OXFORD

Food Quality and Safety, 2020, 4, 159–166
doi:10.1093/fqsafe/fyaa028
Review
Advance Access publication 27 October 2020

Review

CRISPR/Cas9-mediated gene-editing technology in fruit quality improvement

Xin Xu*, Yujin Yuan*, Bihong Feng and Wei Deng***

*Key Laboratory of Plant Hormones and Development Regulation of Chongqing, School of Life Sciences, Chongqing University, Chongqing, China and **College of Agriculture, Guangxi University, Nanning, China.

Correspondence to: Wei Deng, Key Laboratory of Plant Hormones and Development Regulation of Chongqing, School of Life Sciences, Chongqing University, Chongqing 400044, China. E-mail: dengwei1977@cqu.edu.cn and Bihong Feng, College of Agriculture, Guangxi University, Nanning 530004, China. E-mail: fbh@gxu.edu.cn

Received 23 May 2020; Revised 19 October 2020; Editorial decision 19 October 2020.

Abstract

Fruits are an essential part of a healthy, balanced diet and it is particularly important for fibre, essential vitamins, and trace elements. Improvement in the quality of fruit and elongation of shelf life are crucial goals for researchers. However, traditional techniques have some drawbacks, such as long period, low efficiency, and difficulty in the modification of target genes, which limit the progress of the study. Recently, the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technique was developed and has become the most popular gene-editing technology with high efficiency, simplicity, and low cost. CRISPR/Cas9 technique is widely accepted to analyse gene function and complete genetic modification. This review introduces the latest progress of CRISPR/Cas9 technology in fruit quality improvement. For example, CRISPR/Cas9-mediated targeted mutagenesis of RIPENING INHIBITOR gene (*RIN*), Lycopene desaturase (*PDS*), Pectate lyases (*PL*), SIMYB12, and CLAVATA3 (*CLV3*) can affect fruit ripening, fruit bioactive compounds, fruit texture, fruit colouration, and fruit size. CRISPR/Cas9-mediated mutagenesis has become an efficient method to modify target genes and improve fruit quality.

Key words: fruit; CRISPR/Cas9; fruit quality.

Introduction

At present, the genome sequences of many species, including model plants, crops, and medicinal species, have been completed. Researchers have been focussing on the study of gene function, genetic modification, and genetic improvement for decades. Gene modification technology can carry out site-specific knockout, replacement, mutation, and introduction of exogenous genes in genome. There are three main types of gene modification technologies including gene targeting, RNA interference (RNAi), and engineered endonuclease (Zhou *et al.*, 2019). These three kinds of techniques have been widely used in gene modification. However, the techniques have some drawbacks, such as long transformation period, low efficiency, and difficulty in the modification of the target gene. Recently, gene-editing technology, as emerging biotechnology to modify target genes, has been developed rapidly and efficiently.

Gene editing technology mainly includes zinc-finger nucleases (ZFNs; Takatsuji, 1999), transcription activator-like effector nucleases (TALENs; Li *et al.*, 2011), and clustered regularly interspaced short palindromic repeats (CRISPR; Barrangou *et al.*, 2007; Ran *et al.*, 2013; Mao *et al.*, 2019). These technologies usually involve the application of sequence-specific nucleases to identify the sequences connected to the nuclease domain, which can precisely target double-strand DNA to produce double-strand break (DSB). DSB prompts cells to initiate two major DNA damage repair mechanisms: non-homologous end joining (NHEJ) and homology-directed repair (HDR; Moore and Haber, 1996; Haber *et al.*, 2004). In the NHEJ repair, the broken ends of the double strands can be

directly pulled closer to each other by repairing proteins and re-joined with the help of DNA ligase (Budman and Chu, 2005). HDR is a mechanism to repair DNA double-strand damage in cells and can only occur when there are DNA fragments homologous to the damaged DNA in the nucleus (Zha *et al.*, 2009). Therefore, HDR can introduce a specific point mutation sequence by providing exogenous donor template. The repair processes of NHEJ and HDR are shown in Figure 1. ZFNs are the first generation of gene editing nuclease, which is formed by the fusion of transcription factors containing the zinc finger domain and the cutting domain of FokI endonuclease (Mahfouz *et al.*, 2011). FokI protein, a type IIS nuclease, contains an N-terminal DNA-binding domain and a non-specific C-terminal DNA-cleavage domain (Kim *et al.*, 1997). ZFNs technology is a DNA-targeted modification technology, which is widely used in genome targeted modification. But the complex construction process, high cost, high off-target rate, and high toxicity to cells limit the development of ZFNs technique (Gaj *et al.*, 2013). TALENs are nucleic acid endonucleases formed by the fusion of specific DNA-binding domain and non-specific endonuclease FokI cleavage domain, which recognizes the correspondence between single protein and nucleotide (Joung and Sander, 2013). Compared with the ZFNs, TALENs technique is easier to assemble and design and has a lower off-target effect (Mahfouz *et al.*, 2011). CRISPR/Cas9 was first discovered in 1987 in the flanks of the *iap* gene sequence of K12 in *Escherichia coli* (Ishino *et al.*, 1987). In recent decades, CRISPR/Cas9 technology has developed to become the most popular gene-editing technology. CRISPR/Cas9 technology is a new technology that uses specific nucleases to edit the genome under the guidance of specific RNA (Sampson and Weiss, 2014). Compared with the ZFNs and TALENs, CRISPR/Cas9 technique can achieve fixed-point modification of DNA, which is easy to customize and highly efficient in target shooting (Makarova *et al.*, 2015). Therefore, many researchers choose CRISPR/Cas9 gene-editing tool to conduct a qualitative and locational analysis of one or more genes (Puchta, 2017).

