Genome editing for horticultural crop improvement

Jiemeng Xu¹, Kai Hua¹ and Zhaobo Lang¹

Abstract
Horticultural crops provide humans with many valuable products. The improvement of the yield and quality of horticultural crops has been receiving increasing research attention. Given the development and advantages of genome-editing technologies, research that uses genome editing to improve horticultural crops has substantially increased in recent years. Here, we briefly review the different genome-editing systems used in horticultural research with a focus on clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9)-mediated genome editing. We also summarize recent progress in the application of genome editing for horticultural crop improvement. The combination of rapidly advancing genome-editing technology with breeding will greatly increase horticultural crop production and quality.

Introduction
As an important branch of agriculture, horticulture originated thousands of years ago and has developed greatly during the course of human history. Horticultural crops are generally considered to include vegetable and fruit crops as well as floricultural and ornamental plants, which are cultivated for food, for nutritional and medical use, and for esthetic enjoyment¹. Vegetable and fruit crops are low in calories but contain high levels of vitamins and minerals², making them indispensable for balancing our daily diet. Although the supply of horticultural products is increasing, the diversity and nutritional value of the products are decreasing³. These decreases can be partially attributed to the narrow genetic diversity of horticultural crops resulting from domestication and breeding as well as reproductive barriers that inhibit genetic introgression from wild relatives. Therefore, the generation of genetic resources with diverse and desirable characteristics will be of great value for improving horticultural products.

Thousands of years ago, humans began to improve crops by introducing new traits from crossable relatives. The essential goal of this process was the transfer of desirable genetic variations. As late as 1930s, the available variations were generated solely through natural or spontaneous processes. Breeders subsequently learned to produce mutants by using chemical mutagens or radiation⁴. Both spontaneous and induced mutations have significantly increased crop yield and quality⁵. Given the rareness and randomness of these mutations, however, obtaining suitable materials for crop improvement has proved to be laborious and time consuming⁴.

With the rapid progress in molecular biology, DNA sequence-specific manipulation has become a powerful tool. In 1987, several animal scientists invented gene-targeting technology that relies on homologous recombination (HR). This innovative technology enabled researchers to precisely edit (though with a low frequency) an endogenous gene after introducing a donor template into mouse embryonic stem cells⁶,⁷. Similar progress was subsequently reported by plant researchers, but with an extremely low editing frequency of 0.5–7.2 × 10⁻⁴⁸,⁹. DNA double-stranded breaks (DSBs), which commonly result in HR in meiotic chromosomes¹⁰, were later used to increase the HR frequency in gene
targeting\textsuperscript{11}. In addition to HR, DSBs can be repaired through the error-prone nonhomologous end-joining (NHEJ) pathway in somatic cells, which can generate mutations via the small deletions or insertions that occur at a break site\textsuperscript{12}. Scientists have used the following kinds of engineered endonucleases to introduce site-specific DSBs: meganucleases (MNs), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9), and CRISPR from Prevotella and Francisella 1 (CRISPR/Cpf1). These engineered endonucleases have enabled genome editing in various biological systems\textsuperscript{13–16}.

With the advent of CRISPR/Cas9, the application of genome editing to horticultural crops has greatly advanced. In this review, we first introduce and compare the engineered nucleases that are used for genome editing. We then consider their current applications in horticulture. Finally, we discuss the implications and challenges of genome editing for the improvement of horticultural crops.

Genome-editing systems

Sequence-specific DNA binding, such as the interaction between a transcription factor and a promoter, is a common phenomenon. For genome editing, the previously mentioned nucleases can target specific sequences to generate DSBs under the guidance of protein–DNA interaction (for MNs, ZFNs, and TALENs) or RNA–DNA base-pairing (for CRISPR/Cas9 and CRISPR/Cpf1)\textsuperscript{16,17}.

Meganucleases or homing nucleases

The first class of nucleases for genome editing. MNs or homing endonucleases, was discovered in the genomes of microorganisms or organelles. By recognizing DNA sequence elements ranging from 12 to 40 bp, these nucleases cut both strands of DNA in a site-specific manner (Fig. 1a)\textsuperscript{18}. Among MNs, the I-CreI protein has received the most research attention and has been reported to be effective in maize\textsuperscript{19}, but the rare occurrence of recognizable sites limits the ability of I-CreI and other MNs to edit desired target sites\textsuperscript{17}. To broaden the application of MNs, researchers have used mutagenesis or combinatorial assembly to produce MN variants that target the desired DNA sequence\textsuperscript{20,21}. Nevertheless, the overlapping recognition and catalytic domains of modified MNs cause difficulties and often compromise their catalytic activity\textsuperscript{15}. For these reasons, MNs have not been widely used by plant scientists.

ZFNs and TALENs

As suggested by their names, ZFNs or TALENs are generated by fusing the DNA cleavage domain of the endonuclease FokI with zinc fingers (ZFs) or with transcripational activator-like effectors (TALEs). The FokI endonuclease domain mediates independent and non-specific DNA cleavage upon dimerization and is not involved in any sequence recognition\textsuperscript{22}. Therefore, a pair of ZFs or TALEs, each fused with a FokI endonuclease domain, is designed to achieve site-specific cleavage\textsuperscript{23–25}. ZFs are found in transcription factors, with each finger domain recognizing three specific nucleotides. ZFNs typically exhibit an array of 3 or 4 finger domains, which can recognize 18–24 bp sequences when a ZFN occurs as a dimer\textsuperscript{23,25}. Many studies have been conducted to improve ZFN applicability, efficiency, and precision\textsuperscript{26,27}, but there are still concerns about interference from neighboring finger domains and the limited number of recognition sites (Fig. 1b)\textsuperscript{15}.

In contrast to ZFNs, TALENs achieve sequence specificity via the customizable DNA-binding domains of TALEs, which are proteins excreted by the common bacterial plant pathogen Xanthomonas\textsuperscript{28}. During pathogenesis, TALEs bind to a specific sequence of plant promoters to activate gene expression to facilitate infection\textsuperscript{28}. The central binding domain of TALEs consists of 13–28 repeat sequences. Each repeat, which encodes a highly conserved sequence of 34 amino acids, can recognize and bind to one nucleotide through the variable di-residues at the 12th and 13th positions\textsuperscript{29–31}. Such one-to-one pairing, together with the negligible context dependency on neighboring repeats, enables TALENs to target desired sequences (Fig. 1c)\textsuperscript{32,33}. In general, TALENs outperform ZFNs in terms of precision and accessibility.

