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REVIEW

Advances in application of genome editing in tomato and recent development of genome editing technology

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Abstract

Genome editing, a revolutionary technology in molecular biology and represented by the CRISPR/Cas9 system, has become widely used in plants for characterizing gene function and crop improvement. Tomato, serving as an excellent model plant for fruit biology research and making a substantial nutritional contribution to the human diet, is one of the most important applied plants for genome editing. Using CRISPR/Cas9-mediated targeted mutagenesis, the re-evaluation of tomato genes essential for fruit ripening highlights that several aspects of fruit ripening should be reconsidered. Genome editing has also been applied in tomato breeding for improving fruit yield and quality, increasing stress resistance, accelerating the domestication of wild tomato, and recently customizing tomato cultivars for urban agriculture. In addition, genome editing is continuously innovating, and several new genome editing systems such as the recent prime editing, a breakthrough in precise genome editing, have recently been applied in plants. In this review, these advances in application of genome editing in tomato and recent development of genome editing technology are summarized, and their leaving important enlightenment to plant research and precision plant breeding is also discussed.

Keywords CRISPR · Cas9 · *Solanum lycopersicum* · Gene function · Precision plant breeding

Introduction

Food security is a continuous and important global issue, and increasing levels of food production are necessary due to population growth (Pradhan et al. 2015), climate change (Yu and Li 2021), and emergent global events, such as the COVID-19 pandemic (Devereux et al. 2020). With the improvement of human living standards, the increasing demand of high-quality foods with good appearance and abundant nutritional value is also put forward. Thus, technological innovation is required to meet these increasing demands of crop improvement, because conventional breeding is a rather time-consuming practice and is usually accompanied by loss of fitness and genetic diversity (Wang et al. 2019c).

Genome editing is a revolutionary technology in molecular biology and facilitates efficient, precise, and targeted modifications at genomic loci (Zhang et al. 2018). Genome editing may be the greatest innovation in plant breeding, because it allows precision plant breeding and can rapidly produce novel and transgene-free plants that are similar or identical to plants generated by conventional breeding techniques (Araki and Ishii 2015; Zhang et al. 2018). There are three major types of genome editing systems, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) systems (Zhang et al. 2018). Genome editing has been applied in different plants and is revolutionizing basic research and precision breeding in plants (Tiwari et al. 2020; Zhang et al. 2018).

As one of the most cultivated and consumed vegetable crops worldwide, tomato (*Solanum lycopersicum*) is rich in lycopene, vitamins, and minerals, making a substantial nutritional contribution to the human diet (Zhu et al.

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2018). Tomato is also an excellent model plant for fruit biology research, and most of the knowledge about fruit development and ripening on fleshy fruit is from tomato (Pesaresi et al. 2014; Seymour et al. 2013). Genome editing has been applied in tomato since 2014 (Brooks et al. 2014; Lor et al. 2014) and has greatly facilitated the characterization of gene function and precision breeding in tomato. Noticeably, the re-evaluation of tomato genes essential for fruit ripening by using genome editing-mediated targeted mutagenesis (Gao et al. 2020; Ito et al. 2017; Wang et al. 2019b) highlights that several aspects of tomato fruit ripening should be reconsidered. Knockout mutations in several master regulators in tomato ripening generated by CRISPR/Cas9 showed weaker ripening inhibition phenotypes compared to their spontaneous mutants or RNAi plants, indicating that the regulatory network involved in fruit ripening is more complex than previously thought (Wang et al. 2020a). In addition, genome editing has been applied in tomato improvement, mainly including the improvement of fruit yield and quality, the increase of stress resistance, the domestication of wild tomato, and the customization of tomato cultivars for urban agriculture (Kwon et al. 2020; Wang et al. 2019c; Xu et al. 2019).

As a rapidly developing technology applied in basic research, plant and animal breeding, and even clinical research, genome editing is also continuously undergoing innovation (Anzalone et al. 2020), and some new genome editing systems have recently been applied in plants. In this review, these advances in application of genome editing in tomato and recent development of genome editing technology are summarized, and their leaving important enlightenment to plant research and precision plant breeding is also discussed.

Genome editing systems applied in tomato

Most genome editing systems, mainly including the three major types of genome editing systems (ZFN, TALEN, and CRISPR/Cas systems) and cytidine base editor (CBE), which belongs to be one type of DNA base editors, have been applied in tomato for basic research or precision breeding (Brooks et al. 2014; Hilioti et al. 2016; Lor et al. 2014; Shimatani et al. 2017). However, both ZFN and TALEN, which rely on protein–DNA binding to achieve sequence specificity and are generated by fusing the DNA cleavage domain of the endonuclease FokI with zinc fingers or transcriptional activator-like effectors to achieve site-specific cleavage (Xu et al. 2019), are rarely used in tomato compared to the widely used CRISPR/Cas systems.

Widely used and continuously optimized CRISPR/Cas systems

CRISPR/Cas systems are the third-generation genome editing systems, which appeared in 2012 and quickly became a superstar in genome editing tools because of their great simplicity and usability compared to with ZFN and TALEN (Zhang et al. 2018). CRISPR/Cas was originally identified as an effective acquired immune system in bacteria against virus infection and relies on RNA–DNA binding to achieve sequence specificity in genome editing (Zhang et al. 2018). CRISPR/Cas systems can be divided into five major types based on the different combinations of RNA and Cas protein, and the most studied CRISPR/Cas9 belongs to type II (Makarova et al. 2015). The CRISPR/Cas9 system is mainly composed of Cas9 protein and synthetic single-guide RNA (sgRNA) formed by the fusion of CRISPR-RNA (crRNA) and transactivation RNA (tracrRNA), and the Cas9 protein guided by sgRNA can introduce site-specific DNA double-strand breaks (DSBs), thus triggering DNA repair mechanisms (Wang et al. 2019c; Xu et al. 2019) (Fig. 1a). CRISPR/Cas9 has been applied in tomato since 2014 (Brooks et al. 2014) and has become the main genome editing tool used in tomato, from characterizing gene function to precision plant breeding (Wang et al. 2019c; Xu et al. 2019). In addition to CRISPR/Cas9, CRISPR/Cpf1(Cas12a), which belongs to type V, is a novel member of CRISPR/Cas genome editing systems (Makarova et al. 2015). The CRISPR/Cpf1 system has been gradually applied in several plant species, including rice, soybean, tobacco, maize, cotton, and, recently, tomato (Lee et al. 2019; Li et al. 2019; Tang et al. 2017, 2019; Vu et al. 2020; Wang et al. 2018) . Noticeably, the combination of CRISPR/Cpf1 and geminiviral multi-replicons significantly increased (approximately threefold) the homology-directed repair (HDR)-based genome editing efficiency in tomato compared to a Cas9-based single-replicon system (Vu et al. 2020), which demonstrates good prospects of CRISPR/Cpf1 in tomato genome editing.

Although CRISPR/Cas9 has been widely used in various organisms, numerous technological improvements (Table 1), which can improve the editing efficiency, reduce potential off-target effects, accelerate the generation of genome-edited and transgene-free plants, and confer mutations in specific cell types, tissues, or organs, have been realized to optimize the CRISPR/Cas9-mediated genome editing in plants, including tomato. Using multiplex sgRNAs instead of single sgRNA can increase the chance of obtaining large deletions in tomato mutants generated by CRISPR/Cas9 (Santillan Martinez et al. 2020). Adding an expression cassette for overexpressing an anthocyanin intensifying gene *PAP1/MYB75* into the CRISPR/

