CRISPR/Cas9-mediated Gene Editing Technology in Fruit Quality Improvement

Authors

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Abstract

Fruit is an essential part of a healthy, balanced diet and it is particularly important for fiber, essential vitamins and trace element. 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 modification of target genes, which limits the progress of study. Recently, the 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 that 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), SlMYB12 and CLAVATA3 (CLV3) can affect fruit ripening, fruit bioactive compounds, fruit texture, fruit coloration and fruit size. CRISPR/Cas9 mediated mutagenesis has become an efficient method to modify target genes and improve fruit quality.

Keywords: 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 focusing on the study of gene function, genetic modification and genetic improvement for decades. Gene modification technology can carry out site-specific knock out, 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 (EEN) (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 modification of the target gene. Recently, gene editing technology, as an 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; Wang et al., 2017; Mao et al., 2019). These technologies usually involve the application of sequence-specific nucleases (SSNs) 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 et al., 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 rejoined 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 specific point mutation sequence by providing exogenous donor template. The repair processes of NHEJ and HDR are shown in Figure 1. ZFNs is 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 is a nucleic acid endonuclease 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 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 Ecoli (Ishino et al., 1987). In recent decades, CRISPR/Cas 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 has highly efficient in target shooting.
Therefore, many researchers choose CRISPR/Cas gene editing tool to conduct qualitative and locational analysis of one or more genes (Puchta, 2017).

Fruits contain fiber, vitamins, minerals and bioactive compounds, which are major nutrients for human health and preventing the occurrence of many diseases (Singh et al., 2016; Wang et al., 2019; Giovannoni, 2007; Klee and Giovannoni, 2011). 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 external environment and other factors, such as drought, cold and disease. Some fruit crops have 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 sexual hybridization of suitable parents (Hickey et al., 2017). However, this screening method has a long cycle, large randomness and 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 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 study of gene functions and genetic improvement of fruit crop.

![Gene modification in biology](https://academic.oup.com/fqs/advance-article/doi/10.1093/fqsafe/fyaa028/5940658)

**Figure 1. The repair principle of NHEJ and HDR**

**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 DNA sequence, leading to DNA double-strand break (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 signaling 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 work 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).

![CRISPR/Cas system](image)

Figure 2. The mechanism of CRISPR/Cas system

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 mouse, zebrafish and other species. At the same time, the CRISPR/Cas9 system was optimized, including 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 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; Xu et al., 2018; Feng 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, which makes 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, regulation of fruit ripening has become the focus of many scientists (Martin-Pizarro and Pose, 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 mature of tomato fruit, and activates and promotes all physiological processes associated with ripening, including color, hardness and flavor etc. (Fujisawa et al., 2013). The *RIN* gene is knocked out in tomato by CRISPR/Cas9 system and the *rin* mutant is analyzed (Ito et al., 2015). The *rin* knockout mutation does not affect the initiation of ripening and exhibits moderate red coloring, which demonstrates that *RIN* gene is not required for the ripening initiation in fruit (Ito et al., 2017). The *rin* mutant can lead to ripen 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 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., 2018). Osorio et al. (2019) reported that tomato breeders are use of *RIN* mutation to acquire improved hybrids exerts a negative impact on tomato flavour (Osorio et al., 2019). Li et al. (2020) pointed that RIN-deficient 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 maturation of mature 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 RNA interference (RNAi) silencing lines and spontaneous mutants. Wang et al. (2019) 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 was 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 severe blocked ripening phenotype (Marian et al., 2014; Wang et al., 2019). 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 non-ripening (NOR) and colorless 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 a mainly method to re-evaluate the important gene in fruit ripening through generation of null mutant. The tomato nor mutant produces a new protein, 186-amino-acid protein (NOR186), which has prohibitive function that affects ripening. The tomato nor mutant previously been proven to be a gain-of-function mutant, but the specific mechanism of action is still unclear (Wang et al., 2019). 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 virus-induced gene silencing (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 found that the early epigenetic mark is DNA cytosine methylation in the plant genome, which can response to external environmental stress, regulate gene expression and stabilize the genome (Chen et al., 2018). 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 knock down technology, mutants obtained by CRISPR/Cas technology can lead to unexpected weak phenotypes, which indicates 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 biological 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 the accumulation of bioactive substances has been focused 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 can affect in 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 tumors, including prostate cancer, and cardiovascular disease (Tang et al., 2014; Li and Xu, 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. (2018) knocked out SGR1, LCY-E, BLC, LCY-B1 and LCY-B2 by CRISPR/Cas9 method, which inhibited the conversion of lycopene and increased the lycopene content in the fruit about 5.1 times (Li et al., 2018). 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. Rashali 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 the 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 (Takayama and Ezura, 2015; Bachtar et al., 2015). Research has proved that glutamate decarboxylase (GAD) catalyzed 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 SIGAD2 and SIGAD3. The accumulation of GABA in mutant fruits increased by 7 to 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 (Snowden et al., 2015; Bao 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 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 fruit.