CRISPR/Cas9 and CRISPR/Cpf1

Unlike ZFN and TALEN systems, which depend on protein–DNA binding specificity, the CRISPR system relies on RNA–DNA binding to achieve sequence specificity. During the functional elucidation of the CRISPR/Cas system, its involvement in bacterial resistance to viruses was experimentally demonstrated\textsuperscript{34}, and several components, including crRNA, PAM motif, and tracrRNA, were discovered to be necessary for this system\textsuperscript{35–37}. More interestingly, reconstructed key components of the CRISPR/Cas9 system can introduce DSBs in a site-specific way, suggesting the potential use of this programmable RNA-guided CRISPR/Cas9 system for genome editing in organisms other than bacteria\textsuperscript{38,39}. This possibility was soon demonstrated in human and mouse cells\textsuperscript{40–42}, zebrafish\textsuperscript{43}, and plants\textsuperscript{44–48}. In the system, site-specific binding to the target is achieved by RNA-DNA pairing of a 20 nt sequence in the chimeric single-guide RNA (sgRNA) with the target. The other crRNA- and tracrRNA-derived sequences also interact with the target to form an RNA:DNA heteroduplex that is recognized by the collective interactions of several Cas9 domains: PI, REC1, RuvC, and NUC. Thereafter, the RuvC and HNH
Fig. 1 Schematic models of genome-editing systems. 

a A meganuclease can recognize a DNA sequence element of 12–40 bp and cut both strands at specific sites, forming sticky double-stranded breaks (DSBs). 

b In ZFNs, each zinc finger recognizes a 3-bp DNA sequence. Target specificity is achieved by arrays of several zinc fingers. Each DNA strand is bound by one zinc finger array linked with FokI, which in dimer form cuts DNA strands. 

c In TALENs, the central binding domain of each TALE consists of 13–28 repeats. Each repeat (a highly conserved sequence of 34 amino acids) can recognize and bind one nucleotide through the variable di-residues at the 12th and 13th positions. Paired TALENs lead to the dimerization of FokI, and the dimers cut the DNA strands, forming sticky DSBs at the target site. 

d In the CRISPR/Cas9 system, a single guide RNA (sgRNA) pairs with the target sequence upstream of a 5′-NGG-3′ PAM motif (N=A, T, C or G). The Cas9 endonuclease cuts the noncomplementary and complementary DNA strands at a location 3 nucleotides upstream of the PAM motif with RuvC and HNH domains, respectively. The cutting forms a blunt end DSB. 

e In the CRISPR/Cpf1 system, target specificity is achieved by the pairing of crRNA with the DNA strand downstream of a 5′-TTN-3′ PAM motif. The Cpf1 endonuclease uses the RuvC and Nuc domains to cut noncomplementary and complementary DNA strands at different positions, producing DSBs with sticky ends.
domains cut the noncomplementary and complementary DNA strands at a location 3 nucleotides upstream of the PAM motif, respectively (Fig. 1d). The recognizable PAM motif of Cas9 is 5′-NGG-3′ (N=A, T, C, or G), and this G-rich feature prevents the design of sgRNAs in T-rich regions.

Cpf1, another endonuclease in the class 2 Type V CRISPR system, has also been found to be efficient in plant genome editing and to present unique features. First, Cpf1 does not require an additional tracrRNA to form a mature crRNA. Second, unlike Cas9, which recognizes G-rich PAM sequences, Cpf1 recognizes T-rich PAM sequences. Finally, whereas cutting by the Cas9 endonuclease produces blunt ends, cutting by the Cpf1 endonuclease produces cohesive ends (Fig. 1e). In addition to causing site-specific mutations, CRISPR genome-editing systems can be used to achieve gene regulation through the manipulation of the nuclease-inactivated Cas9 (dCas9).

Each of the endonucleases used for genome editing has unique properties because of differences in their underlying mechanisms (Fig. 1 and Table 1; Zhang et al., Knott and Doudna; Knott and Doudna). In addition to generating indel mutations at target sequences, CRISPR/Cas systems have been adapted for precise base editing. Base editors usually consist of an sgRNA-guided Cas9 nickase (nCas9) fused with a deaminase that causes C to T or A to G base conversions. These resources greatly increase the versatility of the tools that can be used for precise manipulation of horticultural crops.

**Current status of genome editing in horticultural crops**

To obtain genetic resources with diverse characteristics for breeding, both spontaneous and induced mutations have been commonly used. The rarity and uncertainty of these mutations have motivated scientists to find ways to introduce precise mutations at target sites. Recently, most genome-editing studies on plants have been carried out in model systems and staple crops, but the application of genome editing to horticultural crops is rapidly increasing. In 2013, the first example of genome editing in a horticultural crop was achieved via a TALEN in *Brassica oleracea*. In the following years, the number of studies involving genome editing in horticulture has exponentially increased (Fig. 2a, Table 2), and CRISPR-based systems now dominate. The functions of genes targeted by genome editing are very diverse, but researchers have focused most on targets affecting development, followed by targets affecting metabolism and stress responses. In addition, studies that focus on the improvement of the CRISPR/Cas9 system in horticultural crops frequently use marker/reporter genes as targets such as *phytoene desaturase* (PDS), whose mutation results in an albino phenotype (Fig. 2b). Among horticultural crops, tomato has received much more attention regarding genome editing than other crops: ~42% of genome-editing studies have involved tomato, whereas ~13% have involved potato. Although most (72%) genome editing with horticultural crops is performed in vegetables (Fig. 2c), some floral and medicinal plants have also been successfully manipulated by genome editing (Fig. 2c).

In tomato, development-related genes have been edited to manipulate flowering patterns and fruit development. The tomato *BLADE-ON-PETIOLE* (BOP) genes, which encode transcriptional cofactors, can regulate inflorescence structure, and knock-out of *SIBOP* genes by gene editing reduces the number of flowers per inflorescence. CRISPR/Cas9-induced mutations in the flowering repressor *self-pruning 5G* lead to rapid flowering and early harvest. In addition, editing of the cis-regulatory region of *SICLV3* or the coding regions of *SIDML2*, *SIORRM4* and the *RIN* locus alters fruit development and ripening. Interestingly, multiplex targeting of several genes that are important for tomato domestication was found to greatly alter the properties of the wild tomato relative *Solanum pimpinellifolium* such that the generated mutants were similar to cultivated tomato. In potato, when the vacuolar invertase gene was disrupted by TALEN, the cold storage and processing of tubers were improved. Another recent study in potato showed the possibility of overcoming self-incompatibility by editing the *S-RNase* gene, which would provide an alternative method of propagation through seeds. In addition to tomato and potato, other horticultural crops have also been edited to obtain desirable traits. Genes related to resistance to plant pathogens such as *Xanthomonas citri* and *Botrytis cinerea* have been manipulated in citrus, apple, and grape. In oilseed crops, genes involved in fatty acid metabolism have been frequently targeted to improve oil quality. The application of genome editing to improve crops is based on knowledge of the association between genes and their controlled traits. In the future, functional characterization of genes in different crops will help to identify valuable targets that could be edited for potential horticultural improvement, such as increased productivity, marketing quality, and nutritional value.

**Possible implications of genome editing in horticulture**

The goal of breeding is to harness genetic variations to introduce desirable traits. These genetic variations can arise in various ways, such as by spontaneous mutation, chemical mutagenesis, and physical mutagenesis. Gene editing could be regarded as biological mutagenesis. In comparison with other approaches, genome-editing technology is superior in terms of versatility, efficiency,
and specificity. For instance, CRISPR-based genome editing can cause many types of mutations in target sequences, including small insertions/deletions, deletions of large fragments, gene replacement, and precise base substitutions\(^\text{16}\). In addition, genome-editing technology is continuously advancing: the endonuclease Cpf1\(^\text{51}\) and newly discovered or designed Cas9 variants\(^\text{80,81}\) can recognize different PAM sequences, thereby broadening the genome-wide sites that can be targeted for editing.

Genome-edited plants are not considered genetically modified organisms (GMOs) in countries such as the U.S. and Japan but are still under strict GMO regulation in Europe. The largest difference between genome-edited plants and GMOs is that the genomes of edited plants can be free of exogenous DNA sequences. The exogenous DNA of the editing tools can be removed through genetic segregation\(^\text{82}\) or may never have to be introduced if CRISPR reagents are delivered as ribonucleoproteins\(^\text{83,84}\).