Fruits contain fibre, vitamins, minerals, and bioactive compounds, which are major nutrients for human health and prevent the occurrence of many diseases (Giovannoni, 2007; Singh *et al.*, 2016; Wang *et al.*, 2019d). Fruit can also be used as a staple food. Bananas and plantains are used as the main food in some tropical regions (Giovannoni *et al.*, 2017). Fruit crops have been threatened by the external environment and other factors, such as drought, cold, and disease. Some fruit crops have a low fruit setting rate and are easy to rot. In order to improve fruit quality and stabilize the supply of fruit, the crops were domesticated from wild plants from generation to generation or selected by the sexual hybridization of suitable parents (Hickey *et al.*, 2017). However, this screening method has a long cycle, large randomness, and a high mutation rate (Yusuff *et al.*, 2016). Transgenic technology is also used for the regulation of gene expression, which may have the characteristics of a long cycle, low safety, and cumbersome process (Martin and Caplen, 2007). Virus-induced gene silencing (VIGS) technology has been widely used in the field for plant gene function research because of its simplicity, high efficiency, and no need to rely on transgenic operations. However, compared with transgenic technology, VIGS is of low cost and fast and many viral silencing vectors only induce a short-term silent phenotype (Ding *et al.*, 2018). In addition, many genetic engineering technologies have emerged that can accurately modify specific target genes in the genome of organisms. Yang *et al.* (2017) used CRISPR/Cas9 to generate *SlORRM4* gene mutants and obtained *slorrm4* mutant lines with delayed fruit ripening. In 2018, CRISPR/Cas9-mediated mutations of long non-coding RNA1459 (*lncRNA1459*) locus resulted in an inhibition of fruit ripening (Li *et al.*, 2018b). Gene-editing technology is an indispensable tool for the study of gene functions and the genetic improvement of fruit crops.

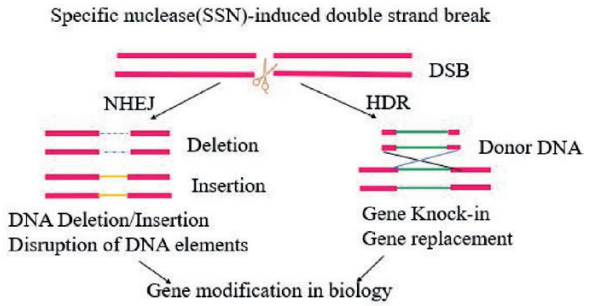

Figure 1. The repair processes of NHEJ and HDR. NHEJ, non-homologous end joining; HDR, homology-directed repair; DSB, double-strand break.

CRISPR/Cas9 Gene Editing Technique

The CRISPR/Cas9 system consists of CRISPR sequence and Cas9 protein. CRISPR sequence is composed of some highly conservative repetitive sequence and interval sequence and Cas9 protein encoded by related genes near the CRISPR sequence. Cas9 protein has nuclease activity and can cut the DNA sequence, leading to DNA DSB (Barrangou, 2013). The earliest known CRISPR/Cas9 system is an autoimmune defense mechanism that can resist foreign DNA invasion (bacteriophages, plasmids, etc.). When exogenous DNA invades, the process of DNA signalling to RNA is disrupted, and CRISPR RNA (crRNA), trans-activated RNA (tracer-RNA), and Cas9 nucleases work together to destroy the binding sites of the invading DNA, thus protecting the host bacteria (Zhou *et al.*, 2020). In general, the CRISPR/Cas9 gene editing mainly consists of three processes: adaptation, obtaining new spacers from invading elements and transferring spacers into the CRISPR site for immunity; production of crRNA, in which the CRISPR locus is transcribed and processed into small interfering crRNA; interference, in which crRNA directs the Cas9 mechanism to specifically clear invasive nucleic acids (Barrangou, 2013). The working principle of the CRISPR/Cas9 of *Streptococcus pyogenes* type II was demonstrated by Jinek *et al.* (2012) and it was proposed that Cas9 could cleave the double strand of target DNA under the guidance of recombinant small RNA molecule (sgRNA; Martin *et al.*, 2012; Figure 2).

Hwang *et al.* (2013) reported that synthetic sgRNAs could guide Cas9 endogenous nucleases to modify zebrafish embryo genes (Hwang *et al.*, 2013). Since 2013, researchers have published several articles on the CRISPR/Cas9 system in *Science* and *Nature Biotechnology*, which reported that precise genetic modification has been successfully achieved in mice, zebrafish, and other species. At the same time, the CRISPR/Cas9 system was optimized, including

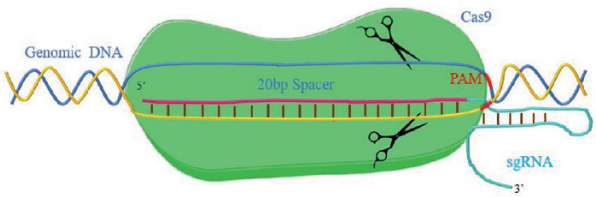

Figure 2. The mechanism of the CRISP/Cas9 system. sgRNA, small RNA molecule.

the optimization of Cas protein, promoter, and sgRNA. Now, in addition to Cas9, Cas12a (Cpf1), Cas13a (C2c2), nCas9, and dCas9 are also used in the optimized CRISPR/Cas9 system for the study of microbial immunity, nucleic acid detection, and plant defense mechanisms (Fauser *et al.*, 2014; Zetsche *et al.*, 2015; Gootenberg *et al.*, 2017). The optimized YAO, SPL, DMC1, and MGE promoters are used in studying the cell division and crop improvement of *Arabidopsis*, citrus, and maize (Mao *et al.*, 2016; Zhang *et al.*, 2017; Feng *et al.*, 2018; Xu *et al.*, 2018). Multiple sgRNA expression cassettes are constructed into one CRISPR/Cas9 vector, and a single polycistron gene is used to produce a large number of sgRNA, which is used to study the gene function in maize and tobacco (Gao *et al.*, 2015; Char *et al.*, 2017). CRISPR/Cas9 system has been applied to the study of fruit ripening and quality.

Application of Gene Editing in Fruit Ripening

The fleshy fruit undergoes a developmental process and ends with an irreversible maturation process. A lot of physiological, biochemical, and structural changes have taken place during the ripening process of fruit, as a result, it can attract more seed spreaders (Gapper *et al.*, 2013). After the fruit reaches the optimum edible stage, the fruit will slowly deteriorate and the fruit quality will be reduced. Therefore, the regulation of fruit ripening has become the focus of many scientists (Martín-Pizarro and Posé, 2018).

