Molecular Biotechnology
https://doi.org/10.1007/s12033-024-01290-8

REVIEW PAPER

# CRISPR-Cas9: Unraveling Genetic Secrets to Enhance Floral and Fruit Traits in Tomato

**S. Bhoomika[1] · Shubham Rajaram Salunkhe[1] · A. R. Sakthi[1] · T. Saraswathi[2] · S. Manonmani[3] · M. Raveendran[1] · M. Sudha[1]**

Received: 2 August 2024 / Accepted: 17 September 2024


## Abstract

Tomato, a globally consumed vegetable, possesses vast genetic diversity, making it suitable for genetic manipulation using various genetic improvement techniques. Tomatoes are grown extensively for their market value and health benefits, primarily contributed by enhanced yield and nutritional value respectively, influenced by floral and fruit traits. Floral morphology is maintained by genes involved in meristem size control, regulation of inflorescence transition, and pollen development. *SP* (*SELF-PRUNING*) and *SP5G* (*SELF-PRUNING 5G*) determine growth habit and flowering time. *RIN* (*RIPENING INHIBITOR*) and *PG* (*POLYGALACTURONASE*) are responsible for the shelf life of fruits. In addition to this, nutrition-enriched tomatoes have been developed in recent times. In this review, we comprehensively discuss the major genes influencing floral morphology, flowering time, fruit size, fruit shape, shelf life, and nutritional value, ultimately resulting in enhanced yield. Additionally, we address the advances in CRISPR/Cas9 applied for the genetic improvement of tomatoes along with prospects of areas in which research development in terms of tomato genetic improvement has to be advanced.

**Keywords** Genome editing · Inflorescence · Architecture · Shelf life · Health

✉ M. Sudha
sudhatamil@gmail.com

S. Bhoomika
bhoomika2921@gmail.com

Shubham Rajaram Salunkhe
shubhamsalunkhe254@gmail.com

A. R. Sakthi
arsakthy@gmail.com

T. Saraswathi
saravel_t@yahoo.co.in

S. Manonmani
manonmanitnau@gmail.com

M. Raveendran
raveendrantnau@gmail.com

[1] Department of Plant Biotechnology, Centre of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India

[2] Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641003, India

[3] Department of Rice, Centre of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India

## Introduction

Tomato (*Solanum lycopersicum* L.,), is a major vegetable crop belonging to *Solanaceae* grown worldwide. Although it originated in South America, tomatoes are now the second most cultivated vegetable with 189 million tonnes grown on a 5.16 Mha land area over the world [1]. It is widely grown owing to its nutritional and economic benefits. The nutritional value of tomatoes is very high, because they accumulate several secondary metabolites which is very much necessary for a complete human diet [2]. Tomatoes contain bioactive compounds like natural antioxidant named lycopene and alpha-tomatine which has antimicrobial, anticancer, and cholesterol-lowering properties [3]. Besides lycopene and tomatine, tomato also provides antioxidants like ß-carotene, nutrients like iron, potassium, folate, vitamin C, and phenolic compounds, such as flavonoids, hydroxycinnamic acid, chlorogenic, homovanillic acid, and ferulic acid [4–6]. Also, the beneficial effects on health including antioxidant, anti-inflammatory, and anti-atherogenic properties, reducing the risk of cardiovascular diseases, have been extensively studied in tomatoes [7, 8]. Economically, tomatoes play a major role in agricultural production and the food

Published online: 08 October 2024

industry. It is a high-market-value crop that supports the livelihoods of millions of people. In addition, tomatoes play a predominant part in the processing industry that attracts consumers globally. Hence, new tomato cultivars need to be bred taking the current consumer demand and processing industry requirements into account and this requires a wide genetic resource comprising several valuable traits [9]. The yield and fruit quality should be improved by genetic improvements to meet the current trend.

Many diverse cultivars of tomato have been developed since the domestication. The genetic diversity of tomatoes is vast and they are spread throughout a wide variety of habitats [10]. The Tomato Genome Consortium in 2012 sequenced the 900 Mb tomato genome creating a forward leap in genetic improvement studies, allowing a better understanding of its genetics. The Solanaceae genome network (SGN) and Tomato Genetic Resource Center (TGRC) contain extensive collections of genotypes and mutants of tomatoes, including genome sequences, gene annotations, and genetic maps which can be assessed by researchers [11–14]. In addition to this, there are several databases like the Tomato Functional Genomics Database (TFGD), Tomato Mutant Database (Mutant DB), the World Vegetable Centre (AVRDC) Tomato Genetic Resources Database, European Variation Archive (EVA), and others that constitute to be useful research resource. Several improved cultivars are being developed by exploiting these resources with the help of genetic advancements in technology. Plants with altered growth habits [15, 16], increased flowering [16], and Vitamin-D-enriched tomatoes [17] are a few among many examples. In this review, we provide a brief overview of the genes responsible for flowering and fruiting traits in tomatoes (Fig. 1) and genetic modifications made by genome editing.

## Flowering and Fruiting Habit of Tomato

Tomato being a self-pollinated crop, has its stigma positioned within the anther cone of the pendant flower. However, cross-pollinated is also facilitated to improve genetic variability. There are two recognized growth habits: determinate and indeterminate growth. The flowers are perfect and hypogynous growing on the short pedicle of the cyme inflorescence. After pollination, fruits develop from the ovules after progressing through several growth stages, including cell division, expansion, and ripening, which spans weeks to months. Berries have many light brown to golden yellow seeds with 2–4 locules and green fruits ripen to become orange, red, or yellow. Tomato plants are categorized based on their fruiting habit, fruit shape, and presence/absence of ridges on fruits [18]. The size and shape of the fruits can vary significantly from small to large size and round to variable shapes [19]. Several genes influence the flowering and fruiting habit in tomatoes.

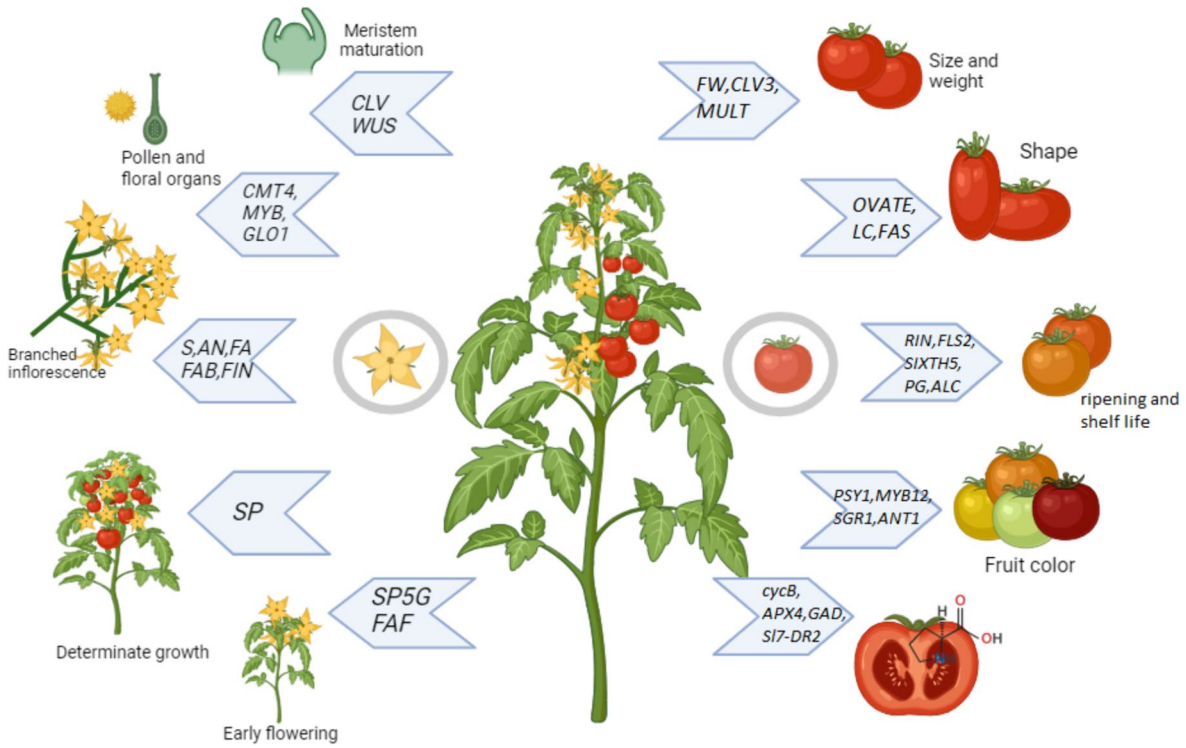


**Fig. 1** Depiction of genes involved in regulating floral and fruit traits

## Approaches for Genetic Improvement in Tomatoes

There are several approaches to crop improvement, of which breeding is considered as efficient tool. Conventional breeding has long been used to develop plants by crossing individual plants with desired targets [20]. However, the genetic diversity of progeny is narrow. Additionally, the possibility of targeting multiple traits is not possible [21]. As technology advances, several breeding methods like pedigree method, hybridization, mutation breeding, pure line selection, mass selection, heterosis breeding, and backcross method have been developed [19]. Next is the introduction of novel genetic alleles by induced mutagenesis. But this resulted in random off-target mutations. Hence to increase the specificity of mutating target genes, genetic engineering approaches were introduced where specific genes responsible for desired traits can be modified [22]. Breeding techniques were also used in combination with genetic engineering technologies, such as marker-assisted selection and molecular breeding [23].

Gene editing tools like RNA interference, ZFN, TALEN, and CRISPR/Cas have been developed to facilitate precise modifications [24]. Virus-induced gene silencing can be used to silence the expression of the target gene relating to stress response and disease resistance (Wang et al.,). Zinc-finger nucleases and Transcription activator-like effector nucleases [25] are seldom used compared with CRISPR/Cas systems. CRISPR/Cas9 system is an adaptive immune system naturally within the bacteria and archaea [26]. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technique uses a single-guide RNA to target the specific location in the desired gene to navigate the Cas9 (CRISPR-Associated Protein 9) which has a nuclease role and creates double-stranded breaks in the genomic sequence. Then, the natural repair mechanisms like NHEJ (Non-Homologous End Joining) or HR (Homologous Recombination) are activated to repair the DNA and create desired mutation [27]. CRISPR/Cas9 is the most flexible, simpler to design and implement, has higher target efficiency, is less expensive, and is easier to handle [27, 28].