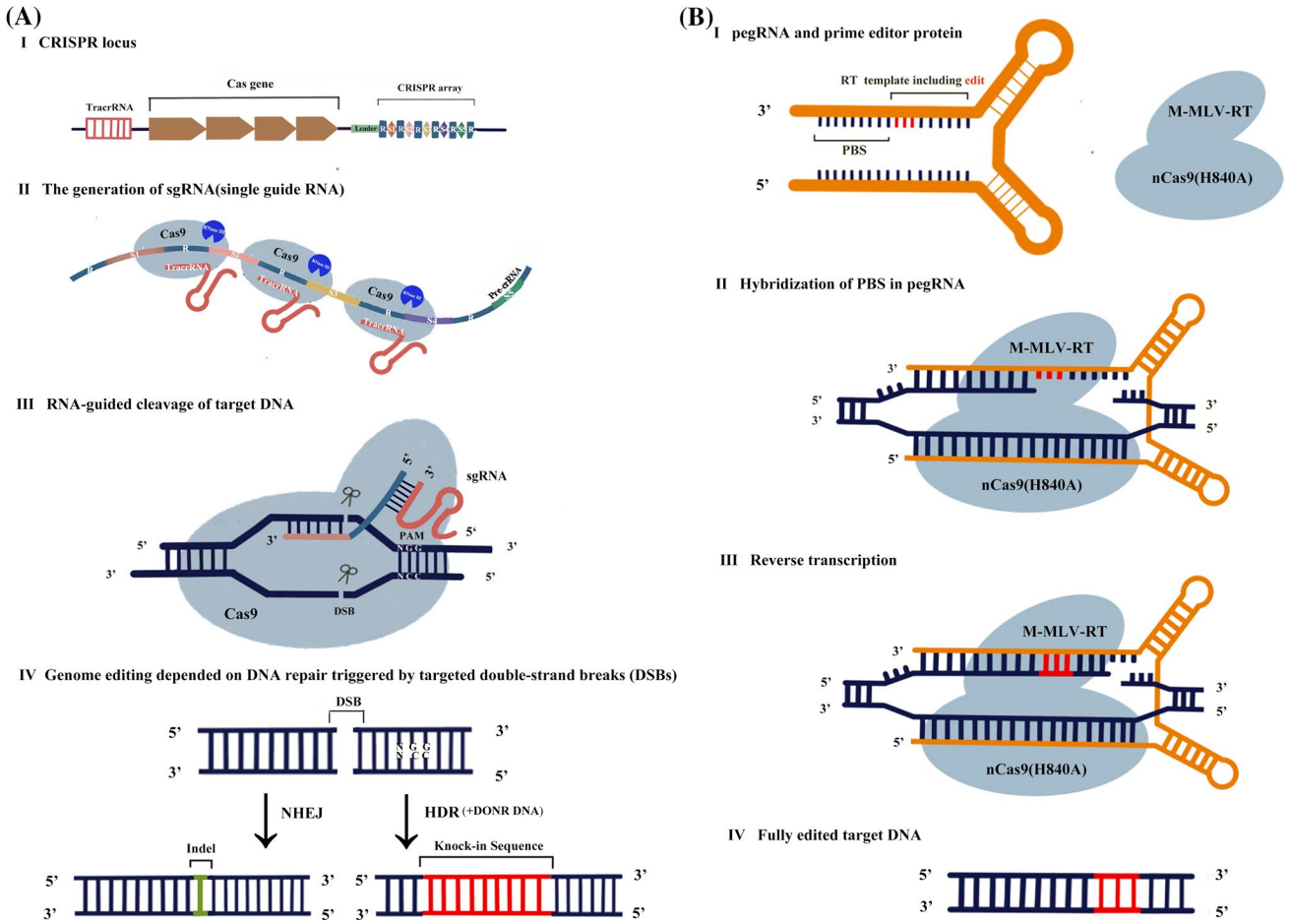

Fig. 1 Working models of conventional CRISPR/Cas9 (**a**) and recent prime editing (**b**). TracrRNA, transactivation RNA; crRNA, CRISPR-RNA; sgRNA, small-guide RNA; PAM, protospacer adjacent motif; NHEJ, non-homologous end joining; HDR, homology-directed repair; pegRNA, prime editing guide RNA; RT, reverse transcription; PBS, primer-binding site; nCas9 (H840A), catalytically impaired Cas9 (H840A) nickase (nCas9); M-MLV-RT, Moloney murine leukemia virus reverse transcriptase

Cas9 construct accelerates the isolation of transgene-free tomato plants, which can be easily screened by the plant color with naked eyes (Hu et al. 2019). A spatiotemporally regulated CRISPR/Cas9 toolkit in which Cas9 expression is driven by a fruit-specific promoter (*phosphoenolpyruvate carboxylase 2* gene promoter) was recently reported to confer fruit-specific gene editing in tomato (Feder et al. 2020), which will significantly accelerate fruit biology research. The potential application of important technological improvements reported in other plants (Table 1), such as the recent establishment of nanoparticle-mediated plant genome editing via a simple foliar spray of nanoparticles coated with CRISPR/Cas9 constructs (Doyle et al. 2019) and the de novo induction of gene-edited meristems for avoiding time-consuming tissue culture (Maher et al. 2020), for characterizing gene function and crop improvement remains to be investigated in tomato.

Increasingly used cytidine base editor

DNA base editors, including cytidine base editors (CBEs) and adenine base editors (ABEs), are CRISPR/Cas9-derived tools and can produce precise single-base substitutions (C-to-T or A-to-G) in genomic DNA without the introduction of DSBs (Komor et al. 2016; Nishida et al. 2016). The CBE genome editing system, which specially confers a C–to–T base conversion in genomic DNA, consists of a cytidine deaminase fused with a Cas9 nickase (nCas9) and a uracil glycosylase inhibitor (Eid et al. 2018). CBE was firstly applied in tomato in 2017 and efficiently edited the two tomato hormone signaling genes *DELLA* and *ETR1* with a base edition efficiency from 26.2% to 53.8% (Shimatani et al. 2017). The *acetolactate synthase* (*ALS*) gene is involved in the biosynthetic pathway for branched-chain amino acid synthesis (McCourt and Duggleby 2006), and

Table 1 Summary of the important technological improvements to optimize CRISPR/Cas9-mediated genome editing in plants, including tomato

Technological improvement	Applied in plants	References
Improving editing efficiency		
Codon optimization of Cas9 protein by utilizing a plant codon-optimized Cas9 gene	*Arabidopsis*, rice and maize	Castel et al. (2019), Ma et al. (2015) and Xing et al. (2014)
Selection of different promoters to drive Cas9 protein	*Arabidopsis* and maize	Castel et al. (2019), Feng et al. (2018), Svitashev et al. (2015) and Xing et al. (2014)
Selection of highly efficient sgRNA	*Arabidopsis* and rice	Castel et al. (2019) and Liang et al. (2016)
Using multiplex sgRNAs instead of single sgRNA	Tomato, rice, and alfalfa	Santillan Martinez et al. (2020), Wang et al. (2016) and Wolabu et al. (2020)
Selection of strong terminator after Cas9	*Arabidopsis*	Castel et al. (2019)
Optimization of T-DNA architecture	*Arabidopsis*	Castel et al. (2019)
Heat treatment	*Arabidopsis*	LeBlanc et al. (2018)
Suppressing RNA silencing	*Arabidopsis*	Mao et al. (2018) and Wang et al. (2019d)
Using chemically modified DNA as donor	Rice	Lu et al. (2020a, b)
Reducing potential off-target effects		
Particle bombardment-mediated transient expression of CRISPR/Cas9 DNA or RNA	Wheat	Zhang et al. (2016)
Biolistic delivery of CRISPR/Cas9 RNPs into plant cells	*Arabidopsis*, tobacco, lettuce, rice, grapevine, apple, maize, wheat, petunia, *Brassica oleracea* and *B. rapa*	Liang et al. (2017), Liang et al. (2018), Malnoy et al. (2016), Murovec et al. (2018), Park and Choe (2019), Subburaj et al. (2016), Svitashev et al. (2016) and Woo et al. (2015)
Nanoparticle-mediated plant genome editing via a simple foliar spray of nanoparticles coating with CRISPR/Cas9 constructs	Wheat	Doyle et al. (2019)
Using Cas9 variants, including xCas9 and Cas9-NG	Rice	Zhong et al. (2019)
Using truncated sgRNA	*Arabidopsis*	Osakabe et al. (2016)
Accelerating the generation of genome-edited plants		
Integration of a cassette containing the GRF4–GIF1 chimera into the CRISPR/Cas9 construct	Wheat and citrus	Debernardi et al. (2020)
De novo induction of gene-edited meristems for avoiding time-consuming tissue culture	*Nicotiana benthamiana*	Maher et al. (2020)
Nanoparticle-mediated plant genome editing via a simple foliar spray of nanoparticles coating with CRISPR/Cas9 constructs	Wheat	Doyle et al. (2019)
Adding an FT expression element into the CRISPR/Cas9 construct	Tobacco	Liu et al. (2019)
Accelerating the generation of transgene-free plants from edited plants		
Agrobacterium-mediated transient expression of CRISPR/Cas9 in plants	Tobacco	Chen et al. (2018)
Biolistic delivery of CRISPR/Cas9 RNPs into plant cells	*Arabidopsis*, tobacco, lettuce, and rice	Luo et al. (2015) and Woo et al. (2015)
Drug-induced elimination of transgenes	Rice	Lu et al. (2017)
Programmed suicide gene-mediated self-elimination of transgenes	Rice	He et al. (2018, 2019)
Visible marker-assisted transgene elimination	*Arabidopsis*, rice, tomato, and tobacco	Chang et al. (2016), Gao et al. (2016), He et al. (2017, 2019, 2020), Liu et al. (2019) and Yu and Zhao (2019)
Conferring mutations in specific cell types, tissues, or organs		
The CRISPR-based tissue-specific knockout system (CRISPR-TSKO)	*Arabidopsis*	Decaestecker et al. (2019)
A fiber-specific or fruit-specific promoter to drive Cas9 expression	Tomato and *Arabidopsis*	Feder et al. (2020) and Liang et al. (2019)

the mutation of the Proline-186 residue in tomato *ALS1* can confer chlorsulfuron resistance (Yu et al. 2010). The CBE-mediated mutation of the tomato *ALS1* gene successfully created chlorsulfuron-resistant tomato plants (Veillet et al. 2019). Noticeably, the precise base edition efficiency at tomato *ALS1* Pro186 codon was up to 71.4%, and 12.9% of these chlorsulfuron-resistant tomato plants were transgene-free (Veillet et al. 2019). These results highlight the feasibility of CBE for tomato genetic improvement.