Mutants generated via genome editing can be directly used for crop production or as prebreeding materials. Through genome editing, desirable traits can be directly introgressed into elite or heirloom lines without compromising other properties, and the resulting lines with targeted improvement will be ready for use in production. The wild relatives of cultivated varieties are also potential materials for genome editing because they generally present unique features in many important traits. For instance, wild species of cultivated tomato are more resistant to unfavorable environments than commercial cultivars\(^\text{85}\). Wild *Solanum pimpinellifolium* was recently domesticated by the editing of several important genes affecting plant architecture and fruit development,
| Species                              | Crop type | Genome editing tool | Targeted gene                                | Gene function or phenotype                          | Classification of targeted gene |
|--------------------------------------|-----------|---------------------|----------------------------------------------|----------------------------------------------------|---------------------------------|
| Solanum lycopersicum                 | Vegetable | CRISPR              | SlALS1                                      | Enhanced herbicide resistance                      | Stress response                 |
|                                      |           |                     | SlJAZ2                                      | Resistance to bacterial spec                        | Stress response                 |
|                                      |           |                     | AP3                                  | ACCAR T2/1, POLYgalacturonase 2/AC2, and betagalacturonase (TBE2) | Cell wall gene, altered fruit color and ripening |
|                                      |           |                     | FUMUL (FUL/VOL and FUL/MAF)                 | Reduced herbicide resistance, and stress response  | Stress response                 |
|                                      |           |                     | Pectate lyase (Pn1), polygalacturonase 2/AC2, | Erbavirin resistance and fruit development         | Development                     |
|                                      |           |                     | and beta-galacturonase (TBE2)              | Development                                         |                                |
|                                      |           |                     | SlNPR1                                      | Reduced drought tolerance                           | Stress response                 |
|                                      |           |                     | SlALS1, SlALS2                            | Enhanced herbicide resistance                      | Stress response                 |
|                                      |           |                     | SlGAI                                      | Gibberellin response and dwarfism                   | Development                     |
|                                      |           |                     | SlEIN2, SlERFE1, SlARF2B, SlGRAS8, SlACS2, SlACS4 | Ethylene response and fruit development            | Development                     |
|                                      |           |                     | SBPase                                     | Leaf senescence (SBPase in primary metabolism)     | Development                     |
|                                      |           |                     | CBF1                                       | Chilling tolerance                                  | Cell wall gene                  |
|                                      |           |                     | POLYGALACTURONASE/PG and PECTATE LYSASE (PGL) | Cell wall gene                                     |                                |
|                                      |           |                     | NPTII                                      | N.A.                                               | Others                          |
|                                      |           |                     | Psy1 and CrtR-b2                           | Carotenoid metabolism                               | Metabolism                      |
|                                      |           |                     | Carotenoid isomerase and Psy1               | NAD Kinase 2A, GA9                                  | Development                     |
|                                      |           |                     | Solyc08g075770                            | Fusarium wilt susceptibility                       | Carotenoid metabolism           |
|                                      |           |                     | PDS                                         | Reduced nucleotide metabolism                      | Carotenoid metabolism           |
|                                      |           |                     | SlDML2                                      | DNA methylation and fruit ripening                 | DNA methylation and fruit ripening |