## Genes Influencing Floral Morphology and Architecture and its Modification through CRISPR

Flowering time, rate, and pattern influence fruit yield. The flowering architecture in tomatoes is sympodial, where the main stem terminates with flowering as the shoot apical meristem on induction gives rise to an inflorescence meristem, which further evolves into floral branches known as sympodial branches, which also terminate with flowering and this pattern continues [29]. These sympodial flowers mature at varied times, resulting in staggered production of fruits. The various floral trait-related genes modified by CRISPR is mentioned in Table 1.

### Inflorescence Architecture and Floral Morphology

Several genes upregulate or downregulate the flowering pattern in tomatoes. Shoot meristems in tomatoes are regulated by arabinosyltransferase genes. The peptides involved in gene signaling pathway of *CLV* (*CLAVATA*) has to be arabinosylated and its proper interaction with *WUSCHEL* is completely required to maintain the meristem size. However, mutations in this gene along with *FAB* (*FASCIATED AND BRANCHED*) and *FIN* (*FASCIATED INFLORESCENCE*) involved in *CLV* pathway result in enlarged meristems, which leads to excess branching in shoot and inflorescence [30]. Also, *SlCLV3* was downregulated by over-expressing *SlLET6* and *SlGIF1*

**Table 1** Manipulation of flowering genes using CRISPR/Cas9

| Cultivar | Target gene | Locus ID | Effect caused | References |
|---|---|---|---|---|
| *Solanum pimpinellifolium* | *SP* | *Solyc06g074350* | Results in determinate plant growth | [101] |
| M82 | *SP5G* | *Solyc05g053850* | Induces early flowering | [16] |
| *Solanum pimpinellifolium* | *SP and SP5G* | *Solyc06g074350* *Solyc05g053850* | Results in determinate plant growth, increased compactness, early and uniform flowering, synchronous ripening of fruits, suitable for mechanical harvest | [101] |
| M82 | *BOP1, BOP2, BOP3* | | Over-expression leads to fusion of floral organs | [39] |
| *Solanum pimpinellifolium* | *CLV3* | *Solyc11g071380* | Enlargement of meristems, increased branching with fasciated flowers | [30] |
| Ailsa Craig | *FAF1, FAF2, FAF3, FAF4* | | Upregulation of *SlFAF1/2b* results in early flowering Downregulation in *SlFAF1/2b*, *SlFAF3/4a* and *SlFAF3/4b* results in early flowering | [40] |

*SP* self-pruning, *BOP* blade-on-petiole, *CLV* clavata, faf fantastic four

(*GRF1-interacting factor 1*), resulting in increased number of carpels in flowers [31] The compound inflorescences are formed as a result of mutation in *S* (*COMPOUND INFLORESCENCE*), *AN* (*ANANTHA*), and *FA* (*FALSIFLORA*) genes [32]. The loss-of-function mutation in *S* (*COMPOUND INFLORESCENCE*) causes delayed meristem maturation. Deletion mutation in *S*, *AN,* and *FA* causes additional branching in inflorescence causing the flowers to be converted to secondary buds [31, 32].

*UNIFLORA* gene has an impact on the inflorescence meristem and its transition to flowers. Studies revealed that mutants of the *UNIFLORA* gene produced a single flower instead of the inflorescence. However, this gene hasn't been mapped and requires further exploration (Dielen et al.,). The knock-out of *SlCMT4* (*Solanum lycopersicum Chromomethylase*) (Solyc08g005400.2) through CRISPR/Cas9 resulted in the induction of two genes Sl*PMEI* (*Solanum lycopersicum Pectin Methyl Esterase Inhibitor*) and *PRALF* (*Pollen-specific Rapid Alkanization Factor*), which causes pollen wall and pollen tube degeneration. The mutants were found to have defective floral morphology with irregular pollen grain and stamen morphology resulting in reduced fruit set, seed number per fruit, and germination rate [33]. Hence, *SlCMT4* positively regulates floral architecture. Similarly, *SlMYB33* (*GAMYB Like gene*) (Solyc06g073640) is a gene that is crucial for pollen development. Studies show that *SlMYB33* on silencing delays flowering and knocking down this gene results in degenerative pollen [34]. *SlGLO1* (*Solanum lycopersicum GLOBOSA*) is a MADS-box protein gene that is expressed in petals and stamens. On silencing this gene, the plants produced are male sterile with stamen to carpel conversion and petal to sepal conversion [35]. *SlMYB72*, expressed in pollen and tapetum, on downregulation by RNA interference, activates the autophagy-related *SlATG7* resulting in inhibition of seed formation as the pollen development is affected [36].

*STM3* (*SISTER OF TM3*) positively regulates the *FUL1* (*FRUITFUL Like*) gene to enhance floral architecture. A mutation in *STM3* downregulates *FUL1*, thereby affecting flowering time and inflorescence morphology [37]. *SlMIR172* (*Solanum lycopersicum microRNA 172*) suppresses *APETALLA2 Like* transcriptional factors posttranscriptionally and regulates flowering time and morphology. CRISPR/Cas9-mediated knock-out of *SlMIR172c* and *SlMIR172d* developed plants with abnormal graded flowers where stamen turned to sepals, thereby indicating its role in maintaining the identity of floral organs [38]. Expression of *SlBOP* (*BLADE-ON-PETIOLE*) occurs during the maturation of meristem and CRISPR/Cas9-generated mutants of these genes had simplified inflorescences yielding single flowers similar to *tmf* (*TERMINATING INFLORESCENCE*) mutants due to its interaction with *TMF* transcriptional factor genes [39].

## Early and Enhanced Flowering

*FAF* (*FANTASTIC FOUR*) family of genes comprising *FAF1, FAF2, FAF3*, and *FAF4* are expressed consecutively during the evolution of shoot apical meristem. It is concluded that it is related to the regulation of flowering time, as early flowering is induced if *SlFAF1/2a* is over-expressed. Flowering is positively regulated by *SlFAF1/2c,* whereas *SlFAF1/2b, SlFAF3/4a,* and *SlFAF3/4b* when negatively regulated induces early flowering [40].

Six *FT* (*FLOWERING LOCUS T*) like genes were identified in tomatoes. On analyzing their role in flowering, *SlSP5G, SlSP5G2*, and *SlSP5G3* repressed flowering, whereas *SlSP3D/SFT* induced flowering. Under long-day conditions, *SlSP5G* was expressed. Contrastingly, *SlSP5G2* and *SlSP5G3* were expressed in short-day conditions. Silencing of these genes led to early flowering under their respective conditions. Phytochrome gene *PHYB1* plays a role in regulating flowering in tomatoes by controlling the expression of *SlSP5G, SlSP5G2*, and *SlSP5G3* [41]. These genes are also controlled by *SlCDF3*, which plays a major role in controlling the flowering time.

*SELF-PRUNING* (*SP*) gene belongs to the PEPB (Phosphatidyl ethanolamine-binding protein) family. *SP* serves as a repressor gene for flowering and is responsible for the transition from vegetative to reproductive growth [42]. In tomato, the main shoot terminates with a terminal inflorescence after 8–10 vegetative nodes. Then, it grows laterally from the axillary bud below the terminal inflorescence. This is called the sympodial shoot, which has three vegetative nodes and again ends with a terminal inflorescence. This pattern is repeated several times to form the complete structure. The growth in wild-type tomatoes is indeterminate, whereas a mutation in *SP* produces determinate plants where sympodial units are terminated by two consecutive inflorescences. If *SP* is over-expressed, it results in increased leaf number and extension in the vegetative phase, thereby replacing flowers with leaves [43]. Another flowering repressor gene paralogue to *SFT* (*SINGLE FLOWER TRUSS*) is *SELF-PRUNING 5G* (*SP5G*) which suppresses flowering in primary and axillary shoots is a paralog of the *SP* gene. When *SP5G* is mutated, it enhances rapid flowering and compactness. This results in the determinate growth pattern, enabling a swift surge in flowering, promoting day neutrality, and in turn giving early yield [16]. Also, all fruits from determinate cultivars usually ripen in a short period from simultaneous flowering, which is beneficial for facilitating mechanical harvest.

*FUL2* (*FRUITFUL Like*) Gene and *MBP20* (MADS-BOX Protein) contribute to flowering and suppressing the branching of inflorescence, owing to their role in the transition of shoot apical meristem to inflorescence meristem. These genes work in combination with genes like *J*

*(JOINTLESS), J2 (SlMBP21), EJ2/MADS1 (ENHANCER OF JOINTLESS)*, and *TM3* (*SOC1*-homolog TOMATO MADS-box gene 3) to regulate flowering. The loss-of-function studies of *FUL2* and *MBP20* through CRISPR/Cas9-generated mutants with delayed flowering, thereby elucidating their role in promoting inflorescence in both primary and sympodial shoots [44].

Hence, several genes upregulate or downregulate the flowering and precise mutations using CRISPR/Cas9 or other genome editing tools in these suitable genes can be exploited to develop cultivars with early and enhanced flowering.

## Genes that Influence Fruiting in Tomato and its Modification Through CRISPR

The characteristics of fruits like weight, size, shape, nutritional content, and shelf life are important criteria that influence the yield and market value of fruiting crops like tomatoes. Several genes regulate the various components of fruits, many of their role being characterized. The major manipulations carried out in these genes are listed in Table 2.