Genome editing-mediated re-evaluation of tomato genes essential for fruit ripening

RNAi-mediated gene silencing or identification of the genes underlying spontaneous mutants was widely used for evaluating gene function in tomato before the emergence of genome editing systems (Wang et al. 2019b). With the establishment of different genome editing systems, especially CRISPR/Cas9, in tomato, tomato mutations can be generated rapidly, and numerous tomato genes involved in development and ripening, fruit yield and quality, and stress responses have been functionally validated using genome editing-mediated targeted mutagenesis (Xu et al. 2019). Noticeably, CRISPR/Cas9-mediated re-evaluation of tomato transcription factors and cell wall modifying enzymes in fruit ripening (Table 2) highlights that several aspects of tomato fruit ripening should be reconsidered.

Revisiting the function of transcription factors in fruit ripening

Tomato is a typical climacteric fruit, and the phytohormone ethylene is critical for tomato fruit ripening (Liu et al. 2020b). There are abundant spontaneous mutants in tomato, and some of these mutants show significant ripening-deficient phenotypes (Giovannoni 2007). The identification of the genes underlying the three important ripening-deficient mutants, including ripening inhibitor (rin), nonripening (nor), and colorless nonripening (Cnr), showed that all *RIN*, *NOR*, and *CNR* genes are transcription factor-encoding genes (Manning et al. 2006; Tigchelaar 1973; Vrebalov et al. 2002). RIN, NOR, and CNR have long been regarded as master regulators in tomato fruit ripening via transcriptional regulation of downstream fruit ripening-related genes, such as other transcription factor-encoding genes, ethylene biosynthesis genes, and cell wall modifying enzyme-encoding genes (Seymour et al. 2013). By mainly using RNAi, a few other tomato transcription factors from different families, such as AP2a, FUL1, FUL2, TAG1, HB1, and MADS1, have been proven to also be important regulators in tomato fruit ripening (Bemer et al. 2012; Cao et al. 2020; Chen et al. 2020a, b; Chung et al. 2010; Dong et al. 2013; Itkin et al. 2009; Karlova et al. 2011; Lin et al. 2008). Thus far, the function of six transcriptional regulators, including RIN, NOR, CNR, AP2a, FUL1, and FUL2, in tomato fruit ripening has been re-evaluated using CRISPR/Cas9-mediated targeted mutagenesis (Table 2). Noticeably, CRISPR/Cas9-induced mutations in the three master regulators (*RIN*, *NOR*, and *CNR*) showed inconsistent ripening phenotypes as spontaneous mutants or RNAi plants (Gao et al. 2020; Ito et al. 2017; Wang et al. 2019b), which indicates that the transcriptional regulatory network involved in tomato fruit ripening should be reconsidered.

RIN has long been believed to be essential for the induction of tomato fruit ripening, because the *rin* spontaneous mutant showed a near-complete inhibition of ripening phenotype and did not produce red pigmentation, soften or induce an ethylene burst (Vrebalov et al. 2002). However, the CRISPR/Cas9-mediated *RIN*-knockout mutation still initiated partial ripening and showed moderate red pigmentation (Ito et al. 2017; Li et al. 2018b), indicating that RIN is not required for tomato ripening initiation. The specific role of RIN in tomato fruit ripening was recently investigated using the *RIN*-knockout mutation generated by CRISPR/Cas9, and RIN was proven to still be essential for the progression of tomato fruit ripening by inducing autocatalytic system-2 of ethylene production and subsequent full ripening (Li et al. 2020d). In addition, the *NOR* mutant in tomato generated by CRISPR/Cas9 also only displayed partial non-ripening phenotype (Gao et al. 2019b; Wang et al. 2019b), which is distinct from the *nor* spontaneous mutant (Kumar et al. 2018; White 2002). The specific role of NOR in fruit ripening was also recently investigated, mainly using the *NOR*-knockout mutation generated by CRISPR/Cas9, indicating that NOR still plays an important role in tomato fruit ripening by interacting with other ripening-related transcription factors and transcriptionally activating ripening-related target genes (*SlACS2*, *SlGgpps2*, and *SlPL*) (Gao et al. 2020). The *nor* spontaneous mutant is a gain-of-function mutation or a dominant-negative mutation by producing a truncated 186-amino acid protein (NOR186), which disrupts the transcriptional activation region and is unable to transcriptionally activate ripening-related target genes (Gao et al. 2020). Noticeably, the overexpression of *NOR* cannot completely restore the ripening phenotype of the *nor* spontaneous mutant (Gao et al. 2020), indicating that there are other unknown factors, resulting in the non-ripening phenotype of the *nor* mutant. For the *CNR* mutant generated by CRISPR/Cas9, it only showed two to three days of delayed ripening, and its fruit obtained the full color finally (Gao et al. 2019b), which is significantly different from the *Cnr* spontaneous mutant and *CNR* silencing plants (Manning et al. 2006). Thus, CNR is not the master regulator of fruit ripening, and its role in tomato fruit ripening remains to be investigated in the future.

Table 2 A list of publications on the genome editing-mediated re-evaluation of tomato genes essential for fruit ripening

Gene	Function shown by genome editing			Function shown by other tools			Con
	Phenotype	Tool	References	Phenotype	Tool	References	
Transcription factors							
RIN	Initiated partial ripening and showed moderate red pigmentation	CRISPR/Cas9	Ito et al. (2017), Ito et al. (2015), Ito et al. (2020) and Li et al. (2018b, 2020d)	A near-complete inhibition of ripening phenotype	Spontaneous mutant	Vrebalov et al. (2002)	No
NOR	Progress of ripening was only partially affected	CRISPR/Cas9	Gao et al. (2019b) and Wang et al. (2019b)	Progress of ripening was totally blocked	Spontaneous mutant	Kumar et al. (2018), Tigchelaar (1973) and White (2002)	No
CNR	Only showed 2–3 days of delayed ripening and its fruit obtained the full color finally	CRISPR/Cas9	Gao et al. (2019b)	A colorless pericarp with strongly reduced ethylene production	Spontaneous mutant and VIGS	Manning et al. (2006)	No
AP2a	Initiated fruit ripening earlier, but did not fully ripen	CRISPR/Cas9	Wang et al. (2019b)	Initiated fruit ripening earlier, but did not fully ripen	RNAi	Chung et al. (2010) and Karlova et al. (2011)	Yes
FUL1/FUL2	Double mutants did not reach a red ripe color	CRISPR/Cas9	Wang et al. (2019b)	Silencing of both genes inhibited fruit ripening	RNAi	Bemer et al. (2012)	Yes
Cell wall modifying enzymes							
TBG4	No effects on fruit firmness	CRISPR/Cas9	Wang et al. (2019a)	Modest effects on fruit firmness	Antisense	Smith et al. (1990, 2002)	No
PL	Significantly enhanced fruit firmness	CRISPR/Cas9	Wang et al. (2019a)	Significantly enhanced fruit firmness	RNAi	Uluisik et al. (2016) and Yang et al. (2017)	Yes
PG2a	No effects on fruit firmness	CRISPR/Cas9	Wang et al. (2019a)	No effects on fruit firmness	Antisense	Smith et al., (1990, 2002)	Yes

Con. consistency between function shown by genome editing and function shown by other tools, *Ref.* references

Different from RIN, NOR, and CNR, three other transcription factors including AP2a, FUL1, and FUL2 have been proven to show consistent ripening phenotypes between CRISPR/Cas9-mediated mutants and RNAi plants (Wang et al. 2019b). Compared to wild-type tomato plants, both tomato *AP2a* mutants and *AP2a*-RNAi plants initiated fruit ripening earlier, but did not fully ripen (Karlova et al. 2011; Wang et al. 2019b), which confirms that AP2a is a negative regulator of tomato fruit ripening initiation. By using CRISPR/Cas9 mutagenesis, FUL1 and FUL2 were proven to show overlapping functions during fruit ripening, because the *FUL1* mutant and *FUL2* mutants showed no apparent differences in final overall fruit color compared with wild-type plants, but the *FUL1*/ *FUL2* double mutants did not reach a red ripe color (Wang et al. 2019b). A similar ripening phenotype was also observed in *FUL1*/*FUL2*-RNAi plants (Bemer et al. 2012). In addition, the *FUL2* mutant generated by CRISPR/Cas9 also showed a fruit development phenotype that had not been described before in *FUL2*-RNAi plants (Wang et al. 2019b), indicating an additional role of FUL2 in fruit development.

