World Applied Sciences Journal 38 (3): 237-249, 2020
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2020.237.249

Applications of CRISPR/CAS9 Technique on Tomato (*Solanum lycopersicum*) Plant

[1,2]*W.G. Shahrour and* [1]*M.T. Sadder*

[1]Department of Horticulture and Crop Science, The University of Jordan, Amman, Jordan
[2]Department of Biotechnology, Al-Balqa Applied University, Al-Salt, Jordan

Abstract: Role of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/CAS9) technology as genome editing tool in plant has been reviewed. The most important applications of CRISPR tool in the tomato plant, from the beginning of CRISPR until now, has been summarized. Development of several physiological features in tomato, editing tomato genes to produce tolerant varieties for harsh environmental conditions, or regenerate resistant varieties against biotic stresses through CRISPR technology has been discussed. History, components and mechanism of the CRISPR system in addition to its efficacy has been presented.

Key words: CRISPR · CAS9 · Tomato · Genome editing · Mutation · Breeding

INTRODUCTION

Development in the agricultural sector is an urgent necessity for continuous production due to the continuous growth of populations in the world. In 2050, crops production is expected to be in shortage and will not meet global demand [1]. To solve this crisis, numerous studies have recommended that agricultural improvement and increase yields are real significant strategies must be applied to achieve food security [2]. Therefore, genetic improvement could be one of the successful solutions for increasing crop production.

Gene editing methods have been applied extensively for crop improvement to have new desirable agronomic traits with high stability in a short time. From these tools: Zinc Finger Nucleases (ZFNs) [3], Transcription Activator-Like Effector Nucleases (TALEN) [4], Targeting Induced Local Lesions in Genomes (TILLING) [5] and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR). In the last years, CRISPR rose as a new, competitive, simple and rapid tool that guides the genetic engineering world to a golden age. It creates loss-of-function mutations and makes valuable contributions to varietal improvement for many crops [6]. It could give more expected results to the traditional breeding pipelines, which need many years of working in multiple generations [7]. CRISPR tool could contribute to plant traits development and expanding croplands that lead definitely to global food security.

Principle of CRISPR System in Genome Editing: CRISPR system is a technique composed of two important parts; the first part is short single guiding (sg) RNA molecule that directed endonucleases protein CAS9 or Cas12 which is the second part of this system [8]. Briefly, as simply illustrated in the scheme of Figure 1, one of the endonucleases cause a double-stranded break (DSB) on the targeted DNA under the direction of sgRNA. sgRNA binds to a recognition site in DNA on the basis of base pairing [6]. Then the DSB is repaired by host cell repair pathways results in indels mutations that can knock out the targeted genes.

Development of CRISPR as a New Genome Editing Tool: The story of CRISPR was started before thirty years, but the real use of it as one of the genome editing techniques was not known before the last eight years as it is outlined in Table 1. CRISPR arrays were firstly known in the genome of *Escherichia coli* since 1987 [9]. But actually in 1993, Francisco Mojica was the first researcher worked on what named now as a CRISPR, he noted a new class of disparate repeat sequences in several prokaryotes with

Corresponding Author: Wesam Shahrour, Department of Horticulture and Crop Science, The University of Jordan, Amman, Jordan. Department of Biotechnology; Al-Balqa Applied University, Al-Salt, Jordan.

Table 1: Time outline of CRISPR history and its development.

Stage	Year	Events of CRISPR development	References
Discovery of CRISPR	1987	CRISPR is known in DNA of *Escherichia coli*	[9]
	1993	Discovery of Short Regularly Spaced Repeats (SRSRs)	[10]
	2002	Releasing of CRISPR term	[11]
Identify CRISPR function	2003	Identification of CRISPR function in bacterial immune system	[12]
	2007	Identify CRISPR role in *Streptococcus thermophiles* bacteria	[14]
Characterize the molecular biology of CRISPR	2012	Demonstrating the primary model of CRISPR/CAS9 in *Streptococcus* bacteria	[15]
CRISPR as genome editing tool	2012	Using CRISPR/CAS9 in engineering DNA	[6]
	2012	Targeting genome in mammalian cells	[16, 17, 18, 19, 20]
	2013	Appling CRISPR/CAS9 technique on model plants	[21, 22]
	2013	Appling CRISPR/CAS9 technique on crop plants	[23]
	2015	First involvement of CPF1 endnuclease in CRISPR system	[24]
		2013-2019 Continuous rapid increase in CRISPR/CAS9 applications	