### Fruit Size

There are almost 30 QTLs characterized to control the fruit size. These include a few loci like (*fruit weight*) *fw1.1,*

**Table 2** Manipulation of fruiting genes using CRISPR

| Trait | Cultivar | Target gene | Locus ID | Effect on flower/fruit | References |
|---|---|---|---|---|---|
| Number | *Solanum pimpinellifolium* | *MULT* | *Solyc02g077390* | Increase in fruit number | [76] |
| Size | *Solanum pimpinellifolium* | *CLV3* | *Solyc11g071380* | Increased in number of locules and fruit weight | [76] |
| | M82 | *CLV3* | *Solyc11g071380* | Increased in number of locules and fruit weight | [102] |
| | *Solanum pimpinellifolium* | *WUS* | *Solyc02g083950* | Increased fruit size and locule number | [101] |
| | *Solanum pimpinellifolium* | *FW3.2* | | Upregulated to increase fruit size | [48] |
| Shape | *Solanum pimpinellifolium* | *OVATE* | *Solyc02g085500* | Downregulated to increase fruit size | [51, 76] |
| | | *LC* | *Solyc02g083950* | Increased fruit size due to gain of function | [51, 102] |
| | | *FAS* | *Solyc11g071810* | Increased fruit size due to loss of function | [53] |
| Color | Ailsa Craig | *PSY1*<br>*MYB12*<br>*SGR1* | | Multiplexed mutation resulting in wide range of colored fruits | [73] |
| | Micro-Tom | *ANT1** | *Solyc10g086360* | High anthocyanin content | [74] |
| Shelf-life | Micro-Tom | *SlPG* | *Solyc10g080210* | Enhanced shelf life | [63] |
| | M82 | *SIXTH5* | *Solyc01g081060* | Delayed softening and increased shelf life | [61] |
| | Ailsa Craig | *RIN* | | Delayed Fruit ripening and delayed softening | [56, 103, 104] |
| | Money Maker | *FLS2* | *Solyc10g007570* | High firmness and increased shelf life | [62] |
| | Micro-Tom | *ORRM4* | | Delayed Fruit ripening | [58] |
| | M82 | *ALC* | | Enhanced shelf life | [105] |
| | | *ETR 7* | | Enhanced shelf life | [59] |
| Parthenocarpy | Micro-Tom, Ailsa Craig | *IAA9* | *Solyc04g076850* | Parthenocarpic seedless fruits | [71, 106] |
| | MP-1 | *AGL6* | | Parthenocarpic seedless fruits | [70] |
| Lycopene | *Solanum pimpinellifolium* | *CycB* | *Solyc04g040190* | Enhanced Lycopene accumulation | [76] |
| GABA | | *SlGAD2, SlGAD3* | | Knocked out to increase GABA content in fruits | [77] |
| Ascorbic acid | | *SlAPX4* | | Increased ascorbate accumulation in fruits | [78] |
| Vitamin D | | *Sl7-DR2* | | Increased provitamin-D3 (7-dehydro carboxylase) accumulation—Vitamin D-enriched tomatoes | [17] |

*Indicates the use of TALEN for genome editing in addition to CRISPR

*MULT* multiflora, *CLV3* Clavata 3, *FW* fruit weight, *LC* locule number, *FAS* fasciated, *PSY1* phytoene synthase 1, *SGR1* stay green 1, ant1 anthocyanin mutant 1, *SlPG* solanum lycopersicum polygalacturonase, Sixth5, Rin ripening inhibitor, *FLS2* firm skin 1, *ORRM4* organelle RNA recognition motif, *ALC* altered lycopene composition, *ETR7* ethylene receptor, *IAA9* auxin-induced 9, *AGL6* agamous like 6, *cycB* lycopene beta cyclase, *GAD* glutamate decarboxylase, *APX4* ascorbate peroxidase 4, *Sl7-DR2* solanum lycopersicum 7-dehydrocholesterol reductase

*fw2.1, fw2.2, fw3.1, fw3.2*, and *fw11.3* [45]. *fw2.2* which is the QTL for fruit weight located on chromosome 2, number 2 negatively regulates the cell multiplication in fruits [46]. Owing to its role in regulating the cell number, *fw2.2* was named *CNR* (*CELL NUMBER REGULATOR*) [47]. Next to *fw2.2*, QTL *fw3.2* encodes for fruit size and weight. *SlKLUH* associated with this QTL, is responsible for increasing the cell number in the septum and pericarp, thereby producing enlarged fruits [48]. QTL *fw11.3* also controls fruit size. *fw11.3* also works in conjunction with *fas (fasciated*) to influence the fruit shape [49]. Recently, fw6.3 has been identified to be a major QTL playing a significant role in controlling fruit size [50].

## Fruit Shape

The fruit shape is determined by four major genes, namely *SUN, OVATE, LC*, and *FAS*. *LC* (*LOCULE NUMBER*) is a major gene that has a significant role in increasing locule number and results in flatter fruit [51]. *SUN* results in elongating fruit shape by encoding a protein [52]. *OVATE*, a member of the gene family containing the IQ67 domain, acts as a down regulator for fruit length, resulting in ellipsoidal fruit [52]. The negative regulation of *FAS* (*FASCIATED*) increases locule number, which in turn causes the fruits to become lengthy and elongated [53]. Recently, *FS8.1* was reported to harm the multiplication of cells in the ovary wall when mutated, resulting in fruits of elongated shape, and thus damage to the fruits was avoided during mechanical harvest [54].

## Delayed Fruit Ripening and Enhanced Shelf Life

*TAGL1* (Tomato *AGAMOUS* Like 1) gene, similar to *SHP* (*SHATTER PROOF*) MADS-Box genes of Arabidopsis, plays a major role in regulating fruit ripening. Suppression of *TAGL1* results in a defective carotenoid metabolism, resulting in orange or yellow fruits with thinner pericarp. This downregulation further affects the ethylene pathway, thereby postponing the fruit maturation. Also, studies show that on ectopically expressing *TAGL1,* the sepals are expanded and lycopene is accumulated [55]. *RIN* (*RIPENING INHIBITOR*) gene plays a critical role in suppressing over ripening but does not initiate fruit ripening. Plants with *RIN* knocked out by CRISPR produced half-ripened fruits with increased softening of flesh and decreased lycopene content. *RIN* gene in combination with *MC* (*MACROCALYX*) and other MADS-Box genes leads to abnormal ripening processes when over-expressed [56, 57]. CRISPR/Cas9-mediated knock-out of *SlORRM4* (*Organelle RNA Recognition Motif*) caused impairment in production of ethylene and respiratory rate, which in turn delayed fruit ripening [58]. The role of *SlETR7* (*Ethylene Receptor*) in enhancing the shelf life of tomato was elucidated by CRISPR-mediated knock-out of *SlETR7*, as it influences the role of ethylene which in turn delays fruit ripening [59].

*SlPL* (*PECTATE LYASE*) on silencing resulted in prolonged shelf life due to the thicker fruits due to the presence of increased cellulose and hemicellulose, but lower levels of water-soluble pectin in fruit wall [60]. Xyloglucan, a hemicellulose is an important component contributing to the firmness of the fruit. On over-expressing *SIXTH5* (*Xyloglucan endotransglucosylase/hydrolase*) depolymerizing effect on xyloglucan is repressed, thereby increasing the fruit firmness. CRISPR-mediated mutation in *SIXTH5* resulted in thicker pericarp-containing fruits with delayed softening and increased shelf life [61]. GA2 oxidase is an enzyme that deactivates gibberellin and is encoded by *FIS2* (*FIRM SKIN 1*). *FIS2* was mutated by the CRISPR method to produce fruits with longer shelf life and increased firmness, owing to the gibberellin compound and biosynthesis of wax and cuticle [62]. The polymer of galacturonic acid, pectin is a major component of fruit cell wall and is degraded by the enzyme polygalacturonase. This enzyme is encoded by *SlPG* (*Polygalacturonase*). Mutation of *SlPG* results in delayed softening and enhanced shelf life of fruits [63].

## Parthenocarpic Fruits

The regulation of auxin and gibberellin influences fruit development and can result in seedless fruits, formed without pollination and fertilization. *SlARF7* (*Auxin-Responsive Factor 7*) of tomato is expressed highly in the unpollinated ovaries and represses the setting of fruits until fertilization happens and also regulates the auxin metabolism. This is proved by silencing *SlARF7* which led to the development of parthenocarpic heart-shaped tomatoes with thicker pericarp [64]. *SlPIN4* (*PIN FORMED*), highly expressed in the ovary plays a significant role in auxin transport, thereby influencing fruit development and inhibiting the precocious development of fruits, as silencing this gene results in impaired auxin metabolism and parthenocarpic fruits. [65]. *SlTIR1* (*Transport Inhibitor Response 1*) similar to *TIR1* of Arabidopsis is expressed in floral organs and plays a key role in auxin regulation. Overexpression of this gene results in parthenocarpic fruits [66]. *DELLA* protein encoded by *PROCERA* was mutated by CRISPR/Cas9 leading to the development of dwarf mutants with increased responsiveness to gibberellin, producing parthenocarpic fruits [67, 68]. The silencing of *SlIAA17* (*Auxin Induced 17*) by RNA interference led to the development of larger fruits with thick pericarp [69]. CRISPR/Cas9-mediated knock-out mutation of *SlAGL6* (*AGAMOUS LIKE 6*) resulted in the production of fruits parthenocarpically [70]. Parthenocarpic seedless fruits are also produced when *SlIAA9* (*Auxin Induced*) was

mutated by CRISPR as a consequence of loss of function [71].

### Fruit Color

The fruit color greatly adds value to the marketability and consumer acceptance and several genes influence the color of the fruit. Few among them are *PSY1*, *MYB12,* and *SGR1*. The loss-of-function mutation in *PSY1* (*Phytoene Synthase 1*) results in yellow-colored fruits due to impaired carotenoid pathway [72]. Pink- and brown-colored fruits were formed when *MYB12* and *SGR1* (*Stay Green 1*) were mutated, respectively. A multiplexed construct was used in CRISPR to jointly mutate all three genes to produce a light green-colored fruit, which was further backcrossed and selfed in the next generation to produce a wide variety of colored fruits, namely pink, pink–brown, yellow, yellow–green, light yellow, and light green [73]. The mutation in ANT1 (Anthocyanin Mutant 1) through TALEN and CRISPR/Cas9 resulted in purple-colored fruits with high anthocyanin accumulation [74, 75].