RIPENING INHIBITOR gene (*RIN*) belongs to the MADS-box gene family and mutations of this gene can inhibit the ripening of tomato fruits (Vrebalov *et al.*, 2002). *RIN* is thought to be the main regulatory gene in the maturation of tomato fruits and activates and promotes all physiological processes associated with ripening, including colour, hardness, and flavour (Fujisawa *et al.*, 2013). The *RIN* gene is knocked out in tomato by CRISPR/Cas9 system and the *rin* mutant is analysed (Ito *et al.*, 2015). The *rin* knockout mutation does not affect the initiation of ripening and exhibits moderate red colouring, which demonstrates that *RIN* gene is not required for the ripening initiation in fruit (Ito *et al.*, 2017). The *rin* mutant can lead to ripening failure, which is caused by the deletion part of the DNA fragment between *rin* and the adjacent gene *MACROCALYX* (*MC*) (Vrebalov *et al.*, 2002). Due to the partial deletion of DNA fragments, the transcription factor MADS-RIN and MADS-MC are fused to form the function of the fusion protein RIN–MC. The RIN–MC fusion protein encodes a new transcription factor, which regulates the expression of downstream genes and inhibits fruit ripening, which has a negative regulatory effect. (Vrebalov *et al.*, 2002; Ito *et al.*, 2017; Li *et al.*, 2018d). Osorio *et al.* (2020) reported that tomato breeders use *RIN* mutation to acquire improved hybrids which exert a negative impact on tomato flavour. Li *et al.* (2020) pointed out that RIN-deficient fruits obtained by CRISPR/Cas9 technology can reduce ethylene production and affect the synthesis of volatile substances and carotenoids. The low ethylene production is due to the fact that RIN-deficient fruits cannot induce the production of ethylene in the autocatalytic system-2. They also lack volatiles and carotenoids and transcripts related to these pathways. Meanwhile, Li *et al.* (2020) supported that the fruit ripening process requires the participation of ERFs, RIN, and ethylene. Ethylene initiates the maturation of green fruit and affects the expression of RIN and other factors, which complete the whole ripening process of fruits (Li *et al.*, 2020).

APETALA2a (AP2a), NON-RIPENING (NOR), and FRUITFULL (FUL1/TDR4 and FUL2/MBP7) play important roles in fruit ripening (Mordy *et al.*, 1998; Chung *et al.*, 2010; Bemer *et al.*, 2012). But these results were derived from analysis of the phenotypes of RNAi silencing lines and spontaneous mutants. Wang *et al.* (2019c) obtained knockout mutants of *AP2a*, *FUL1*, *FUL2*, and *NOR* genes in tomato by CRISPR/cas9 technology. The *nor* spontaneous mutant fruits show green, but the *nor* mutant produced by CRISPR/Cas9 exhibits earlier ripening and orange ripe phenotypes. Compared with spontaneous *nor* mutants, *nor* mutants have a milder phenotype (Wang *et al.*, 2020). The *ful1* or *ful2* double mutant shows a severe blocked ripening phenotype (Marian *et al.*, 2014; Wang *et al.*, 2019b). However, the *ful1* and *ful2* single mutants exhibit normal ripening phenotypes, indicating that *FUL1* and *FUL2* have a redundant function in the fruit ripening process.

The *NOR* and colourless non-ripening (*CNR*) genes are knocked out using CRISPR/Cas9-mediated mutagenesis and the mutants showed only delayed or partial immature phenotypes, which is different from their spontaneous mutants (Gao *et al.*, 2019). CRISPR/Cas9 technology has become the main method to re-evaluate the important gene in fruit ripening through the generation of null mutant. The tomato *nor* mutant produces a new protein, 186-amino-acid protein (NOR186), which has a prohibitive function that affects ripening. The tomato *nor* mutant has previously been proven to be a gain-of-function mutant, but the specific mechanism of action is still unclear (Wang *et al.*, 2019a). In *nor* natural mutants, the NOR186 protein can still enter the nucleus, which can bind but cannot activate the promoters of the key genes *SlACS2*, *SlGgpps2*, and *SlPL* for ethylene biosynthesis, carotenoid accumulation, and fruit softening. The activation effect of NOR protein on the above-mentioned promoter will be inhibited by NOR186. The above research results further prove that the *nor* natural mutant is a gain-of-function mutant, and the truncated protein NOR186 produced by the mutation of the NOR gene has a dominant negative function (Gao *et al.*, 2020). A recent study indicated that VIGS technology can mediate nor-like1 silencing, which can suppress tomato fruit ripening. The inactive mutant of nor-like1 was obtained through CRISPR/Cas9 technology, which delayed fruit ripening and inhibited ethylene, carotenoid synthesis, and fruit softening. The above results indicate that nor-like1 gene plays a positive regulatory role in tomato fruit ripening (Gao *et al.*, 2018).

According to reports, epigenetic modification and fruit ripening are inseparable. It has been found that the early epigenetic mark is DNA cytosine methylation in the plant genome, which respond to external environmental stress, regulate gene expression, and stabilize the genome (Chen *et al.*, 2018b). *SlDML2* is closely related to *Arabidopsis* DNA demethylase gene *ROS1*. *SlDML2* knockout mutants were obtained by the CRISPR/Cas9 system, which inhibits fruit ripening (Zhou *et al.*, 2019).

In summary, compared with traditional knockdown technology, mutants obtained by CRISPR/Cas technology can lead to unexpected weak phenotypes, which indicate that the compensation mechanism in the body may obscure protein function. This requires us to re-evaluate the model of the regulation of ripening, which may involve a complex network of redundant components (Wang *et al.*, 2020). This reminds us to carefully design experimental programs when using CRISPR/Cas technology to evaluate other mechanisms.

Gene Editing in Fruit Bioactive Compounds

Many natural biologically active substances in fresh fruits have anti-inflammatory, anti-cancer, anti-oxidation, and other physiological activities. Lycopene, carotenoid, anthocyanin, and gamma-aminobutyric acid (GABA) are the main functional factors in fresh fruits. Therefore, enhanced accumulation of bioactive substances has been focussed on by numerous studies (Amish *et al.*, 2015).

Carotenoids play a protective role in ROS-mediated disorders and photosensitive or eye-related disorders. Carotenoids are mostly C40 terpenoids, which affect the growth, development, and maturation of plants and improve the oxidative stability of poultry products such as egg and meat (Domonkos *et al.*, 2013; Nisar *et al.*, 2015; Nabi *et al.*, 2020).