Overall, these evidences indicate that the three tomato spontaneous mutants (*rin*, *nor*, and *Cnr*) cannot be used as loss-of-function mutations, and the transcriptional regulatory network involved in tomato fruit ripening is complex and highly redundant. CRISPR/Cas9-mediated targeted mutagenesis can be used to re-evaluate other tomato transcription factors essential for fruit ripening in the future.

Revisiting the function of cell wall modifying enzymes in fruit ripening

As an important feature of fleshy fruit ripening, softening is important for fruit flavor development and overall palatability (Klee and Giovannoni 2011). Over the past 30 years, transgenic plants for silencing tomato genes encoding cell wall-related enzymes, including polygalacturonase (PG) (Cantu et al. 2008; Sheehy et al. 1988; Smith et al. 1990, 1988), pectin methylesterase (Tieman and Handa 1994; Tieman et al. 1992; Wen et al. 2013), galactanase (TBG) (Smith et al. 2002), xyloglucan endo-transglycosylase (Desilva et al. 1994), expansin (Brummell et al. 1999; Cantu et al. 2008), and pectate lyase (PL) (Uluisik et al. 2016; Yang et al. 2017), have been generated to determine which activities are involved in regulating fruit softening. Silencing of these genes usually had no or only modest effects on tomato fruit softening, but suppressing *PL* expression significantly inhibited tomato fruit softening (Uluisik et al. 2016; Yang et al. 2017). The role of three pectin degrading enzyme-encoding genes, including *PL*, *PG2a*, and *TBG4*, in tomato fruit softening was re-evaluated recently using CRISPR/Cas9-mediated targeted mutagenesis (Table 2). The *PL* mutant generated by CRISPR/Cas9 showed consistent ripening phenotypes to *PL*-RNAi plants and conferred significantly enhanced fruit firmness, which highlights a key role of PL in tomato fruit softening (Wang et al. 2019a). Previous studies showed that the silencing of *PG2a* and *TBG4* had no and modest effects on tomato fruit firmness, respectively (Smith et al. 1990, 2002), but both *PG2a* and *TBG4* mutations generated by CRISPR/Cas9 had no effects on tomato fruit firmness (Wang et al. 2019a). Interestingly, mutations in *PG2a* and *TBG4* had effects on other aspects in tomato fruit ripening, including fruit color and carotenoid formation (Wang et al. 2019a), which was not described previously in their silencing plants (Smith et al. 1990, 2002).

Other aspects in fruit ripening remain to be re-evaluated

In addition to transcription factors and cell wall modifying enzymes, other aspects, mainly including ethylene biosynthesis enzymes, ethylene receptors, microRNA (miRNA), and epigenetic regulators, have also been proven to regulate fruit ripening (Chen et al. 2020a, b; Wang et al. 2020c). However, most of these aspects in tomato fruit ripening was only evaluated using the RNAi method. Thus, these aspects in fruit ripening, especially ethylene biosynthesis enzymes and receptors, should be re-evaluated using genome editing, which will provide insights into the regulatory networks of fruit ripening, especially the ethylene-mediated key regulatory hierarchy in climacteric fruit ripening.

Applications of genome editing in tomato improvement

In addition to the basic research, the genetic improvement of tomato has also been greatly promoted by genome editing (Kwon et al. 2020; Wang et al. 2019c). Genome editing has been applied in tomato breeding for improving fruit yield and quality, increasing stress resistance, accelerating the domestication of wild tomato, and customizing tomato cultivars for urban agriculture by mainly using the CRISPR/Cas9 tool for creating precise knockout mutations (Fig. 2); these applications of genome editing in tomato improvement are summarized in Table 3.

Improving tomato fruit yield and quality

As one of the most important agronomic traits in crop breeding, the yield of tomato mainly depends on fruit setting rate, flowering speed, and final cell number and size of fruit, and genetic studies have identified some tomato yield-associated genes (Ariizumi et al. 2013; Zsogon et al. 2017). Inducing mutations in the promoter of the tomato signaling peptide gene *CLV3*, the tomato inflorescence architecture

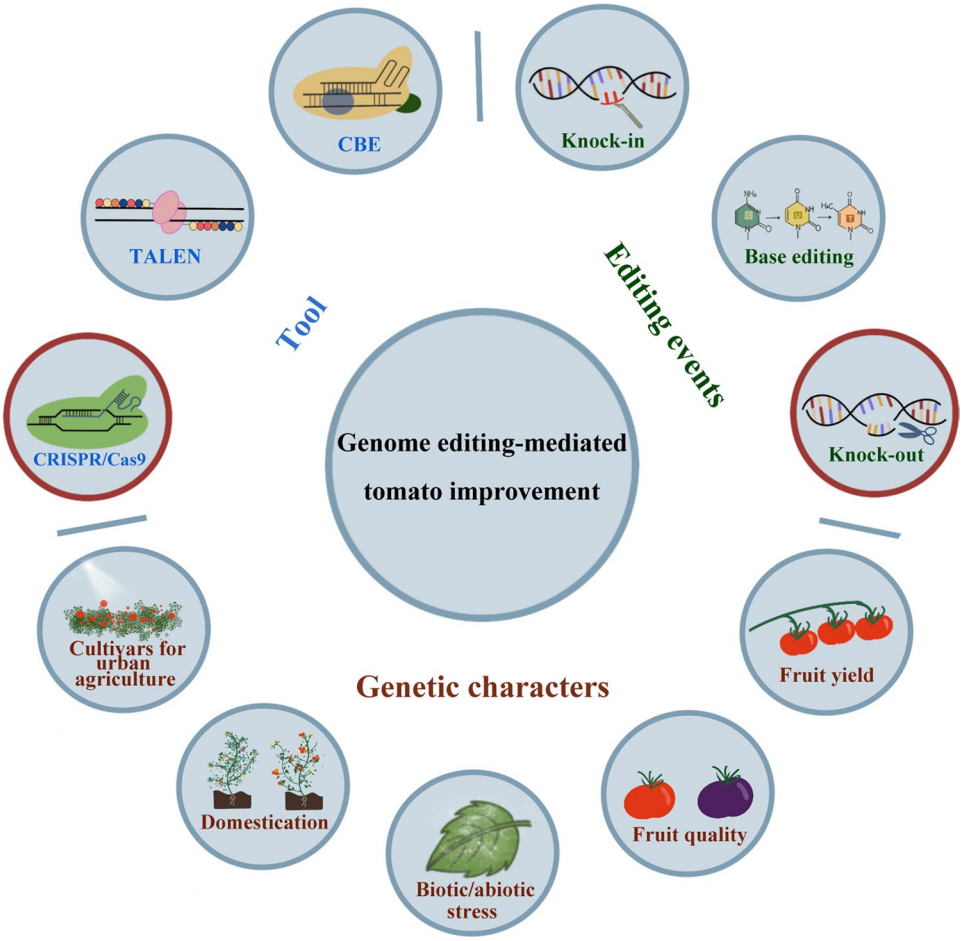

Fig. 2 A chart illustrating the applications of genome editing in tomato improvement

gene *COMPOUND INFLORESCENCE* (*S*), or the tomato architecture gene *SELF PRUNING* (*SP*), was generated by CRISPR/Cas9, and some of these CRISPR/Cas9 *cis*-regulatory mutations showed an increase in the number of floral organs or the size of fruit, conferring enhanced tomato fruit yield (Rodriguez-Leal et al. 2017). Thus, CRISPR/Cas9 drove mutagenesis of promoters will be used as a new plant precision breeding method, especially for engineering quantitative trait variation for crop improvement.