### Nutritional Content of Fruit

The nutritional value of tomato is decided by components like lycopene, ß-carotene, and vitamins. The enzyme Lycopene beta cyclase is responsible for the conversion of lycopene to ß-carotene and this enzyme is regulated by the gene *CycB*. The wild-type plant *Solanum pimpinellifolium* was genetically engineered to produce tomato plants with enlarged fruit size and numbers having increased lycopene accumulation [76].

GABA (Ɣ-Amino Butyric Acid) is a non-protein amino acid present in large quantities in tomatoes, having health benefits like reducing blood pressure. GAD (Glutamate Decarboxylase) is the crucial enzyme involved in the GABA metabolic pathway. This pathway is controlled by 5 GAD genes, namely, *SlGAD1, SlGAD2, SlGAD3, SlGAD4,* and *SlGAD5*. Out of which, the autoinhibitory domain of *SlGAD2* and *SlGAD3* was knocked out by CRISPR/Cas9 to produce tomatoes with increased GABA accumulation with 125 mg/100 g content which is 15 times higher than the wild type. Mutation in *SlGAD2* showed impaired growth, flowering and fruiting, whereas *SlGAD3* mutants were not affected [77].

Vitamin D deficiency has a major impact on the nutritional security of the people. Though Vitamin D can be synthesized in the human body from its precursor 7-DHC (7-Dehydrocholesterol) named Provitamin-D3 on exposure to ultraviolet-B radiation, most of the vitamin D source is from diet consumption. *Sl7-DR2* (7-dehydrocholesterol reductase) helps in the conversion of 7-DHC to cholesterol, which helps in the formation of tomatine. The CRISPR/Cas9-mediated knock-out of the second exon of *Sl7-DR2* leads to increased 7-DHC accumulation, thus developing Provitamin-D3-enriched bio-fortified tomatoes [17].

Ascorbic acid is a major antioxidant present in lower levels in tomatoes that are in yellow stage and gradually decreases as the fruit ripens. *SlAPX4* (*ASCORBATE PEROXIDASE 4*) is involved in the degradation of ascorbate as the fruit ripens. Hence, CRISPR/Cas9-mediated mutation of *SlAPX4* produced gene-edited tomatoes with higher levels of ascorbate accumulation in ripened fruits. However, the mutation had no negative impact on the plant growth or ascorbate content of the leaves [78].

## Advancements in Multi-Trait Improvements

Several advanced biotechnological approaches can be used to improve multiple traits, including floral morphology and architecture, flowering habit, fruit size, shape and color, shelf life, and nutritional value. A modification in transcription factors and promoters of certain genes can result in the combined manipulation of two or more characters. Single or multiple genes can be targeted at the same time using multiple single-guide RNAs, thereby improving the efficacy of gene editing. [79] The golden gate CRISPR kit enables editing multiple genes simultaneously as it can support up to eight sgRNA cassettes. The kit was tested by targeting one site in the *SlEIN2*, *SlERFE1,* and *SlARF2B* genes, 2 sites in the *SlACS2* and *SlACS4* genes, and 3 sites in the *SlGRAS8* gene for improving the shelf life. The mutation efficacy was analyzed and thereby multi-gene editing was validated [80]. CRISPR/Cas9 was used to target six important loci (*SELF-PRUNING, OVATE, FASCIATED, FRUIT WEIGHT, MULTIFLORA, LYCOPENE BETA CYCLASE*) in wild tomato (*Solanum pimpinellifolium*) to improve its fruit size, number, and nutritional value [76]. A pYLCRISPR/Cas9 vector system was constructed with six sgRNA cassettes targeting five genes involved in GABA shunt, namely *GABA-TP1*, *GABA-TP2*, *GABA-TP3*, *CAT9*, and *SSADH*. This multiplex genome editing resulted in mutant plants where GABA accumulation in leaves and fruits were increased as a result of knock-out [81]. CRISPR/Cas9-based knock-out mutations in *MYB12* led to the development of pink-colored fruits, which are quite preferred in Asian countries like Japan and China owing to their better taste [82].

Apart from Cas9, CRISPR/Cas12a (Cpf1) is a similar system for trait modification in tomato. Cas12a was used to mutate an important transcription factor in flavonoid pathway, namely MYB12 leading to the development of pink-colored fruits [83]. This CRISPR/Cas12a system is used to produce various trait modifications apart from floral and fruit characteristics like salinity tolerance due to mutation in *HKT1;2* (*HIGH-AFFINITY* $K^+$ *TRANSPORTER 1;2*) and

herbicide chlorsulfuron resistance by mutation *ALS* (*ACETOLACTATE SYNTHASE)* gene [84]. Cytosine base editors and adenosine base editors were successfully applied through PEG (Polyethylene glycol)-mediated Cas9 delivery into protoplasts to improve plant regeneration by targeting AGO7 in tomato cultivar Micro-Tom [85]. PEG-mediated transfection of diploid and tetraploid protoplast of in vitro shoots using cas9 and RNPs (Ribonucleoproteins) targeting genes involved in small interfering RNAs biogenesis, *RNA-DEPENDENT RNA POLYMERASE 6* (*SpRDR6*), and *SUPPRESSOR OF GENE SILENCING 3* (*SpSGS3*); pathogen-related peptide precursors, *PATHOGENESIS-RELATED PROTEIN-1* (*SpPR-1*), and *PROSYSTEMIN* (*SpProSys*); and fungal resistance (*MILDEW RESISTANT LOCUS O, SpMlo1*) was carried out in wild cultivar *S. peruvianum*, resulting in mutant plants with increased TYLCV (Tomato Yellow Leaf Curl Virus) proliferation, sterility, and mildew attack. This indicates the influence of these genes as a knock-in effect in overall plant regeneration [86]. Similarly, protoplast transfection using Cas9 and RNPs targeting self-pruning genes, *SP,* and *SP5G* resulted in altered determinate plant growth habit, early and synchronous flowering, and plant regeneration [87].

Target-AID (Target-Activation Induced Cytidine Deaminase) represents a base editing technology that facilitates the accurate transformation of particular DNA bases while avoiding the induction of double-strand breaks (DSBs), in contrast to conventional CRISPR methodologies. This technique encompasses the amalgamation of dCas9 (dead Cas9), characterized by the absence of nuclease activity yet retaining the ability to associate specific DNA sequences with a cytidine deaminase enzyme, predominantly PmCDA1. This innovative system enables the direct alteration of cytosine (C) to thymine (T) (or guanine (G) to adenine (A)) at designated target loci, thereby instigating precise point mutations without the necessity of cleaving the DNA [88]. Mutant plants produced by knocking out *SlINVINH1* (Invertase inhibitor) gene by the Target-AID method produced fruits with higher sugar content, without loss in fruit weight and deterioration in plant growth [89]. Similarly, three carotenoid-related genes, namely *SlDDB1*, *SlDET1,* and *SlCYC-B,* were targeted through the Target-AID method to validate the role of these genes in carotenoid accumulation [90]. Advanced biotechnological gene editing approaches like CRISPR systems other than cas9 like Cas12a and Cas13, prime editing, and base editing are all on the verge of development and their efficacy has been tested so far on biotic (viral resistance) and abiotic stress tolerance. Its role in floral and fruit modification is yet to be explored in a wide aspect, to enhance yield and nutritional components. A detailed figure describing the advanced biotechnological approaches in genome editing used for improving several plant characters is given (Fig. 2).

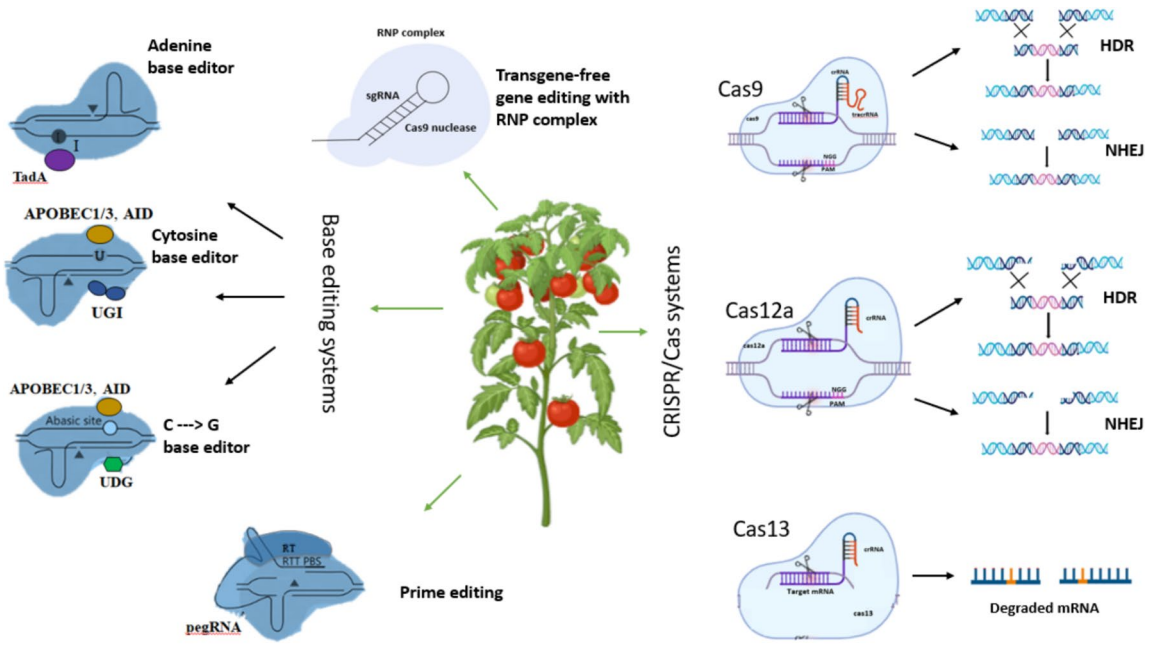


**Fig. 2** Comprehensive figure depicting the overview of advanced CRISPR/Cas-based gene editing systems for genetic improvement. *HDR* Homologous Recombination, *NHEJ* Non-Homologous End Joining, *pegRNA* Prime Editing Guide RNA, *APOBEC* Apolipoprotein B mRNA Editing Catalytic Polypeptide-like family of proteins, *RNP* Ribonucleoprotein