Lycopene is an acyclic carotenoid and red pigment that is abundant in many ripening fruits. Lycopene reduces the risk of a variety of tumours, including prostate cancer, and cardiovascular disease (Li and Xu, 2014; Tang *et al.*, 2014). In the process of fruit ripening, lycopene is decreased due to the conversion to β-carotene and α-carotene. In tomato, Li *et al.* (2018c) knocked out *SGR1*, *LCY-E*, *BLC*, *LCY-B1*, and *LCY-B2* by the CRISPR/Cas9 method, which inhibited the conversion of lycopene and increased the lycopene content in the fruit about 5.1 times. During tomato fruit ripening, phytoene synthase 1 (PSY1) is involved in the formation of lycopene (Fray and Grierson,1993; Giorio *et al.*, 2008). Gene editing of *PSY1* was performed using CRISPR/Cas9 and the impaired *PSY1* gene results in the void of lycopene and yellow fruit, like yellow flesh mutant (D'Ambrosio *et al.*, 2018).

Lycopene desaturase (PDS) is an essential enzyme for the accumulation of lycopene and carotenoids (Bai *et al.*, 2016). The successful mutations PDS1 and PDS2 of banana cv. Rasthali are generated by the CRISPR/Cas9. Gene editing of the two genes resulted in the premature termination of PDS1 and PDS2 protein synthesis by inserting a termination codon into the gene sequences. The mutants exhibited decreased chlorophyll and total carotenoid contents (Kaur *et al.*, 2018).

GABA, as a neuro-suppressant, has the functions of anti-fatigue, sedation, and blood pressure regulation (Bachtiar *et al.*, 2015; Takayama and Ezura, 2015). Research has proved that glutamate decarboxylase (GAD) catalysed the decarboxylation of glutamate to produce GABA (Akihiro *et al.*, 2008). GAD has a C-terminal self-inhibiting region, and deletion of the domain promotes GAD activity (Takayama *et al.*, 2015). In order to increase the content of GABA, Nonaka *et al.* (2017) used the CRISPR/Cas9 method to delete the C-terminal self-inhibitory domains of *SlGAD2* and *SlGAD3*. The accumulation of GABA in mutant fruits increased by 7–15 times, which affects the fruit size and yield in tomato (Nonaka *et al.*, 2017).

Previous studies have demonstrated that GABA transaminase (GABA-TP1, TP2, TP3), succinate semialdehyde dehydrogenase (SSADH), and CAT9 are involved in GABA metabolism (Bao *et al.*, 2015; Snowden *et al.*, 2015). Li *et al.* (2018a) successfully edited the five genes (*GABA-TP1*, *GABA-TP2*, *GABA-TP3*, *SSADH*, and *CAT9*) in tomato genome by using pYLCRISPR/Cas9 vector, which is a multi-locus gene knockout CRISPR/Cas9 system (Ma *et al.*, 2015). The multisite gene mutagenesis resulted in manipulated GABA metabolic pathways and significantly enhanced the GABA content (Li *et al.*, 2018a).

These studies show that CRISPR/Cas9 gene editing can be used as an effective technique to modify bioactive compounds in fruits.

Application of Gene Editing in Fruit Texture

Fruit texture is an indispensable factor in the study of fruit quality and affects the commercial production of the fruit (Preeti *et al.*, 2010). The change of texture may lead to substantial decay of fruit in transportation and storage, which results in the development of typical diseases during post-harvest storage and shelf life (Vicente *et al.*, 2007). These texture changes are related to change the activity of many enzymes, which affect the structure of cell walls (Tucker *et al.*, 2017). Therefore, fruit texture is closely related to fruit quality and shelf life.

Pectate lyases (PL) is an important component of pectinase. It is a depolymerase that can degrade plant cell walls and lead to the softening and even death of plant tissues (Uluisik and Seymour, 2020). The mutation of tomato *PL* gene is induced by CRISPR/cas9, which increases the firmness of the fruit and prolongs the shelf life of the fruit, without negatively affecting other aspects of fruit ripening (Uluisik *et al.*, 2016).

Many ripening spontaneous mutants such as *rin*, *nor*, *crn*, and *alc* can prolong storage time. The *alc* mutant has one base pair mutation of *NOR* gene, resulting in nonsynonymous amino acid change (Yu *et al.*, 2017). Compared with the *rin* and *nor* mutants, the *alc* mutation not only prolongs shelf life, but also has better flavour and better disease resistance. Tomato *ALC* gene mutation is obtained by using the CRISPR/Cas9 method through HDR recombination pathway. The *alc* homozygous mutant without T-DNA insertion exhibits improved storage time and prolonged shelf life (Yu *et al.*, 2017).

Application of Gene Editing in Fruit Colouration

The difference in fruit colour is caused by the change of pigment. Genes affecting pigment synthesis can not only affect the bioactive compounds, but also affect the colour of the fruit. In fruit and vegetable crops, colour is affecting consumer choice. For example, consumers in Europe and the United States prefer red tomatoes, while Asians prefer pink tomatoes (Lin *et al.*, 2014). The study of *SlMYB12* has proven to affect the accumulation of flavonoids. The mutation of *SlMYB12* can produce pink tomato fruits (Ballester *et al.*, 2010). The *ant1* mutation is obtained by the CRISPR/cas9 system that can enhance the accumulation of anthocyanins and produce purple tomato fruits (Čermák *et al.*, 2015). PL, polygalacturonase 2a (PG2a), and β-galactanase (TBG4) are tomato pectin degrading enzymes that affect fruit ripening. Wang *et al.* (2019a) obtained silent mutants of *pl*, *pg2a*, and *tbg4*. Interestingly, *pg2a* and *tbg4* CRISPR strains did not soften but affected the colour of the fruits (Wang *et al.*, 2019a).

As a widely used gene-editing technology, CRISPR/cas9 has great potential in the research of fruit colouration. Delila (*Del*) encodes a transcription factor that has a basic helix-loop-helix (bHLH) domain and Rosea1 (*Ros1*) encodes an MYB-related transcription factor (Goodrich *et al.*, 1992; Schwinn *et al.*, 2006). Butelli *et al.* (2008) expressed the *Del* and *Ros1* genes from snapdragon in tomato, which the anthocyanin content of tomato fruit was significantly higher than the accumulation of tomato anthocyanin previously reported. Engineering *Del* and *Ros1* genes using CRISPR/Cas9 allows the creation of novel mutants and holds great potential for studying the effect of transcription factors on fruit colouration.