Reference: Xu et al. *Horticulture Research* (2019) 6:113
| Species                      | Crop type | Genome editing tool | Targeted gene | Gene function or phenotype                                      | Classification of targeted gene | Reference |
|------------------------------|-----------|---------------------|---------------|-----------------------------------------------------------------|---------------------------------|-----------|
| Solanum lycopersicum         | Vegetable | CRISPR              | PDS and GABA-TP1, GABA-TP2, GABA-TP3, CAT9 and SSADH | γ-aminobutyric acid metabolism | Metabolism                      | 123       |
| Solanum lycopersicum         | Vegetable | CRISPR              | SIMY812       | Pink tomato fruit color                                          | Metabolism                      | 124       |
| Solanum lycopersicum         | Vegetable | CRISPR              | Coat protein, Replicase from TYLCV | Obtained resistance to tomato yellow leaf curl virus | Stress response                 | 125       |
| Solanum lycopersicum         | Vegetable | CRISPR              | RIN           | Ethylene production and fruit ripening                         | Metabolism                      | 126       |
| Solanum pimpinellifolium     | Vegetable | CRISPR              | SP, MULT, FAS, CyCb, OVUTE and FW2.2 | Plant and inflorescence architecture, fruit shape and lycopene biosynthesis | Development, metabolism         | 69        |
| Solanum pimpinellifolium     | Vegetable | CRISPR              | SP, SPS, CLV3 and WUS, GSP1 | Plant architecture, day-length insensitivity, enlarged fruit size and vitamin C | Development, metabolism         | 70        |
| Solanum lycopersicum         | Vegetable | CRISPR              | RIN           | Ethylene production and fruit ripening                         | Development                      | 68        |
| Solanum lycopersicum         | Vegetable | CRISPR              | SORRM4        | RNA editing and fruit ripening                                  | Development                      | 67        |
| Solanum lycopersicum         | Vegetable | CRISPR              | ALC           | Shelf life                                                     | Metabolism                      | 127       |
| Solanum lycopersicum         | Vegetable | CRISPR              | CLAVATA-WUSCHEL | Altered locale number                                         | Development                      | 65        |
| Solanum lycopersicum         | Vegetable | CRISPR              | SIMAPK3       | Drought stress                                                 | Stress response                  | 128       |
| Solanum lycopersicum         | Vegetable | CRISPR              | Glutamate decarboxylase (GAD) | γ-aminobutyric acid metabolism                                | Metabolism                      | 129       |
| Solanum lycopersicum         | Vegetable | CRISPR              | Solyc12g038510 | Jointless mutant, abscission                                   | Development                      | 130       |
| Solanum lycopersicum         | Vegetable | CRISPR              | Multiple genes | Generate a pool of mutants                                     | Others                           | 131       |
| Solanum lycopersicum         | Vegetable | CRISPR              | PSY           | Fruit color                                                    | Development                      | 132       |
| Solanum lycopersicum         | Vegetable | CRISPR              | Solyc12g038510 | Jointless and branching                                       | Development                      | 133       |
| Solanum lycopersicum         | Vegetable | CRISPR              | L1L4          | Involved in fruit metabolism during ripening                  | Metabolism                      | 134       |
| Solanum lycopersicum         | Vegetable | CRISPR              | DELLA and ETR | Hormone response                                              | Development                      | 135       |
| Solanum lycopersicum         | Vegetable | CRISPR              | SfTol1        | Powdery mildew resistance                                     | Stress response                  | 136       |
| Solanum lycopersicum         | Vegetable | CRISPR              | SfIA9         | Parthenocarpic tomato plants                                  | Development                      | 137       |
| Solanum lycopersicum         | Vegetable | CRISPR              | SP5G          | More rapid flowering                                          | Development                      | 64        |
| Solanum lycopersicum         | Vegetable | CRISPR              | Genes involved tomato domestication | Development and plant architecture | Development                      | 138       |
| Species                  | Crop type | Genome editing tool | Targeted gene | Gene function or phenotype                                                                 | Classification of targeted gene | Reference |
|-------------------------|-----------|---------------------|---------------|-------------------------------------------------------------------------------------------|--------------------------------|-----------|
| Solanum lycopersicum    | Vegetable | CRISPR              | SlAGL6        | Production of parthenocarpic fruit under high temperature                                  | Development                     | 139       |
| Solanum lycopersicum    | Vegetable | CRISPR              | N.A           | N.A                                                                                       | Others                          | 140       |
| Solanum lycopersicum    | Vegetable | CRISPR              | SlBOp         | Inflorescence structure                                                                    | Development                     | 63        |
| Solanum lycopersicum    | Vegetable | CRISPR              | L1L4          | Heterochronic phenotype, plant architecture                                                | Development                     | 141       |
| Solanum lycopersicum    | Vegetable | CRISPR              | PDS and PIF   | Albinotype                                                                                | Reporter                        | 142       |
| Solanum lycopersicum    | Vegetable | CRISPR              | N.A           | N.A                                                                                       | Others                          | 143       |
| Solanum lycopersicum    | Vegetable | CRISPR              | RIN           | Fruit ripening                                                                           | Development                     | 145       |
| Solanum lycopersicum    | Vegetable | CRISPR              | PROCERA       | GA response and taller plant                                                              | Development                     | 146       |
| Solanum lycopersicum    | Vegetable | CRISPR              | AGO7          | Leaf morphology                                                                           | Development                     | 147       |
| Solanum tuberosum       | Vegetable | CRISPR              | St16DOX       | Steroidal glycoalkaloids metabolism                                                       | Metabolism                      | 148       |
| Solanum tuberosum       | Vegetable | CRISPR              | GBSS genes    | Starch biosynthesis                                                                       | Metabolism                      | 149       |
| Solanum tuberosum       | Vegetable | CRISPR              | S-RNase       | Self-incompatibility                                                                      | Development                     | 150       |
| Solanum tuberosum       | Vegetable | CRISPR              | Coilin gene   | Enhanced resistance to biotic and abiotic agents                                          | Stress response                 | 151       |
| Solanum tuberosum       | Vegetable | CRISPR              | StALS1, StALS2| Enhanced herbicide resistance                                                             | Stress response                 | 152       |
| Solanum tuberosum       | Vegetable | CRISPR              | GBSS1         | Starch biosynthesis                                                                       | Metabolism                      | 153       |
| Solanum tuberosum       | Vegetable | CRISPR              | S-Rnase       | Self-incompatibility                                                                      | Development                     | 154       |
| Solanum tuberosum       | Vegetable | CRISPR              | Coilin gene   | Enhanced resistance to biotic and abiotic agents                                          | Stress response                 | 155       |
| Solanum tuberosum       | Vegetable | TALEN               | SBE1 and StvacINV22 | Sugar metabolism                                                                         | Metabolism                      | 156       |
| Solanum tuberosum       | Vegetable | CRISPR              | SMYB44        | Phosphorus homeostasis                                                                    | Stress response                 | 157       |
| Solanum tuberosum       | Vegetable | CRISPR              | GBSS          | Starch metabolism and tuber quality                                                      | Metabolism                      | 158       |
| Solanum tuberosum       | Vegetable | TALEN               | StALS1        | Enhanced herbicide resistance                                                             | Stress response                 | 71        |
| Solanum tuberosum       | Vegetable | TALEN               | StALS1        | Enhanced herbicide resistance                                                             | Stress response                 | 71        |
| Solanum tuberosum       | Vegetable | TALEN               | vINV           | Postharvest cold storage and processing                                                   | Metabolism                      | 71        |
| Species                  | Crop type | Genome editing tool | Targeted gene | Gene function or phenotype                   | Classification of targeted gene | Reference |
|-------------------------|-----------|---------------------|---------------|-----------------------------------------------|---------------------------------|-----------|
| Solanum tuberosum      | Vegetable | CRISPR              | StALS1        | Enhanced herbicide resistance                 | Metabolism                      | 159       |
| Solanum tuberosum      | Vegetable | TALEN               | StALS1        | Enhanced herbicide resistance                 | Metabolism                      | 160       |
| Solanum tuberosum      | Vegetable | CRISPR              | StIAA2        | Aux/AA protein, shoot morphogenesis           | Development                     | 161       |
| Brassica oleracea      | Vegetable | CRISPR              | BoCGA4a       | GA response and dwarfism                      | Development                     | 162       |
| Brassica oleracea      | Vegetable | CRISPR              | BoPDS, BoSRK3, BoMS1 | Albino phenotype, self-incompatibility, male sterility | Development | 163       |
| Brassica napus          | Vegetable | CRISPR              | LMI1          | Leaf lobe development                         | Development                     | 164       |
| Brassica oleracea, rapa| Vegetable | CRISPR              | PDS and FRI   | Albino phenotype and flowering                | Reporter, development           | 165       |
| Brassica napus          | Vegetable | CRISPR              | FAD2          | Fatty acid metabolism                         | Metabolism                      | 166       |
| Brassica carinata      | Vegetable | CRISPR              | Fascin-like arabinogalactan protein | Regulation of root hairs under phosphorus stress | Development, stress response  | 167       |
| Brassica napus          | Vegetable | CRISPR              | WRKY11 and WRKY70 | Enhanced biotic resistance                     | Stress response                 | 168       |
| Brassica napus          | Vegetable | CRISPR              | SDG8          | Histone lysine methyltransferase              | Development                     | 169       |
| Brassica napus          | Vegetable | CRISPR              | CLV3 and CLV1, CLV2 | Regulate multicellular seeds                   | Development                     | 170       |