## Long-Term Stability and Ecological Impacts of Gene Editing Systems

Though the CRISPR gene editing technique is effectively used to target specific genes and produce modified plants, care has to be taken to make sure that the edited plants should be free from transgenes to maintain trait stability and also to be approved for commercial production following regulatory rules. Sexual reproduction of the edited plants can result in the removal of transgenes. Also, transgenes can be eliminated and identified through marker-assisted tracking. Further techniques involve sequentially activating CRISPR genes and suicide genes to undergo programmed self-elimination, resulting in transgene-free gene-edited plants. To be precise, non-transgenic approaches like RNP (Ribonucleoprotein) transfection, nano-biotechnology, and transient expression of transgenes without DNA integration can be used, especially for plants that cannot be propagated sexually [91]. Though RNPs do not involve transgenes and can be transformed by particle bombardment or transformation, the real difficulty lies in the regeneration of the plant from a single protoplast, leading to its limited experimentation in laboratories [92]. Chimerically edited plants can be produced by delivering gene editing particles into the meristematic cells using nanoparticles and transgene-free plants can be generated from these edited plants through tissue culture or propagation via cuttings [93]. The Transgene Killer CRISPR technology is a self-eliminating system that has two units, namely a gene editing unit comprising the cas9 and gRNA to facilitate editing of target genes, followed by a suicide genes unit that triggers cell death. This will allow only the transgene-free plants to survive, enabling self-selection [94].

The major concern in CRISPR is the presence of off-target effects, which can be eliminated by efficient guide RNA designing and high-fidelity cas9 variants [95]. Studies have shown that CRISPR/Cas9-induced modifications can be stably inherited across multiple generations in tomatoes, indicating the potential for sustained genetic improvements. Long-term field trials are needed to rule out the effect of environmental factors and epigenetic changes and to ensure the stability of traits passing over to the further generations [96].

Gene editing can be used to produce plants with improved ecological impacts. Plants may be modified genetically to improve their resilience to climate change and resistance to pests and diseases, thereby reducing the use of fertilizers, pesticides, and herbicides. The target traits could be identified and the genes can be modified to increase yield and nutrition, to face the increasing demands, which is the major concern in the current global status. Yet, there are other negative impacts like loss of bio-diversity and effects on non-target species [97, 98]. Further, the enhancement of certain characteristics or morphology of the plant may result in the degradation of other characteristics. For example, mutation in *GABA-TP* (pyruvate-dependent GABA-T) resulted in increased GABA accumulation, but the plants were dwarf in later stages and showed necrosis and abnormal leaf morphology [81].

There are several regulatory frameworks worldwide that debate over the commercial production of gene-edited plants. In the USA, the USDA (United States Department of Agriculture), FDA (Food and Drug Administration), and EPA (Environmental Protection Agency) together have formed guidelines to permit gene-edited crops without any foreign DNA. Whereas, in the European Union, the ECJ (European Court of Justice) brought gene-edited crops under the same regulations as GMOs (Genetically Modified Organisms) and debates are ongoing to modify these frameworks. But, different regulatory frameworks are followed in different countries [99]. In Japan, Sanatech Seed has produced the first ever CRISPR-edited food to enter into the market, naming it Sicilian Rouge, a GABA-enriched tomato [100].

## Conclusion

Concerning the changes in the climatic conditions across the globe, the ecological conditions necessary for the emergence of crops are being affected drastically. In addition to this, the rapid surge in the world population has contributed to the alarming decline in the nutritional security of countries all over the world. The available vast genetic resources for agricultural and horticultural crops have to be meticulously exploited by breeding or genetic engineering approaches to create plants that can meet the current demands of the people. With the advancement in genetic improvement technologies, new-age tools like CRISPR/Cas9 is found to be promising approach to creating specific mutations in the target traits and produce a highly desired plant. In the case of plants like tomatoes, genome editing is used to produce plants with increased yield producing fruits with enhanced shelf life, flavor, aroma, color pigments, antioxidants, and nutrients which need to be further improved. These characteristics, both morphological and physiological, are controlled by several genes. The mechanism by which the genes regulate novel, specific, and complex traits like biotic and abiotic stress tolerance needs to be exhaustively studied. Thorough studies on the role of protein-coding genes in functional mechanisms along with the action of micro-RNAs and long non-coding RNAs on the regulatory mechanism of genes are needed to understand the intricate mechanisms that lie under complex traits. Also, the epigenetic effect on genes

controlling the traits is a broader area yet to be completely exploited. CRISPR can be used to target multiple traits simultaneously by multiplexing and also the production of gene-edited plants takes short period compared to other technologies, but efficiency of transformation, regeneration potential of the plants and success rate are major concerning factors that need to be standardized. Many cultivars have not yet been used for genome editing and protocols have to be standardized for them. Further investigations on the use of other cas variants can also be explored in the near future. CRISPR/CAS9 Ribonucleoproteins can be delivered to plant protoplast to allow DNA-free genome editing methods. Apart from CRISPR, the coming-age gene editing tools like base editing and prime editing, where specific mutations can be created without causing double-stranded breaks must be executed to generate plants of high value. However, proper concern has to be given to the development and release of gene-edited plants as they come under Genetically Modified Organisms and their regulatory framework varies from country to country. This review provides a thorough insight into the major genes involved in the flowering and fruiting habits of tomatoes. The genes *SP, SP5G, CLV,* and *FAF* play an important role in early flowering and altering plant growth and flowering habits. The shelf life of tomatoes is majorly governed by *SlPG, RIN*, *ALC*, and *ETR7*. *MYB12* and *ANT1* are involved in fruit pigmentation, contributing to anthocyanin accumulation. The nutritional value of tomatoes can be increased by modifying genes like *CycB*, *GAD, APX,* and Sl7-DR2. The genes discussed in the above review can be used by researchers and breeders to improve floral and fruit traits. Though tomato genes are being widely studied, in-depth insights need to be given to understand the genes involved and develop plants resistant to fungal and viral diseases, tolerant to biotic and abiotic stresses, modified architecture without the pre-mature dropping of flowers, early yielding, and nutritionally enriched fruits.

**Data availability** There is no data availability statement for this review.

## References


1. FAO. Tomato land and water 2023 https://www.fao.org/land-water/databases-and-software/crop-information/tomato
2. Ali, M. Y., Sina, A. A. I., Khandker, S. S., Neesa, L., Tanvir, E., Kabir, A., Khalil, M. I., & Gan, S. H. (2020). Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. *Foods., 10*(1), 45. https://doi.org/10.3390/foods10010045
3. Friedman, M. (2013). Anticarcinogenic, cardioprotective, and other health benefits of tomato compounds lycopene, α-tomatine, and tomatidine in pure form and in fresh and processed tomatoes. *Journal of Agricultural and Food Chemistry, 61*(40), 9534–9550. https://doi.org/10.1021/jf402654e
4. Borguini, R. G., da Silva, F., & Torres, E. A. (2009). Tomatoes and tomato products as dietary sources of antioxidants. *Food Review International, 25*(4), 313–325. https://doi.org/10.1080/87559120903155859
5. Kumar, K. S., Paswan, S., & Srivastava, S. (2012). Tomato-a natural medicine and its health benefits. *Journal of Pharmacognosy Phytochemistry., 1*(1), 33–43.
6. Toor, R. K., & Savage, G. P. (2005). Antioxidant activity in different fractions of tomatoes. *Food Research International, 38*(5), 487–494. https://doi.org/10.1016/j.foodres.2004.10.016
7. Chaudhary, P., Sharma, A., Singh, B., & Nagpal, A. K. (2018). Bioactivities of phytochemicals present in tomato. *Journal of Food Science and Technology, 55*, 2833–2849. https://doi.org/10.1007/s13197-018-3221-z
8. Fiedor, J., & Burda, K. (2014). Potential role of carotenoids as antioxidants in human health and disease. *Nutrients, 6*(2), 466–488. https://doi.org/10.3390/nu6020466
9. Tiwari, J. K., Rai, N., Singh, M. K., Mishra, L. K., Mishra, G., & Behera, T. (2023). Characterization of tomato lines for yield components, processing traits and disease resistance based on phenotype and molecular markers. *Vegetable Science, 50*(2), 302–309. https://doi.org/10.61180/vegsci.2023.v50.i2.06
10. Razifard, H., Ramos, A., Della Valle, A. L., Bodary, C., Goetz, E., Manser, E. J., Li, X., Zhang, L., Visa, S., Tieman, D., van der Knaap, E., & Caicedo, A. L. (2020). Genomic evidence for complex domestication history of the cultivated tomato in Latin America. *Molecular Biology and Evolution, 37*(4), 1118–1132. https://doi.org/10.1093/molbev/msz297
11. Bauchet, G., & Causse, M. (2012). Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. *Genetic diversity in plants, 8*, 134–162. https://doi.org/10.5772/33073
12. Di Matteo, A., Rigano, M. M., Sacco, A., Frusciante, L., & Barone, A. (2011). Genetic transformation in tomato: Novel tools to improve fruit quality and pharmaceutical production. *Genetic transformation*. https://doi.org/10.5772/24521
13. Sacco, A., Greco, B., Di Matteo, A., De Stefano, R., & Barone, A. (2013). Evaluation of tomato genetic resources for response to water deficit. *American Journal of Plant Sciences*. https://doi.org/10.4236/ajps.2013.4.12A3016
14 Saito, T., Ariizumi, T., Okabe, Y., Asamizu, E., Hiwasa-Tanase, K., Fukuda, N., et al. (2011). TOMATOMA: A novel tomato mutant database distributing Micro-Tom mutant collections. *Plant and Cell Physiology., 52*(2), 283–296. https://doi.org/10.1093/pcp/pcr004
15. Pnueli, L., Gutfinger, T., Hareven, D., Ben-Naim, O., Ron, N., Adir, N., et al. (2001). Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. *The Plant Cell, 13*(12), 2687–2702. https://doi.org/10.1105/tpc.010293
16. Soyk, S., Müller, N. A., Park, S. J., Schmalenbach, I., Jiang, K., Hayama, R., et al. (2017). Variation in the flowering gene *SELF PRUNING 5G* promotes day-neutrality and early yield in tomato. *Nature Genetics, 49*(1), 162–168. https://doi.org/10.1038/ng.3733
17. Li, J., Scarano, A., Gonzalez, N. M., D'Orso, F., Yue, Y., Nemeth, K., et al. (2022). Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nature Plants, 8*(6), 611–616. https://doi.org/10.1038/s41477-022-01154-6
18. Lawal, O., Ayodele, A., & Chukwuka, K. (2007). Morphological studies in Lycopersicon esculentum Mill lines in Southwestern Nigeria. *Journal of Biological Sciences, 7*(5), 737–744. https://doi.org/10.3923/jbs.2007.737.744
19. Sharma, P., Thakur, S., & Negi, R. (2019). Recent advances in breeding of tomato–a review. *International Journal of Current Microbiology and Applied Sciences, 8*(3), 1275–1283. https://doi.org/10.20546/ijcmas.2019.803.151