Summary

The technology of gene editing is a powerful tool for functional genomics in improving the quality and commercial value of fruits. The progresses on CRISPR/Cas9-mediated mutation involved in fruit ripening, fruit bioactive compounds, and fruit texture are summarized in Table 1. The emergence of CRISPR/Cas9 technology provides a new opportunity to accelerate plant molecular breeding. The CRISPR/Cas9 technology used on fruit crops not only provided a shortcut to obtain high yield and good quality of fruit food, but also laid a solid foundation for fruit functional genomics research. An unavoidable problem with all gene-editing tools is the off-target effect of non-specific site dissection of the genome. Off-target effects

Table 1. List of research on fruit improvement by using CRISPR/Cas gene-editing technology.

Species	Gene	Gene function or phenotype	Reference
Tomato	*ALMT9*	Decrease in malate content	Ye *et al.* (2017)
	MPK20	Decrease in sugar content	Chen *et al.* (2018a)
	ARF7	Parthenocarpic fruit	Hu *et al.* (2018)
	GGP1	Ascorbic acid	Li *et al.* (2018a)
	PG2a, *TBG4*	Fruit colour	Wang *et al.* (2019b)
	SlMYB12	Fruit colour	Ballester *et al.* (2010)
	ANT1	Fruit colour	Čermák *et al.* (2015)
	CYCB	Lycopene synthesis	Zsögön *et al.* (2018)
	TBG4	Fruit firmness	Wang *et al.* (2019b)
	CNR, *NOR*	Fruit ripening	Gao *et al.* (2019)
	RIN	Fruit ripening	Ito *et al.* (2017)
	SlEIN2, *SlERFE1*, *SlARF2B*, *SlACS4 SlGRAS8*, *SlACS2*	Fruit ripe and development	Hu *et al.* (2019)
	lncRNA1459	Fruit ripening, lycopene, carotenoid biosynthesis	Li *et al.* (2018b)
	SGR1, *Blc*, *LCY-E*, *LCY-B1*, *LCY-B2*	Increased lycopene content	Li *et al.* (2018c)
	SlORRM4	Fruit ripening	Yang *et al.* (2017)
	L1L4	Fruit metabolism	Gago *et al.* (2017)
	SlAGL6	Parthenocarpic fruit	Klap *et al.* (2017)
	RIN	Fruit ripening	Ito *et al.* (2015)
	AP2a, *FUL1*, *FUL2*, *NOR*	Fruit ripening	Wang *et al.* (2019b)
	SlDML2	Fruit ripening	Zhou *et al.* (2019)
	SGR1, *LCY-E*, *Blc*, *LCY-B1*, *LCY-B2*	Lycopene synthesis	Li *et al.* (2018c)
	PSY1	Lycopene synthesis	D'Ambrosio *et al.* (2018)
	SlGAD2, *SlGAD3*	GABA content	Nonaka *et al.* (2017)
	GABA-TP1, *GABA-TP2*, *GABA-TP3*, *SSADH*, *CAT9*	GABA content	Ma *et al.* (2015)
	PL	Fruit firmness	Uluisik *et al.* (2016)
	ALC	Long shelf life	Yu *et al.* (2017)
	CLV3	Fruit size	Zsögön *et al.* (2018)
	ENO	Fruit size	Yuste-Lisbona *et al.* (2020)
Watermelon	*PDS*	Carotenoid biosynthesis	Wang *et al.* (2019d)
Banana	*PDS1*, *PDS2*	Chlorophyll, Carotenoid	Kaur *et al.* (2018)
Apple	*IdnDH*	Biosynthesis of tartaric acid	Osakabe *et al.* (2018)
Grape	*VvPDS*	Albino phenotype	Nakajima *et al.* (2017)
Groundcherry	*ClV1*	Fruit size	Lemmon *et al.* (2018)
Kiwifruit	*CEN*	Fruit development	Varkonyi-Gasic *et al.* (2019)

RIN, RIPENING INHIBITOR gene; PDS, Lycopene desaturase; PG2a, polygalacturonase 2a; TBG4, β-galactanase; CNR, colourless non-ripening; AP2a, APETALA2a; NOR, NON-RIPENING; PSY1, phytoene synthase 1; PL, Pectate lyases; CLV3, CLAVATA3; PDS, Lycopene desaturase.

disrupt the expression of functional genes, affect gene functions, and eventually produce unpredictable adverse reactions (Guilinger *et al.*, 2014). Schaefer *et al.* (2017) published a peer-reviewed paper in which the authors reported that CRISPR-Cas9 caused unexpected off-target changes in mice (Schaefer *et al.*, 2017). Therefore, gene-editing technology still needs to be further optimized to avoid off-target and increase on-target editing efficiency. It is believed that gene-editing technology will be more widely used in the future and play an important role in fruit quality improvement.

Author Contributions

WD and BFH designed and organized the manuscript. XX and YJY collected and analysed the references. XX, YJY, BHF, and XX wrote the manuscript.

Funding

The research was supported by the National Natural Science Foundation of China (31960618) and the National Key Research and Development Program (2016YFD0400100), China.

Conflict of Interest

The authors declare no conflict of interest.

References

Akihiro, T., Koike, S., Tani, R., *et al.* (2008). Biochemical mechanism on GABA accumulation during fruit development in tomato. *Plant & Cell Physiology*, 49(9): 1378–1389.

Amish, P., Puja, K., Sapan, N. (2015). Color, size and shape feature extraction techniques for fruits: a technical review. *International Journal of Computer Vision*, 130(16): 0975–8887.

Bachtiar, V., Near, J., Johansen-Berg, H., *et al.* (2015). Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation. *Elife*, 4: e08789.

Bai, C., Capell, T., Berman, J., *et al.* (2016). Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor-product balance. *Plant Biotechnology Journal*, 14(1): 195–205.

Ballester, A. R., Molthoff, J., de Vos, R., *et al.* (2010). Biochemical and molecular analysis of pink tomatoes: deregulated expression of the gene encoding transcription factor SlMYB12 leads to pink tomato fruit color. *Plant Physiology*, 152(1): 71–84.

Bao H., Chen X. Y., Lv S. L., *et al*. (2015). Virus-induced gene silencing reveals control of reactive oxygen species accumulation and salt tolerance in tomato by γ-aminobutyric acid metabolic pathway. *Plant, Cell & Environment*, 38(3): 600–613.

Barrangou, R. (2013). CRISPR-Cas systems and RNA-guided interference. *Wiley Interdiscip Rev RNA*, 4(3): 267–278.

Barrangou, R., Fremaux, C., Deveau, H., *et al*. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science (New York, N.Y.)*, 315(5819): 1709–1712.