| Brassica rapa and napus| Vegetable | CRISPR              | AP2a, AP2b    | Sepal to carpal modification                  | Development                     | 171       |
| Brassica napus          | Vegetable | CRISPR              | BnaRGA, BnaDA1, BnaDA2, BnaFUL | Multiple genes involved in plant development | Development                     | 172       |
| Brassica carinata      | Vegetable | CRISPR              | Fascin-like arabinogalactan protein | Root hair development                          | Development                     | 173       |
| Brassica napus          | Vegetable | CRISPR              | ALC           | Valve margin development, seed shattering     | Development                     | 174       |
| Brassica oleracea      | Vegetable | TALEN               | FRIGIDA       | Early flowering phenotype                      | Development                     | 175       |
| Dendrobium officinale  | Flower    | CRISPR              | CSH, C4H, 4Q, CQR, and IRX | Lignocellulose biosynthesis                  | Metabolism                      | 176       |
| Lettuce sativa         | Vegetable | CRISPR              | LsBIN2        | Impaired brassinosteroid response             | Development                     | 177       |
| Lettuce sativa         | Vegetable | CRISPR              | LsNCEQD4      | Thermo-inhibition of seed germination         | Development                     | 178       |
| Cucumis sativus        | Vegetable | CRISPR              | eIF4E         | Enhanced viral resistance                     | Stress response                 | 179       |
| Cucumis sativus        | Vegetable | CRISPR              | CmWIP1        | Gynoecious phenotype                          | Development                     | 180       |
| Musa balbisiana        | Fruit     | CRISPR              | eBSV          | Control of virus pathogenesis                 | Stress response                 | 181       |
| Musa acuminata         | Fruit     | CRISPR              | PDS           | Albino phenotype                              | Reporter                        | 182       |
| Species                  | Crop type | Genome editing tool | Targeted gene | Gene function or phenotype                                                                 | Classification of targeted gene | Reference |
|-------------------------|-----------|---------------------|---------------|-------------------------------------------------------------------------------------------|---------------------------------|-----------|
| *Musa acuminata*        | Fruit     | CRISPR              | PDS           | Albino phenotype                                                                          | Reporter                        | 180       |
| *Actinidia deliciosa*   | Fruit     | CRISPR              | PDS           | Albino phenotype                                                                          | Reporter                        | 181       |
| *Vitis vinifera*        | Fruit     | CRISPR              | VvdPDS        | Albino phenotype                                                                          | Reporter                        | 182       |
| *Vitis vinifera*        | Fruit     | CRISPR              | VvdNRKYS2     | Increased the resistance to *Botrytis cinerea*                                            | Stress response                 | 75        |
| *Vitis vinifera*        | Fruit     | CRISPR              | VvdPDS        | Albino phenotype                                                                          | Reporter                        | 184       |
| *Vitis vinifera*        | Fruit     | CRISPR              | MLO-7         | Powdery mildew resistance                                                                  | Stress response                 | 185       |
| *Vitis vinifera*        | Fruit     | CRISPR              | VvdNRKYS2     | Increased the resistance to *Botrytis cinerea*                                            | Stress response                 | 187       |
| *Citrus sinensis*       | Fruit     | CRISPR              | DMR6          | Huanglongbin resistance                                                                    | Stress response                 | 182       |
| *Citrus sinensis*       | Fruit     | CRISPR              | PDS           | Albino phenotype                                                                          | Reporter                        | 188       |
| *Citrus Paradisi*       | Fruit     | CRISPR              | CsPDS, Cs2g12470 and Cs7g03360 | Albino phenotype                                                                          | Reporter                        | 189       |
| *Citrus sinensis*       | Fruit     | CRISPR              | PDS           | Albino phenotype                                                                          | Reporter                        | 190       |
| *Citrus sinensis*       | Fruit     | CRISPR              | CsLOB1        | Canker resistance                                                                         | Stress response                 | 73        |
| *Citrus sinensis*       | Fruit     | CRISPR              | CsLOB1        | Canker resistance                                                                         | Stress response                 | 74        |
| *Citrus sinensis*       | Fruit     | CRISPR              | CsPDS         | Albino phenotype                                                                          | Reporter                        | 191       |
| *Chrysanthemum montiflorum* | Flower   | CRISPR              | GpYGFP        | Targeted editing of the YGFP reporter gene                                                | Others                          | 192       |
| *Ipomoea nil*           | Flower    | CRISPR              | InDFR-8       | Anthocyanin biosynthesis and white flowers                                                | Metabolism                      | 193       |
| *Ipomoea nil*           | Flower    | CRISPR              | InCCD4        | Altered petal color                                                                       | Development                     | 194       |
| *Petunia inflata*       | Flower    | CRISPR              | PiSSK1        | Self-incompatibility                                                                       | Development                     | 195       |
| *Petunia hybrida*       | Flower    | CRISPR              | PDS           | Albino phenotype                                                                          | Reporter                        | 196       |
| *Citrus lanatus*        | Fruit     | CRISPR              | ALS           | Increased herbicide resistance                                                            | Stress response                 | 197       |
| *Citrus lanatus*        | Fruit     | CRISPR              | PDS           | Albino phenotype                                                                          | Reporter                        | 198       |
| *Salvia miltiorrhiza*   | Medicinal plant | CRISPR              | SmCPS1        | Tanshinone biosynthesis                                                                    | Metabolism                      | 199       |
| *Camelina sativa*       | Vegetable | CRISPR              | FAE1          | Reduced long-chain fatty acids                                                            | Metabolism                      | 77        |
| Species     | Crop type | Genome editing tool | Targeted gene          | Gene function or phenotype                              | Classification of targeted gene | Reference |
|-------------|-----------|---------------------|------------------------|---------------------------------------------------------|--------------------------------|-----------|
| Camelina sativa | Vegetable | CRISPR              | CsDGAT1 or CsPDAT1     | Altered fatty acid composition and reduced oil content  | Metabolism                      | 200       |
| Camelina sativa | Vegetable | CRISPR              | FAD2                   | Reduced levels of polyunsaturated fatty acids           | Metabolism                      | 78        |
| Camelina sativa | Vegetable | CRISPR              | FAD2                   | Decreased polyunsaturated fatty acids                   | Metabolism                      | 79        |
| Malus pumila   | Fruit     | CRISPR              | PDS, TFL1.1            | Albino phenotype, early flowering                       | Development                     | 201       |
| Malus pumila   | Fruit     | CRISPR              | PDS                    | Albino phenotype                                        | Reporter                        | 183       |
| Malus pumila   | Fruit     | CRISPR              | PDS                    | Albino phenotype                                        | Reporter                        | 202       |
| Malus pumila   | Fruit     | CRISPR              | DIPM                   | Blight resistance                                       | Stress response                 | 185       |
| Malus pumila   | Fruit     | ZFN                 | udiA                   | Edited reporter gene                                    | Others                          | 203       |
| Pyrus communis | Fruit     | CRISPR              | TF1L1.1                | Early flowering                                         | Development                     | 201       |
| Daucus carota  | Vegetable | CRISPR              | PDS, MYB13-like        | Albino phenotype                                        | Reporter                        | 204       |
| Daucus carota  | Vegetable | CRISPR              | F3H                    | Altered anthocyanin biosynthesis                       | Metabolism                      | 205       |
| Torenia fournieri | Flower      | CRISPR              | F3H                    | Altered flower pigmentation                             | Metabolism                      | 206       |
| Fragaria vesca | Fruit     | CRISPR              | FveTAA1, FveARF8       | Auxin signaling, plant development                      | Development                     | 207       |
| Fragaria vesca, Fragaria x Ananassa | Fruit | CRISPR              | FvMYB10, FvCHS         | Anthocyanin biosynthesis                                | Metabolism                      | 208       |
| Fragaria x Ananassa | Fruit | CRISPR              | FaTM6                  | Anther development                                      | Development                     | 209       |
| Fragaria vesca, Fragaria x Ananassa | Fruit | CRISPR              | PDS                    | Albino phenotype                                        | Reporter                        | 210,211  |
resulting in new tomato varieties with the desirable properties of cultivated tomato combined with the favorable traits of the wild species. Mutations can generally be introduced in either the coding region or the cis-regulatory region of the targeted gene, and mutations in the cis-regulatory region could be used to generate quantitative variation for breeding selection. In tomato, for example, fruit locule number is determined by several naturally occurring mutations in the cis-regulatory regions of CLAVATA-WUSCHEL. This finding motivated researchers to design a multiplexed CRISPR/Cas9 system targeting the CLAVATA-WUSCHEL promoters to generate tomato lines with a wide range of locule numbers. Quantitative variations have also been observed when the genes responsible for inflorescence and plant architecture are engineered. In addition to regulating gene activity by editing the DNA sequence of the cis-regulatory region, gene activity can be regulated by the its epigenetic status of this region. By integrating genome editing (CRISPR/Cas9) with epigenetic regulation, researchers are able to target a gene of interest and modify its epigenetic status. For instance, an sgRNA-guided fusion protein between the dead Cas9 (dCas9) variant and the catalytic domain of the TEN-ELEVEN TRANSLOCATION1 (TET1cd) demethylase can remove 5mC at specific sites, thereby increasing gene expression. An epigenetic mutant can also be crossed with the corresponding wild type to generate epigenetic recombinant inbred lines (epiRILs). Individuals from these populations are genetically identical but epigenetically distinct. Such populations have been constructed in Arabidopsis and exhibit considerable phenotypic variation. These examples demonstrate that genome editing is an excellent tool for producing new alleles and epialleles, which are important sources of phenotypic variation for crop improvement.