20. Begna, T. (2021). Conventional breeding methods widely used to improve self-pollinated crops. *International Journal of Research Studies in Agricultural Sciences, 7*(1), 1–16. https://doi.org/10.20431/2454-6224.0701001
21. Lamichhane, S., & Thapa, S. (2022). Advances from conventional to modern plant breeding methodologies. *Plant Breed. Biotech., 10*(1), 1–14. https://doi.org/10.9787/PBB.2022.10.1.1
22. Shahwar, D., Ahn, N., Kim, D., Ahn, W., & Park, Y. (2023). Mutagenesis-based Plant Breeding Approaches and Genome Engineering: A Review Focused on Tomato. *Mutation Research/Reviews in Mutation Research*. https://doi.org/10.1016/j.mrrev.2023.108473
23. Hasan, N., Choudhary, S., Naaz, N., Sharma, N., & Laskar, R. A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal of Genetic Engineering and Biotechnology, 19*(1), 1–26. https://doi.org/10.1186/s43141-021-00231-1
24. Zhang, Y., Massel, K., Godwin, I. D., & Gao, C. (2018). Applications and potential of genome editing in crop improvement. *Genome Biology, 19*, 1–11. https://doi.org/10.1186/s13059-018-1586-y
25 Shamshirgaran, Y., Liu, J., Sumer, H., Verma, P. J., & Taheri-Ghahfarokhi, A. (2022). Tools for efficient genome editing; ZFN, TALEN, and CRISPR. *Applications of genome modulation and editing*. https://doi.org/10.1007/978-1-0716-2301-5_2
26. Nussenzweig, P. M., & Marraffini, L. A. (2020). Molecular mechanisms of CRISPR-Cas immunity in bacteria. *Annual Review of Genetics, 54*, 93–120. https://doi.org/10.1146/annurev-genet-022120-112523
27. Rehman, F., Gong, H., Bao, Y., Zeng, S., Huang, H., & Wang, Y. (2022). CRISPR gene editing of major domestication traits accelerating breeding for *Solanaceae* crops improvement. *Plant Molecular Biology, 108*(3), 157–173. https://doi.org/10.1007/s11103-021-01229-6
28. Tyumentseva, M., Tyumentsev, A., & Akimkin, V. (2023). CRISPR/Cas9 landscape: Current state and future perspectives. *International Journal of Molecular Sciences, 24*(22), 16077. https://doi.org/10.3390/ijms242216077
29. Park, S. J., Jiang, K., Schatz, M. C., & Lippman, Z. B. (2012). Rate of meristem maturation determines inflorescence architecture in tomato. *Proceedings of the National Academy of Sciences, 109*(2), 639–644. https://doi.org/10.1073/pnas.1114963109
30. Xu, C., Liberatore, K. L., MacAlister, C. A., Huang, Z., Chu, Y.-H., Jiang, K., Brooks, C., Ogawa-Ohnishi, M., Xiong, G., Pauly, M., Van Eck, J., Matsubayashi, Y., van der Knaap, E., & Lippman, Z. B. (2015). A cascade of arabinosyltransferases controls shoot meristem size in tomato. *Nature Genetics, 47*(7), 784–792. https://doi.org/10.1038/ng.3309
31. Zhang, Y., Sun, H., Tian, L., Miao, Y., Hou, L., Sun, M., Qi, M., & Li, T. (2023). Transcriptome Analysis Identifies Downstream Genes of *CLAVATA3* in Tomato. *Horticulturae, 10*(1), 11. https://doi.org/10.3390/horticulturae10010011
32. Yang, Y., Yang, H., Tan, Y., Zhao, T., Xu, X., Li, J., & Jiang, J. (2021). Comparative Genome Analysis of Genes Regulating Compound Inflorescences in Tomato. *International Journal of Molecular Sciences, 22*(22), 12548. https://doi.org/10.3390/ijms222212548
33. Guo, X., Zhao, J., Chen, Z., Qiao, J., Zhang, Y., Shen, H., & Hu, Z. (2022). CRISPR/Cas9-targeted mutagenesis of *SlCMT4* causes changes in plant architecture and reproductive organs in tomato. *Horticulture Research*. https://doi.org/10.1093/hr/uhac081
34. Zhang, Y., Zhang, B., Yang, T., Zhang, J., Liu, B., Zhan, X., & Liang, Y. (2020). The *GAMYB*-like gene *SlMYB33* mediates flowering and pollen development in tomato. *Horticulture Research*. https://doi.org/10.1038/s41438-020-00366-1
35. Guo, X., Hu, Z., Yin, W., Yu, X., Zhu, Z., Zhang, J., & Chen, G. (2016). The tomato floral homeotic protein FBP1-like gene, *SlGLO1*, plays key roles in petal and stamen development. *Science and Reports, 6*(1), 20454. https://doi.org/10.1038/srep20454
36. Wu, M., Zhang, Q., Wu, G., Zhang, L., Xu, X., Hu, X., Gong, Z., Chen, Y., Li, Z., Li, H., & Deng, W. (2023). *SlMYB72* affects pollen development by regulating autophagy in tomato. *Horticulture. Research., 10*(3), p286. https://doi.org/10.1093/hr/uhac286
37. Wang, X., Liu, Z., Sun, S., Wu, J., Li, R., Wang, H., & Cui, X. (2021). SISTER OF TM3 activates *FRUITFULL1* to regulate inflorescence branching in tomato. *Horticulture Research*. https://doi.org/10.1038/s41438-021-00677-x
38. Lin, W., Gupta, S. K., Arazi, T., & Spitzer-Rimon, B. (2021). *MIR172d* is required for floral organ identity and number in tomato. *International Journal of Molecular Sciences, 22*(9), 4659. https://doi.org/10.3390/ijms22094659
39 Xu, C., Park, S. J., Van Eck, J., & Lippman, Z. B. (2016). Control of inflorescence architecture in tomato by *BTB/POZ* transcriptional regulators. *Genes & Development, 30*(18), 2048–2061. https://doi.org/10.1101/gad.288415.116
40. Shang, L., Tao, J., Song, J., Wang, Y., Zhang, X., Ge, P., Li, F., Dong, H., Gai, W., Grierson, D., Ye, Z., & Zhang, Y. (2023). CRISPR/Cas9-mediated mutations of *FANTASTIC FOUR* gene family for creating early flowering mutants in tomato. *Plant Biotechnology Journal*. https://doi.org/10.1111/pbi.14223
41. Cao, K., Cui, L., Zhou, X., Ye, L., Zou, Z., & Deng, S. (2016). Four tomato *FLOWERING LOCUS* T-like proteins act antagonistically to regulate floral initiation. *Frontiers in Plant Science, 6*, 1213. https://doi.org/10.3389/fpls.2015.01213
42. Carmel-Goren, L., Liu, Y. S., Lifschitz, E., & Zamir, D. (2003). The *SELF-PRUNING* gene family in tomato. *Plant Molecular Biology, 52*, 1215–1222. https://doi.org/10.1023/B:PLAN.0000004333.96451.11
43. Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., & Lifschitz, E. (1998). The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. *Development, 125*(11), 1979–1989. https://doi.org/10.1242/dev.125.11.1979
44. Jiang, X., Lubini, G., Hernandes-Lopes, J., Rijnsburger, K., Veltkamp, V., de Maagd, R. A., Angenent, G. C., & Bemer, M. (2022). *FRUITFULL*-like genes regulate flowering time and inflorescence architecture in tomato. *The Plant Cell, 34*(3), 1002–1019. https://doi.org/10.1093/plcell/koab298
45. Grandillo, S., Ku, H., & Tanksley, S. (1999). Identifying the loci responsible for natural variation in fruit size and shape in tomato. *TAG. Theoretical and Applied Genetics., 99*, 978–987. https://doi.org/10.1007/s001220051405
46. Van der Knaap, E., Chakrabarti, M., Chu, Y. H., Clevenger, J. P., Illa-Berenguer, E., Huang, Z., Keyhaninejad, N., Mu, Q., Sun, L., Wang, Y., & Wu, S. (2014). What lies beyond the eye: The molecular mechanisms regulating tomato fruit weight and shape. *Frontiers in Plant Science, 5*, 227. https://doi.org/10.3389/fpls.2014.00227
47. Guo, M., Rupe, M. A., Dieter, J. A., Zou, J., Spielbauer, D., Duncan, K. E., Howard, R. J., Hou, Z., & Simmons, C. R. (2010). Cell Number Regulator1 affects plant and organ size in maize: Implications for crop yield enhancement and heterosis. *The Plant Cell, 22*(4), 1057–1073. https://doi.org/10.1105/tpc.109.073676
48. Chakrabarti, M., Zhang, N., Sauvage, C., Muños, S., Blanca, J., Cañizares, J., Diez, M. J., Schneider, R., Mazourek, M., McClead, M. C., & van der Knaap, E. (2013). A cytochrome P450 regulates a domestication trait in cultivated tomato. *Proceedings of the National Academy of Sciences, 110*(42), 17125–17130. https://doi.org/10.1073/pnas.1307313110

