 (1): 1–9. https://doi.org/10.1038/s41438-018-0078-2.

Xu, Qiang, Ling Ling Chen, Xiaoan Ruan, Dijun Chen, Andan Zhu, Chunli Chen, Denis Bertrand, et al. 2012. “The Draft Genome of Sweet Orange (Citrus Sinensis).” *Nature Genetics 2012 45:1* 45 (1): 59–66. https://doi.org/10.1038/ng.2472.

Yang, Hung Chih, and Pei Jer Chen. 2018. “The Potential and Challenges of CRISPR-Cas in Eradication of Hepatitis B Virus Covalently Closed Circular DNA.” *Virus Research* 244 (January): 304–10. https://doi.org/10.1016/J.VIRUSRES.2017.06.010.

Yang, Yue, Jin Xu, Shuyu Ge, and Liqin Lai. 2021. “CRISPR/Cas: Advances, Limitations, and Applications for Precision Cancer Research.” *Frontiers in Medicine* 8 (March): 115. https://doi.org/10.3389/FMED.2021.649896/BIBTEX.

Yee, Jiing-Kuan, and -K Yee. 2016. “Off-Target Effects of Engineered Nucleases.” *The FEBS Journal* 283 (17): 3239–48. https://doi.org/10.1111/FEBS.13760.

Zambounis, Antonios, Ioannis Ganopoulos, Filippos Aravanopoulos, Zoe Hilioti, Panagiotis Madesis, Athanassios Molassiotis, Athanasios Tsaftaris, and Aliki Xanthopoulou. 2020. “Genomics Opportunities and Breeding Strategies Towards Improvement of Climate-Smart Traits and Disease Resistance Against Pathogens in Sweet Cherry.” *Genomic Designing of Climate-Smart Fruit Crops*, 385–404. https://doi.org/10.1007/978-3-319-97946-5_10.

Zhang, Fei, Chantal LeBlanc, Vivian F. Irish, and Yannick Jacob. 2017. “Rapid and Efficient CRISPR/Cas9 Gene Editing in Citrus Using the YAO Promoter.” *Plant Cell Reports* 36 (12): 1883–87. https://doi.org/10.1007/S00299-017-2202-4.

Zhang, Yi, Karen Massel, Ian D. Godwin, and Caixia Gao. 2018. “Applications and Potential of Genome Editing in Crop Improvement 06 Biological Sciences 0604 Genetics 06 Biological Sciences 0607 Plant Biology 07 Agricultural and Veterinary Sciences 0703 Crop and Pasture Production.” *Genome Biology* 19 (1): 1–11. https://doi.org/10.1186/S13059-018-1586-Y/FIGURES/2.

Zhang, Yingxiao, Aimee A Malzahn, Simon Sretenovic, and Yiping Qi. 2019. “The Emerging and Uncultivated Potential of CRISPR Technology in Plant Science.” *Nature Plants* 5 (8): 778–94.

Zhou, Junhui, Guoming Wang, and Zhongchi Liu. 2018. “Efficient Genome Editing of Wild Strawberry Genes, Vector Development and Validation.” *Plant Biotechnology Journal* 16 (11): 1868–77. https://doi.org/10.1111/PBI.12922.

Zhu, Haocheng, Chao Li, and Caixia Gao. 2020. “Applications of CRISPR–Cas in Agriculture and Plant Biotechnology.” *Nature Reviews Molecular Cell Biology* 21 (11): 661–77.

Zhu, Qing gang, Yang Xu, Yong Yang, Chang fei Guan, Qiu yun Zhang, Jing wen Huang, Don Grierson, Kun song Chen, Bang chu Gong, and Xue ren Yin. 2019. “The Persimmon (Diospyros Oleifera Cheng) Genome Provides New Insights into the Inheritance of Astringency and Ancestral Evolution.” *Horticulture Research 2019 6:1* 6 (1): 1–15. https://doi.org/10.1038/s41438-019-0227-2.

Zischewski, Julia, Rainer Fischer, and Luisa Bortesi. 2017. “Detection of On-Target and off-Target Mutations Generated by CRISPR/Cas9 and Other Sequence-Specific

 doi:10.20944/preprints202305.1887.v1

Nucleases." *Biotechnology Advances* 35 (1): 95–104. https://doi.org/10.1016/J.BIOTECHADV.2016.12.003.

Zuris, John A., David B. Thompson, Yilai Shu, John P. Guilinger, Jeffrey L. Bessen, Johnny H. Hu, Morgan L. Maeder, J. Keith Joung, Zheng Yi Chen, and David R. Liu. 2014. "Cationic Lipid-Mediated Delivery of Proteins Enables Efficient Protein-Based Genome Editing in Vitro and in Vivo." *Nature Biotechnology 2014 33:1* 33 (1): 73–80. https://doi.org/10.1038/nbt.3081.