Challenges and future perspectives for the improvement of horticultural crops through genome editing

Although genome editing has many advantages over conventional crop breeding, some challenges remain for its application to horticultural crops. In horticultural crops, molecular and genetic studies are difficult, which hinders the identification of genes responsible for desirable traits. Sequencing the genomes of horticultural crops of interest will be important for identifying genes associated with desirable traits. For crops lacking a reference genome, the target sequence could be cloned by using degenerate primers designed for conserved protein motifs with putative functions related to desirable traits. A good example is the mildew-resistance locus (MLO), which has been characterized in detail in barley; the phylogenetically conservative nature of the MLO has facilitated the generation of powdery mildew-resistant plants in wheat, tomato, and strawberry.

Once a gene to be edited has been identified, researchers must take into account the methods used to deliver editing reagents and the procedure for regenerating the edited mutants. To date, more than 25 horticultural plant species have been successfully edited (Table 2), usually with editing reagents delivered via Agrobacteria or virus systems, and the edited plants are regenerated via in vitro tissue culture. Although tissue culture-based transformation and regeneration is most widely used for genome editing, no well-established protocol for transformation and regeneration from tissue culture is available for many horticultural crops. In planta transformation, which is an alternative to in vitro tissue culture-based Agrobacterium transformation, refers to the infection of in vivo explants in which the targeted tissues are apical or auxiliary meristems, stigmas, pollen, or inflorescences. This method has been successfully used to transform tomato and Brassica species and should be further explored for use in horticultural crops that are recalcitrant to traditional genetic transformation. Additionally, successful genetic transformation of horticultural crops requires the consideration of editing efficiency, which is affected by many factors, such as sgRNA number and GC content, the expression levels of sgRNA and Cas9, and the secondary structure of the paired sgRNA and target sequence. In the future, the editing system should be further optimized in different crop species.

The elimination of foreign DNA fragments (transferred T-DNAs) to obtain transgene-free edited plants remains difficult in some highly heterozygous and clonally propagated horticultural species, such as potato, sweet potato, and banana. One possibility is to generate many transformants, followed by high-throughput screening of transgene-free mutants. This approach has been used to generate ~10% of mutants without foreign DNA. Another approach for transgene-free genome editing is to deliver editing reagents as in vitro transcripts or ribonucleoproteins.

In conclusion, mutagenesis via genome editing outperforms spontaneous and induced mutations in terms of precision and efficiency. Although this technology is being increasingly used in many crops, its widespread use in the breeding of horticultural crops will require three challenges to be surmounted. First, clear breeding traits of the horticultural crop in question should be identified via communication among consumers, breeders, and biologists. Second and third, suitable methods must be developed for delivering editing reagents and for subsequently regenerating mutants. Given the great potential of genome editing and the importance of horticultural crops, we expect that these challenges will be overcome in the near future.
Conflict of interest
The authors declare that they have no conflict of interest.

Received: 25 April 2019 Revised: 18 July 2019 Accepted: 13 August 2019
Published online: 08 October 2019

References
1. Ravichandra, N. G. Horticulture and its role in the national economies. Horticultural Nematology (pp. 1–3. Springer, India, 2014).
2. Janick, J. Horticultural plant breeding: past accomplishments, future directions. Acta Hortic. 694, 61–65 (2005). https://doi.org/10.17660/ ActaHortic.2005.694.6.
3. Khoury, C. K. et al. Increasing homogeneity in global food supplies and the implications for food security. Proc Natl Acad Sci USA 111, 4001–4006 (2014).
4. Oladosu, Y. et al. Principle and application of plant mutagenesis in crop improvement: a review. BioTechnol. Biotechnol. Equip. 30, 1–16 (2016).
5. Ahloowalia, B. S. & Maluszynski, M. Induced mutations for genetic improvement. Hort Science 44, 816–821 (2013).
6. Seligman, L. M. et al. Mutations altering the cleavage specificity of host factor RNAse III. Nature 471, 652–657 (2011).
7. Gasiunas, V. et al. CRISPR-Cas9 ribonuclease complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A 109, E2579–E2586 (2012).
8. Shaveh, H. et al. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. J. Bacteriol. 190, 1390–1400 (2008).
9. Brouns, S. J. J. & Mali, P. W. CRISPR RNA-guided Antiviral Defense in Prokaryotes. Science 321, 960–964 (2008).
10. Deltcheva, E. et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNAse III. Nature 471, 652–657 (2011).
11. Voytas, D. F. Plant genome engineering with sequence-specific nucleases. Nature 471, 652–657 (2011).
12. Christiansen, H. et al. Zinc-finger nuclease engineering by context-dependent assembly (CoDA). Nat. Methods. 8, 67–69 (2011).
13. Maeder, M. L. et al. Rapid “open-source” engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol. Cell 31, 294–301 (2008).
14. Kay, S., Hahn, S., Maros, E., Hause, G. & Bonas, U. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. Science 318, 648–651 (2007).
15. Puchta, H. The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. Cell 163, 629–640 (2015).
16. Xu et al. Molecular Plant. 6, 821–826 (2013).
17. Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816–821 (2012).
18. Mali, P. et al. RNA-Guided Human Genome Engineering via Cas9. Science 339, 823–826 (2013).
19. Cong, L. et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. Science 339, 819–823 (2013).
20. Feng, Z. et al. Efﬁcient genome editing in zebraﬁsh using a CRISPR-Cas system. Nat Biotechnol. 31, 227–229 (2013).
21. Liu, J.-F. et al. Multiplex and homologous recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using Cas9 RNA-guided endonuclease. Nat Biotechnol. 31, 691–693 (2013).
22. Zhang, H. et al. Genome editing-principles and applications for functional genomics research and crop improvement. Crit. Rev. Plant Sci. 36, 291–309 (2017).
23. Saedler, H. et al. Zinc-finger nucleases: Custom-designed molecular scissors for genome engineering of plant and mammalian cells. Nucleic Acids Res. 33, 5978–5990 (2005).
24. Wright, D. A. et al. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. Plant J. 44, 693–705 (2005).
25. Wright, J. et al. Targeting DNA double-strand breaks with TAL effector nucleases. Genetica 135, 756–761 (2010).
26. Sander, J. D. et al. Selection-free zinc-finger-nuclease engineering by context-dependent assembly (CoDA). Nat. Methods. 8, 67–69 (2011).
27. Knott, G. J. & Doudna, J. A. CRISPR-Cas guides the future of genetic engineering. Science 361, 866–869 (2018).
28. Komor, A. C., Kim, Y. B., Fakler, M. S., Zuris, J. A. & Liu, D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533, 420–424 (2016).
57. Kim, Y. B. et al. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nat. Biotechnol.* **35**, 371–376 (2017).

58. Gaudelli, N. M. et al. Programmable base editing of A to G in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 (2017).

59. Hua, K. Tao, X. Yuan, F. Wang, D. & Zhu, J. K. Precise A/G to GC Base Editing in the Rice Genome. *Mol. Plant.** 11**, 627–630 (2018).