With the improvement of human living standards, greater fruit quality is increasingly needed by consumers and genome editing has also been used for the genetic improvement of tomato fruit quality (Table 3). Fruit quality is usually composed of color, size, shape, nutrients, sweetness, acidity, aroma, and also shelf life (Bai and Lindhout 2007). Although red tomatoes are the most common, tomatoes with other colors are also needed in the market for different consumers. Pink tomatoes are more popular in Asia than red tomatoes (Zhu et al. 2018), and CRISPR/Cas9-mediated targeted disruption of tomato *MYB12*, a master regulator of tomato flavonoid biosynthesis, in different red tomatoes successfully generated pink tomatoes (Deng et al. 2018; Yang et al. 2019; Zhu et al. 2018). CRISPR/Cas9-mediated knockout of the *carotenoid isomerase* (*CRTISO*) and *phytoene synthase 1* (*PSY1*) genes in tomato from the carotenoid biosynthesis pathway produced orange and yellow tomatoes, respectively (Dahan-Meir et al. 2018). However, these pink, orange, and yellow tomatoes were generated at the expense of some nutrients, such as flavonoids and carotenoids. Purple tomatoes, such as the new developed tomato line "Sun Black," are much-loved by consumers because they are rich in health-promoting anthocyanins (Blando et al. 2019). The targeted insertion of a strong promoter upstream of the endogenous anthocyanin biosynthesis gene *SlANT1* in red-fruited tomatoes using CRISPR/Cas9 or TALEN produced purple tomatoes (Cermak et al. 2015), which is also an excellent case study of genome editing-mediated targeted gene insertion in plant breeding.

In addition to change visual fruit color, genome editing has also been used to improve the intrinsic quality of tomato fruit (Table 3). Tomato fruit contains abundant nutrients, such as lycopene, vitamins, and minerals, making a substantial nutritional contribution to our daily diet (Zhu et al. 2018). Multiplex editing of five genes associated with the carotenoid metabolic pathway of tomato, including *stay-green 1* (*SGR1*), *lycopene ε-cyclase* (*LCY-E*), *beta-lycopene*

Table 3 A list of publications on genome editing-mediated tomato improvement

Targeted gene	Genome editing tool	Phenotype	References
To improve tomato fruit yield and quality			
CLV3, *S or SP*	CRISPR/Cas9 (cis-regulatory mutations)	An increase in floral organ number or fruit size, conferring enhanced tomato fruit yield	Rodriguez-Leal et al. (2017)
MYB12	CRISPR/Cas9	Pink tomatoes	Deng et al. (2018), Yang et al. (2019) and Zhu et al. (2018)
CRTISO or *PSY1*	CRISPR/Cas9	Orange tomatoes and yellow tomatoes, respectively	Dahan-Meir et al. (2018)
SlANT1	TALEN and CRISPR/Cas9 (targeted insertion of a strong promoter)	Purple tomatoes	Cermak et al. (2015)
SGR1, *LCY-E, Blc, LCY-B1,* and *LCY-B2*	CRISPR/Cas9	5.1-fold increase in the lycopene content	Li et al. (2018d)
SlDDB1, SlDET1 and *SlCYC-B*	CBE	Increased carotenoid, lycopene, and β-carotene	Hunziker et al. (2020)
SlGAD2 and *SlGAD3*	CRISPR/Cas9	sevenfold to 15-fold increase in GABA accumulation	Nonaka et al. (2017)
GABA-TP1,GABA-TP2, GABA-TP3, *CAT9* and *SSADH*	CRISPR/Cas9	1.34-fold to 3.50-fold in GABA accumulation	Li et al. (2018a)
SlIAA9 or *SlAGL6*	CRISPR/Cas9	Parthenocarpy	Ueta et al. (2017)
SlARF7 and *SlARF5*	CRISPR/Cas9	Parthenocarpy	Hu et al. (2018)
ALC	CRISPR/Cas9	Extend long-shelf	Yu et al. (2017)
PL	CRISPR/Cas9	Extend long-shelf	Uluisik et al. (2016) and Wang et al. (2019a)
To increase tomato resistance to stresses			
SlMlo1	CRISPR/Cas9	Improved resistance to powdery mildew fungus *Oidium neolycopersi*	Nekrasov et al. (2017)
SLPMR4	CRISPR/Cas9	Improved resistance to powdery mildew fungus *O. neolycopersi*	Santillan Martinez et al. (2020)
ACET1a and *ACET1b*	CRISPR/Cas9	Improved resistance to necrotrophic fungus *Botrytis cinerea*	Jeon et al. (2020)
SlDMR6-1	CRISPR/Cas9	Improved resistance to different oomycete or bacterial pathogens	de Toledo Thomazella et al. (2016)
SlJAZ2	CRISPR/Cas9	Improved resistance to bacterial *Pto* DC3000	Ortigosa et al. (2019)
eIF4E1	CRISPR/Cas9	Improved resistance to potyvirus PepMoV	Yoon et al. (2020)
TYLCV genome	CRISPR/Cas9	Improved resistance to yellow leaf curl virus TYLCV	Tashkandi et al. (2018)
SlLBD40	CRISPR/Cas9	Enhanced drought tolerance	Liu et al. (2020a)
ALS	CBE	Conferring resistance to sulfonylurea herbicide chlorsulfuron	Danilo et al. (2019) and Veillet et al. (2019)
To accelerate domestication of wild tomato			
FW2.2, *FAS*, *MULT* and *CycB*	CRISPR/Cas9	Threefold increase in fruit size and a tenfold increase in fruit number	Zsogon et al. (2018)
SP5G, *SP*, *CLAVATA3*, *WUSCHEL* and *SlGGP1*	CRISPR/Cas9	Conferred domesticated phenotypes yet retained parental disease resistance and salt tolerance	Li et al. (2018c)
To customize tomato cultivars for urban agriculture			
SP, *SP5G* and *SlER*	CRISPR/Cas9	Compactness and growth cycle of tomato plants were dramatically increased and shortened, respectively	Kwon et al. (2020)
HEL	CRISPR/Cas9	Vine-like growth of tomato	Yang et al. (2020)

cyclase (*Blc*), lycopene β-cyclase 1 (*LCY-B1*), and *LCY-B2*, was engineered by CRISPR/Cas9, and the multiplex-edited tomato fruit showed an approximately 5.1-fold increase in the lycopene content (Li et al. 2018d). CBE-mediated nucleotide substitutions in three other tomato genes responsible for carotenoid accumulation, including *DNA Damage UV Binding protein 1* (*SlDDB1*), *deetiolated1* (*SlDET1*), and *Lycopene beta cyclase* (*SlCYC-B*), also showed a significant increase in total carotenoid, lycopene, and β-carotene (Hunziker et al. 2020). Tomato fruit contains a large amount of gamma-aminobutyric acid (GABA) (Takayama and Ezura 2015), which is a non-protein amino acid and a health-promoting functional compound as an inhibitory neurotransmitter (Bachtiar et al. 2015). Multiplex-edited tomato fruit by targeting five genes involved in GABA metabolism, including three *GABA-T* genes (*GABA-TP1*, *GABA-TP2*, and *GABA-TP3*), *CAT9* and *SSADH*, showed an increase (1.34- to 3.50-fold) in the content of GABA (Li et al. 2018a). Glutamate decarboxylase (GAD) is a key enzyme in GABA biosynthesis, and CRISPR/Cas9-induced mutations of the two tomato GAD genes *SlGAD2* and *SlGAD3* significantly increased GABA accumulation by sevenfold to 15-fold in tomato fruit (Nonaka et al. 2017). These results suggest that genome editing-mediated knockout or nucleotide substitutions can be used to effectively improve plant nutrients by regulating their synthesis and metabolism pathways.

Parthenocarpy is an important trait of horticultural crops because seedless fruit taste better and also have various industrial purposes (Gorguet et al. 2005; Ueta et al. 2017). Parthenocarpy is controlled by several phytohormones, especially auxin (Pandolfini 2009). CRISPR/Cas9-mediated mutations of the auxin/indole-3-acetic acid (Aux/IAA)-encoding gene *SlIAA9* and two AUXIN RESPONSE FACTOR (ARF) transcription factor-encoding genes, including *SlARF7* and *SlARF5*, produced seedless tomato fruit (Hu et al. 2018; Ueta et al. 2017), confirming the key role of auxin in parthenocarpy. Noticeably, mutations of the MADS-box gene *SlAGAMOUS-LIKE 6* (*SlAGL6*) generated by CRISPR/Cas9 were also parthenocarpic and even showed improved yielding under heat (Klap et al. 2017). Thus, *SlAGL6* is a novel and attractive gene of breeding for seedless fruit, and the specific regulation role of *SlAGL6* in parthenocarpy remains to be investigated. Shelf life is also an important component of fruit quality, especially from the consumer's point of view. CRISPR/Cas9-induced mutagenesis and replacement of tomato *ALC* identified from tomato spontaneous mutant *alcobaca* (*alc*) and proven to be an allele of the *NOR* gene (Bota et al. 2014; Garg et al. 2008), conferred a long shelf life for tomato fruit (Yu et al. 2017). The CRISPR/Cas9-induced mutation of the tomato cell wall modifying enzyme-encoding gene *PL* significantly improved the shelf life of tomato fruit by enhancing fruit firmness (Uluisik et al. 2016; Wang et al. 2019a). Noticeably, other fruit characteristics of *AL*- and *CPL*-edited plants were not sacrificed (Wang et al. 2019a; Yu et al. 2017), and the *alc* spontaneous mutant and *PL*-RNAi fruit even showed increased resistance to plant diseases (Bota et al. 2014; Yang et al. 2017). Thus, both the transcription factor-encoding gene *ALC* and the pectate lyase gene *PL* are valuable genetic resources for the genetic improvement of tomato.