Bemer, M., Karlova, R., Ballester, A. R., *et al*. (2012). The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. *The Plant Cell*, 24(11): 4437–4451.

Budman, J., Chu, G. (2005). Processing of DNA for nonhomologous end-joining by cell-free extract. *The EMBO Journal*, 24(4): 849–860.

Butelli, E., Titta, L., Giorgio, M., *et al*. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature Biotechnology*, 26(11): 1301–1308.

Čermák, T., Baltes, N. J., Čegan, R., *et al*. (2015). High-frequency, precise modification of the tomato genome. *Genome Biology*, 16: 232.

Char, S. N., Neelakandan, A. K., Nahampun, H., *et al*. (2017). An *Agrobacterium*-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize. *Plant Biotechnology Journal*, 15(2): 257–268.

Chen, L. F., Yang, D. D., Zhang, Y. W., *et al*. (2018a). Evidence for a specific and critical role of mitogen-activated protein kinase 20 in uni-to-binucleate transition of microgametogenesis in tomato. *The New Phytologist*, 219(1): 176–194.

Chen, Y. R., Yu, S., Zhong, S. (2018b). Profiling DNA methylation using bisulfite sequencing (BS-Seq). *Methods in Molecular Biology (Clifton, N.J.)*, 1675: 31–43.

Chung, M. Y., Vrebalov, J., Alba, R., *et al*. (2010). A tomato (*Solanum lycopersicum*) APETALA2/ERF gene, SlAP2a, is a negative regulator of fruit ripening. *The Plant Journal: for Cell and Molecular Biology*, 64(6): 936–947.

D'Ambrosio, C., Stigliani, A. L., Giorio, G. (2018). CRISPR/Cas9 editing of carotenoid genes in tomato. *Transgenic Research*, 27(4): 367–378.

Ding, X. S., Mannas, S. W., Bishop, B. A., *et al*. (2018). An improved brome mosaic virus silencing vector: greater insert stability and more extensive VIGS. *Plant Physiology*, 176(1): 496–510.

Domonkos, I., Kis, M., Gombos, Z., *et al*. (2013). Carotenoids, versatile components of oxygenic photosynthesis. *Progress in Lipid Research*, 52(4): 539–561.

Fauser, F., Schiml, S., Puchta, H. (2014). Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*. *The Plant Journal: for Cell and Molecular Biology*, 79(2): 348–359.

Feng, C., Su, H. D., Bai, H., *et al*. (2018). High-efficiency genome editing using a dmc1 promoter-controlled CRISPR/Cas9 system in maize. *Plant Biotechnology Journal*, 16(11): 1848–1857.

Fray, R. G., Grierson, D. (1993). Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. *Plant Molecular Biology*, 22(4): 589–602.

Fujisawa, M., Nakano, T., Shima, Y., *et al*. (2013). A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. *The Plant Cell*, 25(2): 371–386.

Gago, C., Drosou, V., Paschalidis, K., *et al*. (2017). Targeted gene disruption coupled with metabolic screen approach to uncover the LEAFY COTYLEDON1-LIKE4 (L1L4) function in tomato fruit metabolism. *Plant Cell Reports*, 36(7): 1065–1082.

Gaj, T., Gersbach, C. A., Barbas, C. F. 3rd. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology*, 31(7): 397–405.

Gao, J. P., Wang, G. H., Ma, S. Y., *et al*. (2015). CRISPR/Cas9-mediated targeted mutagenesis in *Nicotiana tabacum*. *Plant Molecular Biology*, 87(1–2): 99–110.

Gao, Y., Wei, W., Zhao, X. D., *et al*. (2018). A NAC transcription factor, NOR-like1, is a new positive regulator of tomato fruit ripening. *Horticulture Research*, 5: 75.

Gao, Y., Zhu, N., Zhu, X. F., *et al*. (2019). Diversity and redundancy of the ripening regulatory networks revealed by the fruit ENCODE and the new CRISPR/Cas9 CNR and NOR mutants. *Horticulture Research*, 6: 39.

Gao, Y., Wei, W., Fan, Z. Q., *et al*. (2020). Re-evaluation of the nor mutation and the role of the NAC-NOR transcription factor in tomato fruit ripening. *Journal of Experimental Botany*, 71(12): 3560–3574.

Gapper, N. E., McQuinn, R. P., Giovannoni, J. J. (2013). Molecular and genetic regulation of fruit ripening. *Plant Molecular Biology*, 82(6): 575–591.

Giorio, G., Stigliani, A. L., D'Ambrosio, C. (2008). Phytoene synthase genes in tomato (*Solanum lycopersicum* L.)—new data on the structures, the deduced amino acid sequences and the expression patterns. *The FEBS Journal*, 275(3): 527–535.

Giovannoni, J. J. (2007). Fruit ripening mutants yield insights into ripening control. *Current Opinion in Plant Biology*, 10(3): 283–289.

Giovannoni, J., Nguyen, C., Ampofo, B., *et al*. (2017). The epigenome and transcriptional dynamics of fruit ripening. *Annual Review of Plant Biology*, 68: 61–84.

Goodrich, J., Carpenter, R., Coen, E. S. (1992). A common gene regulates pigmentation pattern in diverse plant species. *Cell*, 68(5): 955–964.

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., *et al*. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science (New York, N.Y.)*, 356(6336): 438–442.

Guilinger, J. P., Pattanayak, V., Reyon, D., *et al*. (2014). Broad specificity profiling of TALENs results in engineered nucleases with improved DNA-cleavage specificity. *Nature Methods*, 11(4): 429–435.

Haber, J. E., Ira, G., Malkova, A., *et al*. (2004). Repairing a double-strand chromosome break by homologous recombination: revisiting Robin Holliday's model. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 359(1441): 79–86.

Hickey, J. M., Chiurugwi, T., Mackay, I., *et al*.; (2017). Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. *Nature Genetics*, 49(9): 1297–1303.

Hu, J., Israeli, A., Ori, N., *et al*. (2018). The interaction between DELLA and ARF/IAA mediates crosstalk between gibberellin and auxin signaling to control fruit initiation in tomato. *The Plant Cell*, 30(8): 1710–1728.

Hu, N., Xian, Z. Q., Li, N., *et al*. (2019). Rapid and user-friendly open-source CRISPR/Cas9 system for single- or multi-site editing of tomato genome. *Horticulture Research*, 6: 7.