49. Huang, Z., & van der Knaap, E. (2011). Tomato fruit weight 11.3 maps close to fasciated on the bottom of chromosome 11. *Theoretical and Applied Genetics, 123*, 465–474. https://doi.org/10.1007/s00122-011-1599-3
50. Ning, Y., Wei, K., Li, S., Zhang, L., Chen, Z., Lu, F., Yang, P., Yang, M., Liu, X., Liu, X., Wang, X., Cao, X., Wang, X., Guo, Y., Liu, L., Li, X., Du, Y., Li, J., & Huang, Z. (2023). Fine mapping of fw6. 3, a major-effect quantitative trait locus that controls fruit weight in tomato. *Plants, 12*(11), 2065. https://doi.org/10.3390/plants12112065
51. Rodríguez, G. R., Muños, S., Anderson, C., Sim, S.-C., Michel, A., Causse, M., McSpadden Gardener, B. B., Francis, D., & van der Knaap, E. (2011). Distribution of *SUN, OVATE, LC*, and *FAS* in the tomato germplasm and the relationship to fruit shape diversity. *Plant Physiology, 156*(1), 275–285. https://doi.org/10.1104/pp.110.167577
52. Xiao, H., Jiang, N., Schaffner, E., Stockinger, E. J., & van der Knaap, E. (2008). A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science, 319*(5869), 1527–1530. https://doi.org/10.1126/science.115304
53. Cong, B., Barrero, L. S., & Tanksley, S. D. (2008). Regulatory change in *YABBY*-like transcription factor led to evolution of extreme fruit size during tomato domestication. *Nature Genetics, 40*(6), 800–804. https://doi.org/10.1038/ng.144
54. Zhu, Q., Deng, L., Chen, J., Rodríguez, G. R., Sun, C., Chang, Z., Yang, T., Zhai, H., Jiang, H., Topcu, Y., Francis, D., Hutton, S., Sun, L., Li, C. B., van der Knaap, E., & Li, C. (2023). Redesigning the tomato fruit shape for mechanized production. *Nature Plants, 9*(10), 1659–1674. https://doi.org/10.1038/s41477-023-01522-w
55. Vrebalov, J., Pan, I. L., Arroyo, A. J. M., McQuinn, R., Chung, M., Poole, M., Rose, J., Seyour, G., Grandillo, S., Giovannoni, J., & Irish, V. F. (2009). Fleshy fruit expansion and ripening are regulated by the tomato *SHATTERPROOF* gene *TAGL1*. *The Plant Cell, 21*(10), 3041–3062. https://doi.org/10.1105/tpc.109.066936
56. Ito, Y., Sekiyama, Y., Nakayama, H., Nishizawa-Yokoi, A., Endo, M., Shima, Y., Nakamura, N., Kotake-Nara, E., Kawasaki, S., Hirose, S., & Toki, S. (2020). Allelic mutations in the ripening-inhibitor locus generate extensive variation in tomato ripening. *Plant Physiology, 183*(1), 80–95. https://doi.org/10.1104/pp.20.00020
57. Li, S., Xu, H., Ju, Z., Cao, D., Zhu, H., Fu, D., Grierson, D., Qin, G., Luo, Y., & Zhu, B. (2018). The *RIN-MC* fusion of *MADS*-box transcription factors has transcriptional activity and modulates expression of many ripening genes. *Plant Physiology, 176*(1), 891–909. https://doi.org/10.1104/pp.17.01449
58. Yang, Y., Zhu, G., Li, R., Yan, S., Fu, D., Zhu, B., Tian, H., Luo, Y., & Zhu, H. (2017). The RNA editing factor *SlORRM4* is required for normal fruit ripening in tomato. *Plant Physiology, 175*(4), 1690–1702. https://doi.org/10.1104/pp.17.01265
59 Chen, Y., Hu, G., Rodriguez, C., Liu, M., Binder, B. M., & Chervin, C. (2020). Roles of *SlETR7*, a newly discovered ethylene receptor, in tomato plant and fruit development. *Journal Horticulture Research*. https://doi.org/10.1038/s41438-020-0239-y
60. Yang, L., Huang, W., Xiong, F., Xian, Z., Su, D., Ren, M., & Li, Z. (2017). Silencing of *SlPL*, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life and reduced susceptibility to grey mould. *Plant Biotechnology Journal, 15*(12), 1544–1555. https://doi.org/10.1111/pbi.12737
61. Wang, D., Lu, Q., Wang, X., Ling, H., & Huang, N. (2023). Elucidating the role of *SlXTH5* in tomato fruit softening. *Horticulture Plant Journal, 9*(4), 777–788. https://doi.org/10.1016/j.hpj.2022.12.005
62. Li, R., Sun, S., Wang, H., Wang, K., Yu, H., Zhou, Z., Xin, P., Chu, J., Zhao, T., Wang, H., Li, J., & Cui, X. (2020). *FIS1* encodes a GA2-oxidase that regulates fruit firmness in tomato. *Nature Communications, 11*(1), 5844. https://doi.org/10.1038/s41467-020-19705-w
63. Nie, H., Shi, Y., Geng, X., & Xing, G. (2022). CRISRP/Cas9-mediated targeted mutagenesis of tomato polygalacturonase gene (*SlPG*) delays fruit softening. *Frontiers in Plant Science, 13*, 729128. https://doi.org/10.3389/fpls.2022.729128
64. De Jong, M., Wolters-Arts, M., Feron, R., Mariani, C., & Vriezen, W. H. (2009). The *Solanum lycopersicum* auxin response factor 7 (*SlARF7*) regulates auxin signaling during tomato fruit set and development. *The Plant Journal, 57*(1), 160–170. https://doi.org/10.1111/j.1365-313X.2008.03671.x
65. Mounet, F., Moing, A., Kowalczyk, M., Rohrmann, J., Petit, J., Garcia, V., Maucourt, M., Yano, K., Deborde, C., Aoki, K., Berges, H., Granell, A., Fernie, A. R., Bellini, C., Rothan, C., & Lemaire-Chamley, M. (2012). Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. *Journal of Experimental Botany, 63*(13), 4901–4917. https://doi.org/10.1093/jxb/ers167
66. Ren, Z., Li, Z., Miao, Q., Yang, Y., Deng, W., & Hao, Y. (2011). The auxin receptor homologue in *Solanum lycopersicum* stimulates tomato fruit set and leaf morphogenesis. *Journal of Experimental Botany, 62*(8), 2815–2826. https://doi.org/10.1093/jxb/erq455
67. Martí, C., Orzáez, D., Ellul, P., Moreno, V., Carbonell, J., & Granell, A. (2007). Silencing of *DELLA* induces facultative parthenocarpy in tomato fruits. *The Plant Journal, 52*(5), 865–876. https://doi.org/10.1111/j.1365-313X.2007.03282.x
68. Tomlinson, L., Yang, Y., Emenecker, R., Smoker, M., Taylor, J., Perkins, S., Smith, J., MacLean, D., Olszewski, N. E., & Jones, J. D. G. (2019). Using CRISPR/Cas9 genome editing in tomato to create a gibberellin-responsive dominant dwarf *DELLA* allele. *Plant Biotechnology Journal, 17*(1), 132–140. https://doi.org/10.1111/pbi.12952
69. Su, L., Bassa, C., Audran, C., Mila, I., Cheniclet, C., Chevalier, C., Bouzayen, M., Roustan, J. P., & Chervin, C. (2014). The auxin Sl-*IAA17* transcriptional repressor controls fruit size via the regulation of endoreduplication-related cell expansion. *Plant and Cell Physiology, 55*(11), 1969–1976. https://doi.org/10.1093/pcp/pcu124
70 Klap, C., Yeshayahou, E., Bolger, A. M., Arazi, T., Gupta, S. K., Shabtai, S., Usadel, B., Salts, Y., & Barg, R. (2017). Tomato facultative parthenocarpy results from Sl AGAMOUS-LIKE 6 loss of function. *Plant Biotechnology Journal., 15*(5), 634–647. https://doi.org/10.1111/pbi.12662
71. Ueta, R., Abe, C., Watanabe, T., Sugano, S. S., Ishihara, R., Ezura, H., Osakabe, Y., & Osakabe, K. (2017). Rapid breeding of parthenocarpic tomato plants using CRISPR/Cas9. *Scientific Reports, 7*(1), 507. https://doi.org/10.1038/s41598-017-00501-4
72 Filler Hayut, S., MelamedBessudo, C., & Levy, A. A. (2017). Targeted recombination between homologous chromosomes for precise breeding in tomato. *Nature Communications, 8*(1), 15605. https://doi.org/10.1038/ncomms15605
73. Yang, T., Ali, M., Lin, L., Li, P., He, H., Zhu, Q., et al. (2023). Recoloring tomato fruit by CRISPR/Cas9-mediated multiplex gene editing. *Horticulture Research, 10*(1), 214. https://doi.org/10.1093/hr/uhac214
74 Čermák, T., Baltes, N. J., Čegan, R., Zhang, Y., & Voytas, D. F. (2015). High-frequency, precise modification of the tomato genome. *Genome Biology., 16*, 1–15. https://doi.org/10.1186/s13059-015-0796-9
75. Menconi, J., Perata, P., & Gonzali, S. (2024). In pursuit of purple: Anthocyanin biosynthesis in fruits of the tomato clade. *Trends in Plant Science*. https://doi.org/10.1016/j.tplants.2023.12.010
76. Zsögön, A., Čermák, T., Naves, E. R., Notini, M. M., Edel, K. H., Weinl, S., Freschi, l, Voytas, D. F., Kudla, J., & Pereira, L.