Increasing tomato resistance to stresses

Plants are constantly subjected to numerous biotic stresses (i.e., fungi, bacteria, viruses, and nematodes as well as other plants) and abiotic stresses (i.e., drought, extreme temperatures, and chemical reagents), and there are multiple genes involved in plant responses to stresses (Ashrafi-Dehkordi et al. 2018; Bai et al. 2018). Genetic improvement of tomato for resistance to biotic and abiotic stresses is always a major objective for tomato breeders (Tieman et al. 2017). Powdery mildew, caused by the obligate biotrophic fungus *Oidium neolycopersi*, is a severe fungal disease for both greenhouse- and field-grown tomatoes worldwide (Jones et al. 2001), and CRISPR/Cas9-induced mutations of disease susceptibility (S) genes has been used to generate powdery mildew-resistant tomato plants (Table 3). S genes, which are required for successful pathogen infection, have received increased attention, because disabling plant S genes by genome editing is a novel breeding strategy for the genetic improvement of plant disease resistance (Pavan et al. 2010; Zaidi et al. 2018). *MILDEW-RESISTANT LOCUS O* (*Mlo*), which encodes a membrane-associated protein with seven transmembrane domains, is a conserved S gene conferring susceptibility to powdery mildew fungus in both monocots and dicots (Acevedo-Garcia et al. 2014). There are 16 *Mlo* genes (*SlMlo1* to *SlMlo16*), and transgenic RNAi showed that *SlMlo1* is the major contributor to powdery mildew susceptibility (Zheng et al. 2016). The CRISPR/Cas9-induced mutation of *SlMlo1* significantly reduced powdery mildew susceptibility and the transgene-free powdery mildew-resistant tomato can be generated in less than 10 months (Nekrasov et al. 2017), which highlights the efficiency of genome editing in plant precision breeding. There are four *Arabidopsis* S genes conferring susceptibility to powdery mildew fungus, *Powdery Mildew Resistant 1* (*PMR1*) to *PMR4* (Vogel and Somerville 2000), and CRISPR/Cas9-induced mutation of *SlPMR4*, the closest tomato ortholog of *Arabidopsis PMR4* (*SlPMR4*), significantly improved tomato resistance to the powdery mildew fungus *O. neolycopersi* (Santillan Martinez et al. 2020). Grey mold caused by the ubiquitous necrotrophic fungus *Botrytis cinerea* is another severe fungal disease for field- and greenhouse-grown tomatoes and can also cause serious post-harvest losses for tomato (Williamson et al.

2007). However, genome editing-mediated tomato resistance to biotic *B. cinerea* remains relatively unexploited. Recently, CRISPR/Cas9-induced mutations of both tomato acetylenase-encoding genes *ACET1a* and *ACET1b* showed increased resistance to *B. cinerea* (Jeon et al. 2020). Several candidate S genes conferring susceptibility to *B. cinerea* have been identified from tomato by transgenic RNAi or overexpression (Cai et al. 2020; Gonorazky et al. 2016; Sun et al. 2017), and the application of genome editing-induced mutations of these candidate S genes in breeding resistant tomato plants is promising.

Genome editing has also been used to increase tomato resistance to other different types of pathogens, including oomycetes, bacteria, and viruses (Table 3). The CRISPR/Cas9-mediated mutation of *SlDMR6-1*, a tomato orthologue of the *Arabidopsi*s S gene *DMR6 (Downy Mildew Resistant 6)*, resulted in improved resistance to different pathogens, including the oomycete pathogen *Phytophthora capsici*, and the bacterial pathogens *Pseudomonas syringae* pv. *Tomato* DC3000 (*Pto* DC3000) and *Xanthomonas* spp (de Toledo Thomazella et al. 2016). The CRISPR/Cas9-mediated mutation of *SlJAZ2*, the closest tomato ortholog of *Arabidopsis* JAZ (JASMONATE ZIM DOMAIN) repressor *AtJAZ2*, also significantly increased tomato resistance to *Pto* DC3000, the causal agent of tomato bacterial speck disease (Ortigosa et al. 2019). Pepper mottle virus (PepMoV) is a potyvirus that can infect tomato plants (Melzer et al. 2012), and the CRISPR/Cas9-mediated mutation of tomato *eIF4E1*, which encodes a eukaryotic translation initiation factor and is a recessive resistance gene against potyviruses (Wang 2015), conferred tomato resistance to PepMoV (Yoon et al. 2020). Interestingly, a stably engineered CRISPR/Cas9 system by targeting virus genes encoding coat protein (CP) or replicase (Rep) has been generated in tomato, and these tomato transgenic plants also showed significantly increased resistance to the infection of tomato yellow leaf curl virus (TYLCV) (Tashkandi et al. 2018).

Compared to genome editing-mediated tomato resistance to biotic stresses, the genetic improvement of tomato for resistance to abiotic stresses by genome editing remains relatively unexploited (Table 3). Drought is one of the most destructive abiotic stresses in plants, and many tomato cultivars are highly sensitive to drought (Zhou et al. 2017b). Recently, the CRISPR/Cas9-mediated mutation of LATERAL ORGAN BOUNDARIES DOMAIN (LBD) transcription factor-encoding gene *SlLBD40* in tomato significantly enhanced tomato drought tolerance (Liu et al. 2020a), which highlights the usability of genome editing in breeding for drought tolerance in tomato. In addition, the mutation of the tomato branched-chain amino acid synthesis gene *ALS1* generated by CRISPR/Cas9 and CBE showed increased resistance to the sulfonylurea herbicide chlorsulfuron (Danilo et al. 2019; Veillet et al. 2019).

Accelerating the domestication of wild tomato

Wild relatives contain some beneficial traits, such as stress tolerance, which are usually lost in conventional breeding (van de Wouw et al. 2010); thus, the de novo domestication of wild species has been proposed as an important strategy of crop improvement (Yin et al. 2017; Zsogon et al. 2017). *Solanum pimpinellifolium*, the putative ancestral progenitor of modern tomato varieties, is remarkably tolerant to biotic and abiotic stresses (Zuriaga et al. 2009), and genome editing has recently been used to accelerate the domestication of the wild tomato (Li et al. 2018c; Zsogon et al. 2018). Multiplex editing of four yield- and productivity-related genes, including *FRUIT WEIGHT 2.2* (*FW2.2*), *FASCIATED* (*FAS*), *MULTIFLORA* (*MULT*), and *LYCOPENE BETA CYCLASE* (*CycB*), in the wild tomato *S. pimpinellifolium* was engineered by CRISPR/Cas9 (Zsogon et al. 2018). Noticeably, the multiplex-edited tomato plants showed a threefold increase in fruit size, a tenfold increase in fruit number, and a fivefold increase in fruit lycopene accumulation (Zsogon et al. 2018). In addition, CRISPR/Cas9-mediated multiplex editing of tomato genes, including flowering repressor *SELF-PRUNING 5G* (*SP5G*), *SELF PRUNING* (*SP*), the small-peptide-encoding gene *CLV3* (*CLAVATA3*), the homeobox-encoding gene *WUS* (*WUSCHEL*), and the vitamin C-biosynthetic enzyme-encoding gene *SlGGP1*, also conferred domesticated phenotypes, yet retained parental disease resistance and salt tolerance in tomato plants (Li et al. 2018c). These two studies on de novo domestication of wild tomato provide a good demonstration of the domestication of wild plants using genome editing. Noticeably, genome editing has also been used for the de novo domestication of the orphan *Solanaceae* crop "groundcherry" (*Physalis pruinosa*) (Tiwari et al. 2020) and the recent allotetraploid rice (Yu et al. 2021). Based on the roadmap for de novo domestication (Fernie and Yang 2019), genome editing will significantly accelerate the de novo domestication of other wild relatives of important crops and semi- or non-cultivated plant species.