Hwang, W. Y., Fu, Y., Reyon, D., *et al*. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology*, 31(3): 227–229.

Ishino, Y., Shinagawa, H., Makino, K., *et al*. (1987). Nucleotide sequence of the *iap* gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *Journal of Bacteriology*, 169(12): 5429–5433.

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

Ito, Y., Nishizawa-Yokoi, A., Endo, M., *et al*. (2017). Re-evaluation of the *rin* mutation and the role of RIN in the induction of tomato ripening. *Nature Plants*, 3(11): 866–874.

Jinek, M., Chylinski, K., Fonfara, I., *et al*. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science (New York, N.Y.)*, 337(6096): 816–821.

Joung, J. K., Sander, J. D. (2013). TALENs: a widely applicable technology for targeted genome editing. *Nature Reviews. Molecular Cell Biology*, 14(1): 49–55.

Kaur, N., Alok, A., Shivani, *et al*. (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.

Kim, Y. G., Shi, Y., Berg, J. M., *et al*. (1997). Site-specific cleavage of DNA-RNA hybrids by zinc finger/FokI cleavage domain fusions. *Gene*, 203(1): 43–49.

Klap, C., Yeshayahou, E., Bolger, A. M., *et al*. (2017). Tomato facultative parthenocarpy results from SlAGAMOUS-LIKE 6 loss of function. *Plant Biotechnology Journal*, 15(5): 634–647.

Lemmon, Z. H., Reem, N. T., Dalrymple, J., *et al*. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. *Nature Plants*, 4(10): 766–770.

Li, T., Huang, S., Jiang, W. Z., *et al*. (2011). TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. *Nucleic Acids Research*, 39(1): 359–372.

Li, X., Xu, J. (2014). Meta-analysis of the association between dietary lycopene intake and ovarian cancer risk in postmenopausal women. *Scientific Reports*, 4: 4885.

Li, R., Li, R., Li, X. D., *et al*. (2018a). Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ-aminobutyric acid levels in *Solanum lycopersicum*. *Plant Biotechnology Journal*, 16(2): 415–427.

Li, R., Fu, D. Q., Zhu, B. Z., *et al*. (2018b). CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. *The Plant Journal: for Cell and Molecular Biology*, 94(3): 513–524.

Li X. D., Wang, Y. N., Chen, S., *et al*. (2018c). Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. *Frontiers in Plant Science*, 9: 559.

Li, S., Xu, H. J. L., Ju, Z., *et al*. (2018d). The RIN-MC fusion of MADS-Box transcription factors has transcriptional activity and modulates expression of many ripening genes. *Plant Physiology*, 176(1): 891–909.

Li, S., Zhu, B., Pirrello, J., *et al*. (2020). Roles of RIN and ethylene in tomato fruit ripening and ripening-associated traits. *The New Phytologist*, 226(2): 460–475.

Lin, T., Zhu, G., Zhang, J., *et al*. (2014). Genomic analyses provide insights into the history of tomato breeding. *Nature Genetics*, 46(11): 1220–1226.

Ma, X., Zhang, Q., Zhu, Q., *et al*. (2015). A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Molecular Plant*, 8(8): 1274–1284.

Mahfouz, M. M., Li, L., Shamimuzzaman, M., *et al*. (2011). De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks. *Proceedings of the National Academy of Sciences of the United States of America*, 108(6): 2623–2628.

Makarova, K. S., Wolf, Y. I., Alkhnbashi, O. S., *et al*. (2015). An updated evolutionary classification of CRISPR-Cas systems. *Nat Rev Microbiol*, 13(11): 722–736.

Mao, Y., Zhang, Z., Feng, Z., *et al*. (2016). Development of germ-line-specific CRISPR-Cas9 systems to improve the production of heritable gene modifications in *Arabidopsis*. *Plant Biotechnology Journal*, 14(2): 519–532.

Mao, Y., Botella, J. R., Liu, Y, *et al*. (2019). Gene editing in plants: progress and challenges. *National Science Review*, 6(3): 421–437.

Marian, B., Rumyana, K., Ana, R. B., *et al*. (2014). The tomato FRUITFULL homologs *TDR4/FUL1* and *MBP7/FUL2* regulate ethylene-independent aspects of fruit ripening. *The Plant Cell*, 24(11): 4437–4451.

Martin, S. E., Caplen, N. J. (2007). Applications of RNA interference in mammalian systems. *Annual Review of Genomics and Human Genetics*, 8: 81–108.

Martin, J., Krzysztof, C., Ines, F., *et al*. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096): 816–821.

Martín-Pizarro, C., Posé, D. (2018). Genome editing as a tool for fruit ripening manipulation. *Frontiers in Plant Science*, 9: 1415.

Moore, J. K., Haber, J. E. (1996). Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 16(5): 2164–2173.

Mordy, A. A., Mikal, E. S., Adel, S. E. (1998). Saline growing conditions induce ripening of the non-ripening mutants nor and rin tomato fruits but not of Nr fruit. *Postharvest Biology and Technology*, 13(3): 225–234.

Nabi, F., Arain, M. A., Rajput, N., *et al*. (2020). Health benefits of carotenoids and potential application in poultry industry: a review. *Journal of Animal Physiology and Animal Nutrition (Berl)*, 2020: 1439–0396.

Nakajima, I., Ban, Y., Azuma, A., *et al*. (2017). CRISPR/Cas9-mediated targeted mutagenesis in grape. *PLoS One*, 12(5): e0177966.

Nisar, N., Li, L., Lu, S., *et al*. (2015). Carotenoid metabolism in plants. *Molecular Plant*, 8(1): 68–82.

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

Osakabe, Y., Liang, Z., Ren, C., *et al*. (2018). CRISPR-Cas9-mediated genome editing in apple and grapevine. *Nature Protocols*, 13(12): 2844–2863.

Osorio, S., Carneiro, R. T., Lytovchenko, A., *et al*. (2020). Genetic and metabolic effects of ripening mutations and vine detachment on tomato fruit quality. *Plant Biotechnology Journal*, 18(1): 106–118.

Preeti, K. D., Samuel, V. K., Bansal, K. C. (2010). Fruit-specific over-expression of *LeEXP1* gene in tomato alters fruit texture. *Journal of Plant Biochemistry and Biotechnology*, 19(2): 177–183.

Puchta, H. (2017). Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. *Current Opinion in Plant Biology*, 36: 1–8.