60. Boglioli, E. & Richard, M. Rewriting The Book Of Life: A New Era in Precision Gene Editing. (2015).

61. Karkute, S. G., Singh, A. K., Gupta, O. P., Singh, P. M. & Singh, B. CRISPR/Cas9 mediated genome engineering for improvement of horticultural crops. *Front. Plant Sci.* **8**, 1–6 (2017).

62. Sun, Z. et al. Site-Specific Gene Targeting Using Transcription Activator-Like Effector (TALE)-Based Nuclease in *Brassica oleracea*. *Integr. Plant Biol.* **55**, 1092–1103 (2013).

63. Xu, C., Park, S. J., Van Eck, J. & Lippman, Z. B. Control of inflorescence architecture in tomato by BT87/PQ2 transcriptional regulators. *Genes Dev.* **30**, 2048–2061 (2016).

64. Soyka, S. et al. Mutagenization in the flowering gene SELF PRUNING 3 in soybean. *Nat. Genet.* **49**, 162–168 (2017).

65. Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E. & Lippman, Z. B. Engineering quantitative trait variation for crop improvement by genome editing. *Cell** 171**, 470–480.e8 (2017).

66. Lang, Z. et al. Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit. *Proc. Natl. Acad. Sci. USA** 114**, E4511–E4519 (2017).

67. Yang, Y. et al. The RNA editing factor SIORRM4 is required for normal fruit ripening in tomato. *Plant Physiol.* **175**, pp.01265.2017 (2017).

68. Ito, Y. et al. Re-evaluation of the ir mutation and the role of RN in the induction of tomato ripening. *Nat. Plants** 3**, 566–574 (2017).

69. Zsögön, A. et al. De novo domestication of wild tomato using genome editing. *Nat. Biotechnol.* **36**, 1211–1216 (2018).

70. Li, T. et al. Domestication of wild tomato is accelerated by genome editing. *Nat. Biotechnol.* **36**, 1160–1163 (2018).

71. Clasen, B. M. et al. Improving cold storage and processing traits in tomato through targeted gene knockdown. *Plant Biotechnol. J.* **14**, 169–176 (2016).

72. Ye, M. et al. Generation of self-compatible diploid potato by knock-out of S-Rhase. *Nat. Plants** 4**, 651–654 (2018).

73. Peng, A. et al. Engineering canker-resistant plants through CRISPR/Cas9 targeted editing of the susceptibility gene *ClO81* in citrus. *Plant Biotechnol. J.* **15**, 1509–1519 (2017).

74. Jia, H. et al. Genome editing of the disease susceptibility gene *ClO81* in citrus confers resistance to citrus canker. *Plant Biotechnol. J.* **15**, 817–823 (2017).

75. Wang, X. et al. CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. *Plant Biotechnol. J.* **16**, 844–855 (2018).

76. Okuza, A. et al. CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica rapa*. *Plant Physiol. Biochem.* **131**, 63–69 (2019).

77. Onosya, M. E., Kang, J., Mu, X. & Lu, C. Mutagenesis of the tomato fruit. *Cell** 2048, 1148 (2014).

78. Niazian, M., Sadatmoori, S. A., Galuszka, P. & Mortazavian, S. M. S. M. Tissue culture-based *Agrobacterium*-mediated and in plants transformation methods. *Front. Cell. Plant Genet.* **53**, 133–143 (2017).

79. Shah, S. H., Ali, S., Jan, S. A., Jalal-Uddin & Ali, G. M. Piercing and incubation method of in planta transformation producing stable transgenic plants by overexpressing *DREB1A* gene in tomato (*Solanum lycopersicum Mill*). *Plant Tissue Organ Cult.* **120**, 1139–1157 (2015).

80. Verma, S. S., Chinnusamy, V. & Bansal, C. A. Simplified floral dip method for transformation of *Brassica napus* and B. *campestris*. *J. Plant. Biochem. Biotechnol.* **17**, 197–200 (2008).

81. Hu, N. et al. Rapid and user-friendly open-source CRISPR/Cas9 system for single- or multi-site editing of tomato genome. *Hortic. Res.* **6**, 7 (2019).

82. Kumeleme, J., Pietralia, J., Hensel, G., Pacher, M. & Puchta, H. The CRISPR/Cas revolution continues: From efficient gene editing for crop breeding to plant synthetic biology. *J. Integr. Plant Biol.* **60**, 1127–1153 (2018).

83. Nadakuduti, S. S., Buell, C. R., Voytas, D. F., Starker, C. G. & Douches, D. S. Genome editing for crop improvement – applications in clonally propagated polyplids with a focus on potato (*Solanum tuberosum L*). *Front. Plant Sci.* **9**, 1607 (2018).

84. Chen, L. et al. A method for the production and expedient screening of CRISPR/Cas9-mediated non-transgenic mutant plants. *Hortic. Res.* **5**, 13 (2018).

85. Veillet, F. et al. Transgene-free genome editing in tomato and potato plants using *agrobacterium*-mediated delivery of a CRISPR/Cas9 cytokine base editor. *Int. J. Mol. Sci.* **20**, 402 (2019).

86. Zhang, Y. et al. Efficient and transpose-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nat. Commun.* **7**, 12617 (2016).

87. Danila, B. et al. Efficient and transpose-free gene targeting using Agrobacterium-mediated delivery of the CRISPR/Cas9 system in tomato. *Plant Cell Rep.* **38**, 459–462 (2019).

88. Ortigosa, A., Grimenez-Ibanez, S., Leonhardt, N. & Solano, R. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of *SIA22*. *Plant Biotechnol. J.* **17**, 665–673 (2019).

89. Wang, R. et al. Re-evaluation of transcription factor function in tomato fruit development and ripening with CRISPR/Cas9-mutagenesis. *Sci. Rep.* **9**, 1696 (2019).

90. Wang, D. et al. Characterisation of CRISPR mutants targeting genes modulating pectin degradation in ripening tomato. *Plant Physiol.* **pp.01187.2018**, https://doi.org/10.1104/pp.18.01187 (2018).

91. Li, R. et al. CRISPR/Cas9-Mediated *SINPR1* mutagenesis reduces tomato plant drought tolerance. *BMC Plant Biol.* **19**, 88 (2019).

92. Tomlinson, L. et al. Using CRISPR/Cas9 genome editing in tomato to create a gibberellin-responsive dominant dwarf DELA allele. *Plant Biotechnol. J.* **17**, 132–140 (2019).

93. Ding, F., Wang, M. & Zhang, S. Sedoheptulose-1,7-Bisphosphatase is Involved in Methyl Jasmonate- and Dark-Induced Leaf Senescence in Tomato Plants. *Int. J. Mol. Sci.* **19**, 3673 (2018).

94. Li, R. et al. Reduction of tomato-plant chilling tolerance by CRISPR-Cas9-mediated *SICEB1* mutagenesis. *J. Agric. Food Chem.* **66**, 9042–9051 (2018).
Ma, C. et al. CRISPR/Cas9-mediated multiple gene editing in Brassica oleracea var. capitata using the endogenous tRNA-processing system. *Hortic. Res.* **6**, 20 (2019).

Hu, L. et al. Promoter variations in a homeobox gene, Bn1MLO111, determine lobed leaves in raptured (*Brassica napus* L.). *Theor. Appl. Genet.* **131**, 2699–2708 (2018).