Customizing tomato cultivars for urban agriculture

The cultivation of crops in urban environments is an ideal approach for developing sustainable agriculture in the future because of the loss of arable land worldwide (Pearson et al. 2010). Multiplex editing of three tomato genes, including the classical flowering repressor gene *SELF PRUNING* (*SP*), the paralog of *SP SP5G*, and the regulator of tomato stem length *ERECTA* (*SlER*), was generated by CRISPR/Cas9, and the compactness and growth cycle of the triple *sp*, *sp5g*, and *sler* mutant were dramatically increased and shortened, respectively (Kwon et al. 2020). Noticeably, the triple-determinate mutant in cherry tomato produced its first ripe fruit

at less than 40 days after transplantation (Kwon et al. 2020). Meanwhile, harvest index of the triple-determinate mutant was not significantly affected, and it grew well in indoor farm systems, including a light-emitting diode (LED) growth chamber and a self-contained, climate-controlled LED hydroponic vertical farm system (Kwon et al. 2020). Thus, these vine-like tomato plants with compact and early yielding characteristics (Kwon et al. 2020) can be developed to accommodate the plant size and space restrictions of urban agriculture. Recently, the CRISPR/Cas9-mediated mutation of the tomato *HEL* gene identified in a spontaneous tomato helical (*hel*) mutant also caused vine-like growth of tomato (Yang et al. 2020), which provides a new idea for developing urban agriculture in the future.

New genome editing systems applied in plants

Genome editing technology continuously undergoes innovation (Anzalone et al. 2020), and some new genome editing systems have recently been established and applied in plants. Herein, we mainly introduce three different types of genome editing systems, including mitochondria-targeted transcription activator-like effector nucleases (mitoTALENs) (Kazama et al. 2019), APOBEC–Cas9 fusion-induced deletion systems (AFIDs) (Wang et al. 2020b), and prime editing systems (Lin et al. 2020), because they will likely further revolutionize basic research and precision breeding in plants.

mitoTALENs for mitochondrial genome editing

In addition to nuclei, plastids (chloroplasts) and mitochondria also harbor DNA, and many important genes are present in mitochondria and plastids (Bock and Knoop 2012). Although stable transformation of plant mitochondrial genomes is still infeasible, two recent reports successfully achieved stable and heritable targeted modification of the mitochondrial genes in rice, rapeseed, and *Arabidopsis* by using nuclear transformation and mitoTALENs which are composed of a TALEN nuclease and a mitochondria localization signal (Arimura et al. 2020; Kazama et al. 2019). The mitochondrial gene *atp6-1* encoding an ATP synthase subunit also has a copy in the nuclear genome, and mitoTALEN-mediated editing of *atp6-1* resulted into mutations only in its mitochondrial copy (Arimura et al. 2020), which confirms the specificity of mitoTALEN-mediated mitochondrial genome editing. To optimize mitoTALEN-mediated mitochondrial genome editing in plants, three different promoters (i.e., CaMV35S, Ubiquitin1, and RPS5A) and two types of TALENs (i.e., conventional TALEN and compact TALEN) were compared, and the RPS5A promoter and conventional TALEN were proven to be the most efficient (Arimura et al. 2020). There are approximately 60 genes and abundant non-coding DNA in plant mitochondrial genomes (Gualberto and Newton 2017), and mitoTALEN will further unravel the role of mitochondrial genomes in plant cellular processes. In addition, engineering plastid genomes, which are also organelle genomes, has been successfully used to improve many economic and agronomic traits in different plants (Li et al. 2021). Thus, the potential application of mitoTALEN-mediated mitochondrial genome editing in crop improvement is highly anticipated.

AFIDs for precise and predictable multi-nucleotide deletions

The widely used genome editing system CRISPR/Cas9 usually results in frequent small indels, including short insertions and short deletions, which likely cannot destroy the small functional regulatory elements and domains in genomic DNA (Wang et al. 2020b). To overcome this limitation, larger deletions should be produced by genome editing systems. The recently developed APOBEC–Cas9 fusion-induced deletion systems (AFIDs), which mainly include AFID-1, AFID-2 and AFID-3, were proven to efficiently produce large (multi-nucleotide) deletions in the genomes of rice and wheat (Wang et al. 2020b). Compared to other strategies of genome editing for producing large deletions, AFIDs, especially AFID-3, have significant advantages in producing predictable deletions (Wang et al. 2020b). AFID-3 has the most additional elements including uracil DNA-glucosidase from *Escherichia coli* and apurinic or apyrimidinic site lyase (AP lyase) from *E. coli*, and approximately one-third of the deletions produced by AFID-3 are predictable (Wang et al. 2020b). These AFID systems confer precise and predictable multi-nucleotide deletions in plant genomes and will significantly accelerate the study of small functional regulatory elements and domains in genomic DNA. In addition, increasing evidences indicate that small functional regulatory elements and domains in genomic DNA, such as upstream open reading frames (uORFs) and other *cis*-regulatory elements, are also promising targets for crop improvement (Li et al. 2020a; Xu et al. 2017; Zhang et al. 2020; Zhou et al. 2017a), and some transcription factors function in different physiological functions by selective binding to different *cis*-regulatory elements (Xiao et al. 2013). Thus, AFIDs will accelerate precise plant breeding by fine-tuning of the expression of target genes, mainly at the transcriptional and translational levels.

Prime editing, a breakthrough in precise genome editing

Although genome editing is revolutionizing basic research and precision breeding in plants, most genome editing

systems cannot confer precision genome editing by installing desired substitutions, insertions and deletion (Li et al. 2020c). CRISPR/Cas-mediated HDR is a previous major strategy of precision genome editing in plants (Li et al. 2020c), but HDR-mediated precision genome editing remains challenging because of the low efficiency of producing HDR and delivering DNA templates in plants (Lin et al. 2020). Prime editing, a breakthrough in precise genome editing, was recently developed in human cells (Anzalone et al. 2019) and was then quickly applied in plants by multiple research groups worldwide (Table 4) (Butt et al. 2020; Hua et al. 2020; Jiang et al. 2020b; Li et al. 2020b; Lin et al. 2020; Lu et al. 2020b; Tang et al. 2020; Xu et al. 2020a, b). Prime editing mainly consists of two parts, including the fusion of a catalytically impaired Cas9 (H840A) nickase (nCas9) and a reverse transcriptase M-MLV-RT (Moloney murine leukemia virus reverse transcriptase), and an engineered guide RNA, pegRNA (prime editing guide RNA) (Anzalone et al. 2019). pegRNA is composed of a sgRNA for targeting specific sites, a reverse transcription (RT) template for conferring predefined edits, and a primer-binding site (PBS) for allowing the 3′ end of the nicked DNA strand to hybridize to the pegRNA (Anzalone et al. 2019). As a search-and-replace genome editing tool, prime editing specifically searches the target site in genomic DNA by using the sgRNA in pegRNA and replaces it in the nCas9 (H840A)-mediated nicked DNA strand by using the reverse transcription reverse and the PBS and RT template in pegRNA (Fig. 1b) (Anzalone et al. 2019). Prime editing can produce all 12 kinds of base substitutions, which is obviously more omnipotent than DNA base editors, and can result in predefined multiple base substitutions, insertions, and deletions (Lin et al. 2020). Based on the four prime editing systems established in human cells, including PE1, PE2, PE3, and PE3b (Anzalone et al. 2019), multiple prime editing systems (Table 4), which usually undergo the optimization of codons and promoters or add other components, such as nuclear localization signals, have been established and applied in different plants. Noticeably, no off-target effects were found in prime editing in plants (Li et al. 2020b). However, these prime editing systems usually have relatively low or variable editing efficiency in plants (Table 4). The length of PBS or the RT template in pegRNA and the position of the nicking sgRNA have significant effects on the editing efficiency of prime editing systems in rice (Lin et al. 2020), and the enhancement of pegRNA expression conferred much higher prime editing efficiency (up to 53.2%) in maize (Jiang et al. 2020b). Three computational models, namely DeepPE, PE_type, and PE_position, were recently developed to predict the efficiency of pegRNA in human cells (Kim et al. 2020), and designing pegRNA using these computational models and upgrading prime editing vectors will further optimize prime editing systems.

Site-directed mutations are essential for identifying the function sites of targeted genes and for studying the biological significance of single nucleotide variants (SNVs) or single nucleotide polymorphisms (SNPs) in plants (Angaji 2009; Henikoff and Comai 2003; Schilbert et al. 2020). Compared to the time-consuming transgenic overexpression of mutated genes in the gene mutant (Jiang et al. 2020a), prime editing-mediated base substitution can conveniently create a site-directed mutation in plants. Based on this significant advantage, prime editing systems will revolutionize basic plant research by increasing the depth of research. In addition, prime editing also seems to be the best genome editing tool for achieving plant precision breeding, and prime editing-mediated introduction of elite alleles into commercialized cultivars will significantly accelerate crop improvement.