E. (2018). De novo domestication of wild tomato using genome editing. *Nature Biotechnology, 36*(12), 1211–1216. https://doi.org/10.1038/nbt.4272

77. Nonaka, S., Arai, C., Takayama, M., Matsukura, C., & Ezura, H. (2017). Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Scientific Reports, 7*(1), 7057. https://doi.org/10.1038/s41598-017-06400-y
78. Do, J. H., Park, S. Y., Park, S. H., Kim, H. M., Ma, S. H., Mai, T. D., Shim, J. S., & Joung, Y. H. (2022). Development of a genome-edited tomato with high ascorbate content during later stage of fruit ripening through mutation of *SlAPX4*. *Frontiers in Plant Science, 13*, 836916. https://doi.org/10.3389/fpls.2022.836916
79 Chaudhuri, A., Halder, K., & Datta, A. (2022). Classification of CRISPR/Cas system and its application in tomato breeding. *Theoretical and Applied Genetics, 135*(2), 367–387. https://doi.org/10.1007/s00122-021-03984-y
80. Hu, N., Xian, Z., Li, N., Liu, Y., Huang, W., Yan, F., Su, D., Chen, J., & Li, Z. (2019). Rapid and user-friendly open-source CRISPR/Cas9 system for single- or multi-site editing of tomato genome. *Horticulture Research*. https://doi.org/10.1038/s41438-018-0082-6
81. Li, R., Li, R., Li, X., Fu, D., Zhu, B., Tian, H., Luo, Y., & Zhu, H. (2018). Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ-aminobutyric acid levels in *Solanum lycopersicum*. *Plant Biotechnology Journal, 16*(2), 415–427. https://doi.org/10.1111/pbi.12781
82. Yang, T.-x, Deng, L., Zhao, W., Zhang, R., Jiang, H.-l, Ye, Z., Li, C. B., & Li, C. (2019). Rapid breeding of pink-fruited tomato hybrids using the CRISPR/Cas9 system. *Journal of Genetics and Genomics, 46*(10), 505–508. https://doi.org/10.1016/j.jgg.2019.10.002
83. Fernandez-Moreno, J.-P., Tzfadia, O., Forment, J., Presa, S., Rogachev, I., Meir, S., Orzaez, D., Aharoni, A., & Granell, A. (2016). Characterization of a new pink-fruited tomato mutant results in the identification of a null allele of the *SlMYB12* transcription factor. *Plant Physiology, 171*(3), 1821–1836. https://doi.org/10.1104/pp.16.00282
84 Larriba, E., Yaroshko, O., & Pérez-Pérez, J. M. (2024). Recent advances in tomato gene editing. *International Journal of Molecular Sciences, 25*(5), 2606. https://doi.org/10.3390/ijms25052606
85. Sretenovic, S., Liu, S., Li, G., Cheng, Y., Fan, T., Xu, Y., Zhou, J., Zheng, X., Coleman, G., Zhang, Y., & Qi, Y. (2021). Exploring C-To-G base editing in rice, tomato, and poplar. *Frontiers in Genome Editing, 3*, 756766. https://doi.org/10.3389/fgeed.2021.756766
86. Lin, C.-S., Hsu, C.-T., Yuan, Y.-H., Zheng, P.-X., Wu, F.-H., Cheng, Q.-W., Wu, Y.-L., Wu, T.-L., Lin, S., Yue, J.-J., Cheng, Y.-H., Lin, S.-I., Shih, M.-C., Sheen, J., & Lin, Y.-C. (2022). DNA-free CRISPR-Cas9 gene editing of wild tetraploid tomato *Solanum peruvianum* using protoplast regeneration. *Plant Physiology, 188*(4), 1917–1930. https://doi.org/10.1093/plphys/kiac022
87. Liu, Y., Andersson, M., Granell, A., Cardi, T., Hofvander, P., & Nicolia, A. (2022). Establishment of a DNA-free genome editing and protoplast regeneration method in cultivated tomato (*Solanum lycopersicum*). *Plant Cell Reports, 41*(9), 1843–1852. https://doi.org/10.1007/s00299-022-02893-8
88. Shimatani, Z., Kashojiya, S., Takayama, M., Terada, R., Arazoe, T., Ishii, H., Teramura, H., Yamamoto, T., Komatsu, H., Miura, K., Ezura, H., Nishida, K., Ariizumi, T., & Kondo, A. (2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nature Biotechnology, 35*(5), 441–443. https://doi.org/10.1038/nbt.3833
89. Kawaguchi, K., Takei-Hoshi, R., Yoshikawa, I., Nishida, K., Kobayashi, M., Kusano, M., Lu, Y., Ariizumi, T., Ezura, H., Otagaki, S., Matsumoto, S., & Shiratake, K. (2021). Functional disruption of cell wall invertase inhibitor by genome editing increases sugar content of tomato fruit without decrease fruit weight. *Science and Reports, 11*(1), 21534. https://doi.org/10.1038/s41598-021-00966-4
90 Hunziker, J., Nishida, K., Kondo, A., Ariizumi, T., & Ezura, H. (2022). Phenotypic characterization of high carotenoid tomato mutants generated by the target-AID base-editing technology. *Frontiers in Plant Science., 13*, 848560. https://doi.org/10.3389/fpls.2022.848560
91. He, Y., & Zhao, Y. (2020). Technological breakthroughs in generating transgene-free and genetically stable CRISPR-edited plants. *Abiotechnology, 1*(1), 88–96. https://doi.org/10.1007/s42994-019-00013-x
92. Lin, C. S., Hsu, C. T., Yang, L. H., Lee, L. Y., Fu, J. Y., Cheng, Q. W., Wu, F. H., Hsiao, H. C. W., Zhang, Y., Zhang, R., Chang, W. J., Yu, C. T., Wang, W., Liao, L. J., Gelvin, S. B., & Shih, M. C. (2018). Application of protoplast technology to CRISPR/Cas9 mutagenesis: From single-cell mutation detection to mutant plant regeneration. *Plant Biotechnology Journal, 16*(7), 1295–1310. https://doi.org/10.1111/pbi.12870
93. Wang, P., Zhao, F.-J., & Kopittke, P. M. (2019). Engineering crops without genome integration using nanotechnology. *Trends in Plant Science, 24*(7), 574–577. https://doi.org/10.1016/j.tplants.2019.05.004
94. Yubing, H., Min, Z., Lihao, W., Junhua, W., Qiaoyan, W., Rongchen, W., & Yunde, Z. (2019). Improvements of TKC technology accelerate isolation of transgene-free CRISPR/Cas9-edited rice plants. *Rice Science, 26*(2), 109–117. https://doi.org/10.1016/j.rsci.2018.11.001
95. Pan, C., Ye, L., Qin, L., Liu, X., He, Y., Wang, J., Chen, L., & Lu, G. (2016). CRISPR/Cas9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations. *Scientific Reports, 6*(1), 24765. https://doi.org/10.1038/srep24765
96. Xia, X., Cheng, X., Li, R., Yao, J., Li, Z., & Cheng, Y. (2021). Advances in application of genome editing in tomato and recent development of genome editing technology. *Theoretical and Applied Genetics, 134*(9), 2727–2747. https://doi.org/10.1007/s00122-021-03874-3
97. Noack, F., Engist, D., Gantois, J., Gaur, V., Hyjazie, B. F., Larsen, A., Mgonigle, L. K., Missirian, A., Qaim, M., Sargent, R. D., Souza-Rodrigues, E., & Kremen, C. (2024). Environmental impacts of genetically modified crops. *Science, 385*(6712), 9340. https://doi.org/10.1126/science.ado9340
98. Rissler, J., & Mellon, M. G. (1996). *The ecological risks of engineered crops*. MIT Press.
99. Chandrasekaran, M., Boopathi, T., & Paramasivan, M. (2021). A status-quo review on CRISPR-Cas9 gene editing applications in tomato. *International Journal of Biological Macromolecules, 190*, 120–129. https://doi.org/10.1016/j.ijbiomac.2021.08.169
100 Waltz, E. (2022). GABA-enriched tomato is first CRISPR-edited food to enter market. *Nature Biotechnology, 40*(1), 9–11. https://doi.org/10.1038/d41587-021-00026-2
101. Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., Dong, H., Dong, W., Gao, C., & Xu, C. (2018). Domestication of wild tomato is accelerated by genome editing. *Nature Biotechnology, 36*(12), 1160–1163. https://doi.org/10.1038/nbt.4273
102 Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E., & Lippman, Z. B. (2017). Engineering quantitative trait variation for crop improvement by genome editing. *Cell, 171*(2), 470–480. https://doi.org/10.1016/j.cell.2017.08.030
103. Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, M., Shima, Y., Nakamura, N., Kotake-Nara, E., Kawasaki, S., & Toki, S.

(2017). Re-evaluation of the rin mutation and the role of *RIN* in the induction of tomato ripening. *Nature Plants, 3*(11), 866–874. https://doi.org/10.1038/s41477-017-0041-5

104. Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, M., & Toki, S. (2015). CRISPR/Cas9-mediated mutagenesis of the *RIN* locus that regulates tomato fruit ripening. *Biochemical and Biophysical Research Communications, 467*(1), 76–82. https://doi.org/10.1016/j.bbrc.2015.09.117
105. Yu, Q.-h, Wang, B., Li, N., Tang, Y., Yang, S., Yang, T., Xu, J., Guo, C., Yan, P., Wang, Q., & Asmutola, P. (2017). CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. *Scientific Reports, 7*(1), 11874. https://doi.org/10.1038/s41598-017-12262-1
106. Nguyen, C. C., Van Vu, T., Shelake, R. M., Nguyen, N. T., Khanh, T. D., Kim, W.-Y., & Kim, J.-Y. (2024). Generation of parthenocarpic tomato plants in multiple elite cultivars using the CRISPR/Cas9 system. *Molecular Breeding, 44*(2), 13. https://doi.org/10.1007/s11032-024-01452-1