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 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 flavor and better disease resistance. Tomato ALC gene mutation is obtained by using 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 Coloration

The difference in fruit color is caused by the change of pigment. Genes affecting pigment synthesis can not only affect the bioactive compounds, but also affect the color of fruit. In fruit and vegetable crops, color 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 obtained by the CRISPR/cas9 system that can enhance the accumulation of anthocyanins and produce purple tomato fruits (Cermak et al., 2015). PL, Polygalacturonase 2a (PG2a) and β-galactanase (TBG4) are tomato pectin degrading enzyme that affect fruit ripening. Wang et al. (2019) obtained silent mutants of pl, pg2a and tbg4. Interestingly, the fruits of the pg2a and tbg4 CRISPR strains did not soften but affected the color of the fruits (Wang et al., 2019).

As a widely used gene editing technology, CRISPR/cas9 has great potential in the research of fruit coloration. Delila (Del) encodes a transcription factor that has a basic-helix-loop-helix (bHLH) domain and Rosea1 (Ros1) encodes a 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 (Butelli et al., 2008). 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 Coloration.

Summary

The technology of gene editing is a powerful tool for functional genomics in improving the quality and commercial value of fruit. 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 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, the 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.

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. (2018) |
|         | ARF7 | Parthenocarpic fruit      | Hu et al. (2018) |
|         | GGP1 | Ascorbic acid             | Li et al. (2018) |
|         | PG2a, TBG4 | Fruit color | Wang et al. (2019) |
|         | SIMYB12 | Fruit color            | Ballester et al. (2010) |
|         | ANT1 | Fruit color              | Cermak et al. (2015) |
|         | CYCB | Lycopene synthesis       | Zsögön et al. (2018) |
|         | TBG4 | Fruit firmness           | Wang et al. (2019) |
|         | CNR, NOR | Fruit ripening        | Gao et al. (2019) |
|         | RIN  | Fruit ripening           | Ito et al. (2017) |
|         | SIEIN2, SIERFE1, SIARF2B, SIACS4 SIGRAS8, SIACS2 | Fruit ripe and development | Hu et al. (2019) |
|         | IncRNA1459 | Fruit ripening, lycopene, Carotenoid biosynthesis | Li et al. (2018b) |
|         | SGR1, Bic, LCY-E, LCY-B1, LCY-B2 | Increased lycopene content | Li et al. (2018) |
|         | SIORRM4 | Fruit ripening         | Yang et al. (2017) |
|         | L1L4 | Fruit metabolism         | Gago et al. (2017) |
|         | SIAGL6 | Parthenocarpic fruit    | Klap et al. (2017) |
|         | RIN  | Fruit ripening           | Ito et al. (2015) |
| Gene       | Function                        | Reference               |
|------------|---------------------------------|-------------------------|
| AP2a, FUL1, FUL2, NOR | Fruit ripening                   | Wang et al. (2019)      |
| SIDM1L2    |                                 |                         |
| SGR1, LCYE, Blc, LCY-B1, LCY-B2 | Fruit ripening                | Zhou et al. (2019)      |
| PSY1       | Lycopene synthesis               | Li et al. (2018)        |
|            |                                 | D’Ambrosio et al. (2018)|
| SIGAD2, SIGAD3 | GABA content                     | Nonaka et al. (2017)    |
| GABA-TP1, GABA-TP2, GABA-TP3, SSADH, CAT9 | GABA content | Ma et al. (2015)        |
| PL         |                                 |                         |
| ALC        |                                 |                         |
| CLV3       | Fruit size                       | Zsogon et al. (2018)    |
| ENO        | Fruit size                       | Yuste et al. (2020)     |
| Watermelon PDS | Carotenoid biosynthesis         | Wang et al. (2019)      |
|            | Chlorophyll, Carotenoid          | Kaur et al. (2018)      |
| Banana     | iDnDH                           | Biosynthesis of tartaric acid | Osakabe et al. (2018) |
| Apple      | VvPDS                           | Albino phenotype        | Nakajima et al. (2017)|
| Grape      | CLV1                            | Fruit size              | Lemmon et al. (2018)  |
| Groundcherry | CEN                             | Fruit development       | Varkonyi et al. (2019)|

**Author Contributions**

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

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**Conflicts of Interest**

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.
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