Ran, F. A., Hsu, P. D., Wright, J., *et al*. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature Protocols*, 8(11): 2281–2308.

Sampson, T. R., Weiss, D. S. (2014). Exploiting CRISPR/Cas systems for biotechnology. *Bioessays: News and Reviews in Molecular, Cellular and Developmental Biology*, 36(1): 34–38.

Schaefer, K. A., Wu, W. H., Colgan, D. F., *et al*. (2017). Unexpected mutations after CRISPR-Cas9 editing in vivo. *Nature Methods*, 14(6): 547–548.

Schwinn, K., Venail, J., Shang, Y., *et al*. (2006). A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus *Antirrhinum*. *The Plant Cell*, 18(4): 831–851.

Singh, B., Singh, J. P., Kaur, A., *et al*. (2016). Bioactive compounds in banana and their associated health benefits—a review. *Food Chemistry*, 206: 1–11.

Snowden, C. J., Thomas, B., Baxter, C. J., *et al*. (2015). A tonoplast Glu/Asp/GABA exchanger that affects tomato fruit amino acid composition. *The Plant Journal: for Cell and Molecular Biology*, 81(5): 651–660.

Takatsuji, H. (1999). Zinc-finger proteins: the classical zinc finger emerges in contemporary plant science. *Plant Molecular Biology*, 39(6): 1073–1078.

Takayama, M., Ezura, H. (2015). How and why does tomato accumulate a large amount of GABA in the fruit? *Frontiers in Plant Science*, 6: 612.

Tang, L., Lee, A. H., Su, D., *et al*. (2014). Fruit and vegetable consumption associated with reduced risk of epithelial ovarian cancer in southern Chinese women. *Gynecologic Oncology*, 132(1): 241–247.

Tucker, G., Yin, X. R., Zhang, A. D., *et al*. (2017). Ethylene and fruit softening. *Food Quality and Safety*, 1(4): 253–267.

Uluisik, S., Chapman, N. H., Smith, R., *et al*. (2016). Genetic improvement of tomato by targeted control of fruit softening. *Nature Biotechnology*, 34(9): 23955–6900.

Uluisik, S., Seymour, G. B. (2020). Pectate lyases: their role in plants and importance in fruit ripening. *Food Chemistry*, 309: 125559.

Varkonyi-Gasic, E., Wang, T., Voogd, C., *et al*. (2019). Mutagenesis of kiwifruit CENTRORADIALIS-like genes transforms a climbing woody perennial with long juvenility and axillary flowering into a compact plant with rapid terminal flowering. *Plant Biotechnology Journal*, 17(5): 869–880.

Vicente, A. R., Saladié, M., Rose, J. KC, *et al*. (2007). The linkage between cell wall metabolism and fruit softening: looking to the future. *Journal of the Science of Food and Agriculture*, 87: 1435–1448.

Vrebalov, J., Ruezinsky, D., Padmanabhan, V., *et al*. (2002). A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. *Science (New York, N.Y.)*, 296(5566): 343–346.

Wang, D. D., Samsulrizal, N. H., Yan, C., *et al*. (2019a). Characterization of CRISPR mutants targeting genes modulating pectin degradation in ripening tomato. *Plant Physiology*, 179(2): 544–557.

Wang, R., Tavano, E. C. D. R., Lammers, M., *et al*. (2019b). Re-evaluation of transcription factor function in tomato fruit development and ripening with CRISPR/Cas9-mutagenesis. *Scientific Reports*, 9(1): 1696.

Wang, Y. C., Wang, D. S., Wang, X. J., *et al*. (2019c). Highly efficient genome engineering in *Bacillus anthracis* and *Bacillus cereus* using the CRISPR/Cas9 system. *Frontiers in Microbiology*, 10: 1932.

Wang, T., Zhou, H. Y., Zhu, H. L. (2019d). CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. *Horticulture Research*, 6: 77.

Wang, R., Angenent, G. C., Seymour, G., *et al*. (2020). Revisiting the role of master regulators in tomato ripening. *Trends in Plant Science*, 25(3): 291–301.

Xu, P., Su, H., Chen, W., *et al*. (2018). The application of a meiocyte-specific CRISPR/Cas9 (MSC) system and a suicide-MSC system in generating inheritable and stable mutations in *Arabidopsis*. *Frontiers in Plant Science*, 9: 1007.

Yang, Y. F., Zhu, G. N., Li, R., *et al*. (2017). The RNA editing factor SlORRM4 is required for normal fruit ripening in tomato. *Plant Physiology*, 175(4): 1690–1702.

Ye, J., Wang, X., Hu, T. X., *et al*. (2017). An InDel in the promoter of Al-activated malate transporteR9 selected during tomato domestication determines fruit malate contents and aluminum tolerance. *The Plant Cell*, 29(9): 2249–2268.

Yu, Q. H., Wang, B., Li, N., *et al*. (2017). CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. *Scientific Reports*, 7(1): 11874.

Yuste-Lisbona, F. J., Fernández-Lozano, A., Pineda, B., *et al*. (2020). ENO regulates tomato fruit size through the floral meristem development network. *Proceedings of the National Academy of Sciences of the United States of America*, 117(14): 8187–8195.

Yusuff, O., Mohd, Y. R., Norhani, A., *et al*. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment*, 30(1): 1314–3530.

Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., *et al*. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, 163(3): 759–771.

Zha, S., Boboila, C., Alt, F. W. (2009). Mre11: roles in DNA repair beyond homologous recombination. *Nature Structural & Molecular Biology*, 16(8): 798–800.

Zhang, F., LeBlanc, C., Irish, V. F., *et al*. (2017). Rapid and efficient CRISPR/Cas9 gene editing in Citrus using the YAO promoter. *Plant Cell Reports*, 36(12): 1883–1887.

Zhou, L. L., Tian, S. P., Qin, G. Z. (2019). RNA methylomes reveal the m6A-mediated regulation of DNA demethylase gene SlDML2 in tomato fruit ripening. *Genome Biology*, 20(1): 156.

Zhou, J. H., Li, D. D., Wang, G. M., *et al*. (2020). Application and future perspective of CRISPR/Cas9 genome editing in fruit crops. *Journal of Integrative Plant Biology*, 62(3): 269–286.

Zsögön, A., Čermák, T., Naves, E. R. (2018). De novo domestication of wild tomato using genome editing. *Nature Biotechnology*, 36: 1211–1216.