Murovecj, J., Galčík, K., Bohnaneck, B., Avbelj, M. & Jeralja, R. DNA-Free Genome Editing of Brassica oleracea and *B. rapa* Protoplasts Using CRISPR/Cas9 Ribonuclease Iproteins Complexes. *Front. Plant Sci.* **9**, 1594 (2018).

Kirchner, T. W. et al. Molecular background of Pi deficiency-induced root hair growth in *Brassica campestris* - a fasciin-like arabinogalactan protein is involved. *Front. Plant Sci.* **9**, 1372 (2018).

Sun, Q. et al. CRISPR/Cas9-mediated multiplex genome editing of the BnMYC1 and BnMYC201 genes in *Brassica napus* L. *Int. J. Mol. Sci.* **19**, 2716 (2018).

Jiang, L. et al. Histone lysine methyltransferases BnsSOS3A and BnsSOS3C are involved in the floral transition in *Brassica napus*. *Plant J.* **95**, 672–685 (2018).

Yang, Y. et al. Precise editing of CLAVATA genes in *Brassica napus* L. regulates multicellular silique development. *Plant Biotechnol.* **16**, 1332–1335 (2018).

Zhang, Y. et al. Defective APETALA2 genes lead to sepal modification in *Brassica* Crops. *Front. Plant Sci.* **9**, 367 (2018).

Yang, H., Wu, J.-J., Tang, T., Liu, K.-D. & Dai, C. CRISPR/Cas9-mediated genome editing efficiently creates specific mutations at multiple loci using one sgRNA in *Brassica napus* L. *Plant Sci.* **142**, 1–8 (2018).

Kirchner, T. W., Niehaus, M., Debener, T., Schenk, M. K. & Herde, M. Efficient generation of mutations mediated by CRISPR/Cas9 in the hairy root transformation system of *Brassica campestris*. *PLoS ONE* **12**, e0185429 (2017).

Braatz, J. et al. CRISPR-Cas9 targeted mutagenesis leads to simultaneous oral transition in *Brassica napus*. *Front. Plant Sci.* **9**, 1025 (2018).

Bertier, L. D. et al. High-resolution analysis of the expression system of *Brassica carinata*. *Plant Biotechnol. J.* **16**, 7489–7497 (2018).

Kurai, L. et al. Building a genetic manipulation tool box for orchid biology: identification of constitutive promoters and application of CRISPR/Cas9 in the Orchid, *Dendrobium officinale*. *Front. Plant Sci.* **7**, 2036 (2016).

Charrier, A. et al. Efficient CRISPR/Cas9-based gene knockout in watermelon. *Plant Cell Rep.* **37**, 1353–1356 (2018).

Tian, S. et al. Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. *Plant Cell Rep.* **37**, 1–9 (2018).

Charron, A. et al. Efficient targeted mutagenesis in apple and first time edition of pear using the CRISPR-Cas9 system. *Front. Plant Sci.* **10**, 40 (2019).

Nihtianu, C. et al. Efficient genome editing in apple using a CRISPR/Cas9 system. *Sci. Rep.* **6**, 31481 (2016).

Peer, R. et al. Targeted mutagenesis using zinc-finger nucleases in perennial fruit trees. *Planta* **241**, 941–951 (2013).

Xu, Z.-S., Feng, K. & Xiong, A.-S. CRISPR/Cas9-mediated multiple targeted mutagenesis in orange and purple fruit cantaloupe. *Mol. Biotechnol.* **61**, 191–199 (2019).

Klimk-Chodacka, M., Oleszkiewicz, T., Lowder, L. G., Qi, Y. & Baranski, R. Efficient CRISPR/Cas9-based genome editing in carrot cells. *Plant Cell Rep.* **37**, 575–586 (2018).

Nishihiro, M., Higuchi, A., Watanabe, A. & Tasaki, K. Application of the CRISPR/Cas9 system for targeted genome mutagenesis in *Petunia* (Carvata) (2017). e89086 (2014).

Watanabe, K. et al. Gene editing the phytoene desaturase alleles of Cavendish banana (*Musa* cv. *AAA*). *Sci. Rep.* **7**, 10028 (2017).

Watanabe, K., Oda-Yamazico, C., Sage-Ono, K., Ohmiya, A. & Ono, M. Alteration of flower colour in (*Musa* cv. *AAA*) through CRISPR/Cas9-mediated mutagenesis of *carotenoid cleavage dioxygenase 4*. Transgenic Res. https://doi.org/10.1007/s12148-017-0051-0 (2017).

Sun, L. & Kao, T.-H. CRISPR/Cas9-mediated knockout of *PSK1* reveals essential role of S-locus F-box protein-encoding *SFC* complexes in recognition of non-self S-RNases during cross-compatible pollination in self-incompatible *Petunia inflata*. *Plant Reprod.* https://doi.org/10.1007/s00497-017-0914-7 (2017).

Zhang, B., Yang, X., Yang, C., Li, M. & Guo, Y. Exploiting the CRISPR/Cas9 system for targeted genome mutagenesis in *Petunia*. *Sci. Rep.* **6**, 20315 (2016).

Charron, A. et al. Efficient targeted mutagenesis in apple and first time edition of pear using the CRISPR-Cas9 system. *Front. Plant Sci.* **10**, 40 (2019).

Nihtianu, C. et al. Efficient genome editing in apple using a CRISPR/Cas9 system. *Sci. Rep.* **6**, 31481 (2016).

Peer, R. et al. Targeted mutagenesis using zinc-finger nucleases in perennial fruit trees. *Planta* **241**, 941–951 (2013).

Xu, Z.-S., Feng, K. & Xiong, A.-S. CRISPR/Cas9-mediated multiple targeted mutagenesis in orange and purple fruit cantaloupe. *Mol. Biotechnol.* **61**, 191–199 (2019).

Klimk-Chodacka, M., Oleszkiewicz, T., Lowder, L. G., Qi, Y. & Baranski, R. Efficient CRISPR/Cas9-based genome editing in carrot cells. *Plant Cell Rep.* **37**, 575–586 (2018).

Nishihiro, M., Higuchi, A., Watanabe, A. & Tasaki, K. Application of the CRISPR/Cas9 system for modification of flower color in *Torenia fournieri*. * BMC Plant Biol.* **18**, 331 (2018).

Zhou, J., Wang, G. & Liu, Z. Efficient genome editing of wild strawberry genes, vector development and validation. *Plant Biotechnol.* **16**, 1151–1162 (2019).

Xing, S. et al. CRISPR/Cas9-introduced single and multiple mutagenesis in strawberry. *J. Genet. Genomics* **45**, 685–687 (2018).

Martin-Pizarro, C., Triviño, J. C. & Pézé, D. Functional analysis of the *TaMADS* box gene in the octoploid strawberry by CRISPR/Cas9-directed mutagenesis. *J. Exp. Bot.* **70**, 885–889 (2019).

Wilson, F. M., Harrison, K., Armitage, A. D., Simkin, A. J. & Harrison, R. J. CRISPR/Cas9-mediated mutagenesis of phytoene desaturase in diploid and octoploid strawberry. *Plant Methods* **15**, 45 (2019).

Ren, X. et al. Enhanced specificity and efficiency of the CRISPR/Cas9 system with optimized sgRNA parameters in *Drosophila* *Cell Rep.* **9**, 1151–1162 (2014).