Outlook on genome editing-mediated precision breeding in tomato

Conventional breeding is a rather time-consuming practice and is usually accompanied by a loss of fitness and genetic diversity (Wang et al. 2019c), and the revolutionary genome editing technology substantially accelerates precision plant breeding (Chen et al. 2019). Tomato is one of the most important vegetable crops worldwide, and genome editing has also been applied in tomato genetic improvement (Fig. 2; Table 3). Noticeably, these applications of genome editing in the genetic improvement of tomato were performed by mainly using the CRISPR/Cas9 tool for creating precise knockout mutations (Fig. 2; Table 3). Prime editing, which is a breakthrough in precise genome editing and can install desired substitutions, insertions, and deletion, has also recently been established in tomato (Lu et al. 2020b). Thus, the precise knock-in and replacement mutations generated by other genome editing tools, such as prime editing, will further accelerate precision breeding in tomato. In addition, multiplex editing of a series of related genes usually provides better results during genome editing-mediated tomato improvement (Table 3), but CRISPR/Cas9 does not always confer successful editing in all desired tomato genes (Li et al. 2018c; Zsogon et al. 2018). The recent Cas Hybrid for Multiplexed Editing and Screening Applications (ChyMErA) genome editing system established in human cells (Gonatopoulos-Pournatzis et al. 2020) has unique advantages over multiplex editing, and thus, it is a promising approach for achieving precision tomato breeding.

Although there are numerous applications of genome editing in tomato breeding for improving several agronomic traits, mainly including yield, nutritional quality, and stress responses (Table 3), there is still a long way to go for genome editing-mediated precision breeding in tomato.

Table 4 Summary of the prime editing systems established in different plants

Systems	Component description	Editing efficiencies	Applied in plants	References
PPE2	Corresponding to PE2 in human cells: optimization of codons and promoters for cereal plants	2.6%–21.8%	Wheat and rice	Lin et al. (2020)
PPE3	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants			
PPE3b	Corresponding to PE3b in human cells: optimization of codons and promoters for cereal plants			
PE3	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, and adding two nuclear localization signals (NLSs)	2.22%–9.38%	Rice	Li et al. (2020b)
PPE3-V01	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, and adding two NLSs	0.05%–1.55%	Rice	Tang et al. (2020)
PPE3-V02	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, and adding three NLSs			
PPE2-V02	Corresponding to PE2 in human cells: optimization of codons and promoters for cereal plants, and adding three NLSs			
PE-P1	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, and adding two NLSs	0%–26%	Rice	Xu et al. (2020b)
PE-P2	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, adding two NLSs and a hygromycin phosphotransferase (Hpt), and using enhanced esgRNA			
pPE2	Corresponding to PE2 in human cells: optimization of codons and promoters for cereal plants, and adding four NLSs	0%–31.3%	Rice	Xu et al. (2020a)
pPE3	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, and adding four NLSs and a Hpt			
pPE3b	Corresponding to PE3b in human cells: optimization of codons and promoters for cereal plants, and adding four NLSs and a Hpt			
Surrogate pPE2	Corresponding to PE2 in human cells: optimization of codons and promoters for cereal plants, and adding four NLSs and a Hpt^{-ATG}			
Sp-PE2	Corresponding to PE2 in human cells: optimization of promoters for cereal plants and adding two NLSs	0%–17.1%	Rice	Hua et al. (2020)
Sp-PE3	Corresponding to PE3 in human cells: optimization of promoters for cereal plants and adding two NLSs			
Sa-PE3	Corresponding to PE3 in human cells: optimization of promoters for cereal plants, adding two NLSs, and using SaCas9 and Sa sgRNA			
PE2	Corresponding to PE2 in human cells: optimization of promoters for cereal plants	0.26%–2%	Rice	Butt et al. (2020)
PE3	Corresponding to PE3 in human cells: optimization of promoters for cereal plants			
pCXPE01	Corresponding to PE3 in human cells: optimization of promoters to drive pegRNA and gRNA, and adding two NLSs	0.025%–1.66%	Tomato	Lu et al. (2020b)
pCXPE02	Corresponding to PE3 in human cells: optimization of promoters to drive pegRNA and gRNA, adding two NLSs, and using a plant codon-optimized M-MLV-RT			
pCXPE03	Corresponding to PE3 in human cells: optimization of all promoters, adding two NLSs, and using a plant codon-optimized M-MLV-RT			
pZ1PE3	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants	6.5%–53.2%	Maize	Jiang et al. (2020b)
pZ1PE3b	Corresponding to PE3b in human cells: optimization of codons and promoters for cereal plants			

Table 4 (continued)

Systems	Component description	Editing efficiencies	Applied in plants	References
pZ1WS	Corresponding to PE3 in human cells: optimization of codons and promoters for cereal plants, doubling the number of pegRNA expression cassettes, and using two promoter systems to drive pegRNA expression			
pZ1PE3-Csy4	On the basis of pZ1PE3, a Csy-type (CRISPR system yersinia) ribonuclease 4 (Csy4) was added			
pZ1PE3b-Csy4	On the basis of pZ1PE3b, a Csy4 was added			
pZ1WS-Csy4	On the basis of pZ1WS, a Csy4 was added			

The deterioration in flavor quality of modern commercial tomato varieties relative to heirloom tomato varieties is a serious problem according to consumer feedback, which can be partially attributed to the excessive concern for yield and external appearance in past tomato domestication and breeding (Tieman et al. 2017; Xu et al. 2019). The achievement of tomato genome sequencing (Sato et al. 2012), tomato pan-genome construction using genome sequences of 725 tomato phylogenetically and geographically representative accessions (Gao et al. 2019a), and recent panSV (structural variant) genome for 100 diverse tomato lines (Alonge et al. 2020) identify a vast number of genes probably controlling fruit characteristics, including fruit flavor. These abundant and valuable gene resources are of great significance for genome editing-mediated precision breeding in tomato.

Concluding remarks and future perspectives

Genome editing is revolutionizing basic research in plants because of its great simplicity and usability in creating plant mutations in genomic DNA. However, other classical research methods, including RNAi-mediated gene silencing and the identification of genes underlying spontaneous mutants, are still widely used for characterizing gene function in plants, especially in non-model plants. A series of research reports indicate that CRISPR/Cas9-induced mutations in several tomato genes essential for fruit ripening show inconsistent ripening phenotypes compared to previous spontaneous mutants or RNAi plants (Table 2), and a similar phenomenon was also reported in rice (Li et al. 2016; Yu et al. 2020). These results highlight the significance of revisiting plant genes essential for different physiological processes using genome editing. Genome editing has been successfully applied in all types of plants from monocots to dicots, and the main reason for the unsuccessful application of genome editing in some plants is the lack of a stable genetic transformation system. The latest establishment of nanoparticle-mediated plant genome editing and the de novo induction of gene-edited meristems for avoiding time-consuming tissue culture (Table 1) bring great hope for the usability of genome editing in these plants. Thus, genome editing will be the standard method for characterizing gene function in plants, especially in those plants that have successfully established genome editing systems.

Genome editing has been applied in the breeding of tomato (Fig. 2) and other important crops (Tiwari et al. 2020), and, noticeably, most of the mutants generated by genome editing are loss-of-function mutations via the knockout of targeted genes. Loss-of-function mutants have unique advantages for characterizing gene function, but most of them do not usually produce agriculturally useful phenotypes (Zhu and Qian 2020). On the contrary, gain-of-function mutations via base replacement or targeted transgene insertion show great potential for direct application in crop improvement (Zhu and Qian 2020). The DNA base editor CBE-mediated base substitution and CRISPR/Cas9-mediated targeted transgene insertion of exogenous valuable genes have been reported to increase tomato resistance to abiotic stresses and to create purple tomatoes (Table 3). In addition, the recent prime editing systems, which can confer precision genome editing by installing desired substitutions and insertions, are applied in different plants (Table 4), and using chemically modified DNA as a donor in CRISPR/Cas9 can substantially improve the efficiency of gene insertion (Table 1). Thus, broad applications of genome editing-mediated gain-of-function mutations in precision plant breeding are promising.

Although genome editing has been used to improve agronomic traits, mainly including yield, nutritional quality, and stress responses, in many different crops (Tiwari et al. 2020), several less-developed or new breeding directions need to be explored in future genome editing-mediated precision plant breeding, including (1) genome editing-mediated extension of the shelf life of horticultural products to reduce postharvest loss; (2) genome editing of plant growth genes to produce compact, synchronized ripening, and rarely dropping fruit and vegetables, which are more suitable for mechanical harvesting; (3) genome editing-mediated de novo domestication of other wild relatives of important crops and semi- or non-cultivated plant species; (4) the application of genome editing in plant molecular farming to produce high-value

recombinant pharmaceutical proteins by modifying recombinant pharmaceutical proteins or directly targeting insertion of exogenous pharmaceutical protein-encoding genes; (5) the application of genome editing in plant breeding for diversification, such as conceived pungent tomatoes (Naves et al. 2019). Finally, we believe that genome editing is a key tool for achieving the long-term breeding goal of creating "smart crops" (Yu and Li 2021), which show rapid adaptation to climate change and high resistance to extreme weather conditions, as well as high yield and quality.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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