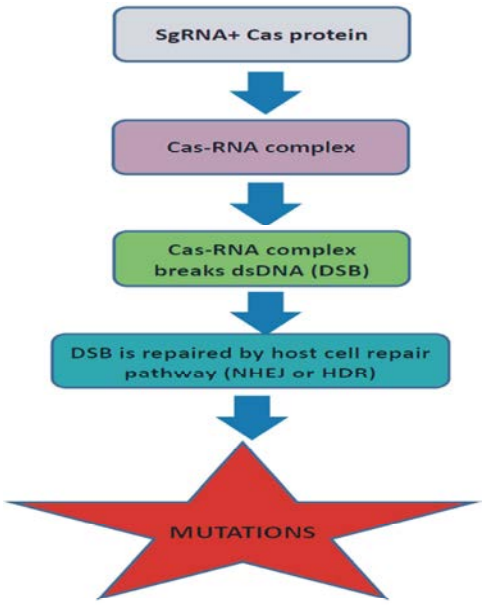

Fig. 1: Scheme to the principle of CRISPR/CAS in genome editing.

common set of properties and called it at that time Short Regularly Spaced Repeats (SRSRs) [10]. Then the term CRISPR was coined through correspondence between Francisco Mojica and Ruud Jansen whose was the first one used CRISPR term in writing in 2002 [11].

The biological function of CRISPR was identified as RNA-guided endonucleases defense system in bacterial immunity against bacteriophage [12]. These sequences were founded laterally in about 47% of archaea and bacteria [13]. Then in 2007, a researcher called Rodolphe Barrangou proved that *Streptococcus thermophiles*, a lactic acid bacteria in milk, was resistance to phage infection through using sequences spacers similar to infectious viral genome (CRISPR) [14]. The primary model of CRISPR's role in genome editing was demonstrated by Jennifer Doudna in 2012 through studying CAS9 protein in *Streptococcus* bacteria [15]. Interestingly in 2010, the breakthrough of CRISPR began when it exceeded its natural biological role to become a powerful tool in DNA editing [6]. Subsequently, the development of CRISPR/CAS9 was very rapid in several fields and it is difficult to definitely know who's the first user of CRISPR in each organism, but for sure it is considered as the pioneering genome engineering tool all over the world. The time outline of CRISPR history and stages of its development are summarized in Table 1 depending on published data from different literatures.

Using CRISPR as Genome Editing Tool in Biological Organisms: CRISPR/CAS9 technique proved to be a functional genome editing tool in many organisms. It has been used in human, mouse and zebrafish [16-18]. In Plant, several independent trials established in 2013 and got promising results. First reports using CRISPR system were in the model plants *Nicotiana benthamiana* and *Arabidopsis thaliana* [21] which were transformed by *Agrobacterium tumefaciens* to express the endonucleases and sgRNA. Also, it successfully directed to edit genomes of two important cultivated crop: rice (*Oryza sativa*) [23] and wheat (*Triticum aestivum*) [24].

Subsequently, CRISPR/CAS9 technology has been applied to numerous dicot and monocot plant species [25, 26] and generated a large number of beneficial mutants lines in minimal efforts and lower cost. Interestingly, this technique somehow similar in its action to the natural mutations. The resulted edited plants are free from any foreign genetic materials and are transgene-free mutants [27]. Recently, Ming M. *et al.,* [8] reported that CAS9 is the first promising system for plant genetic engineering in CRISPR, followed by Cas12a and then Cas12b. Theoretically, any gene can be edited or knocked out by CRISPR.

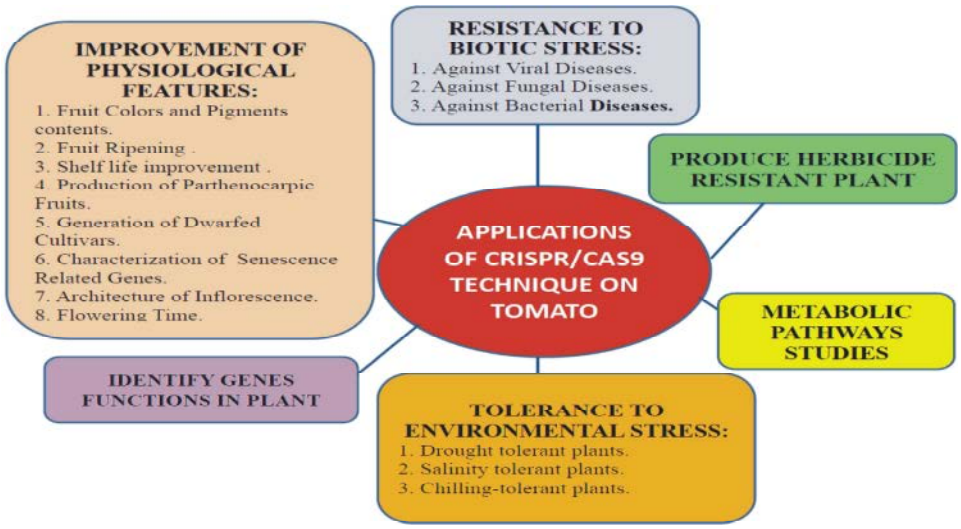

Fig. 2: Main fields for CRISPR applications on tomato plant.

Tomato Plant and CRISPR/CAS9 Technique: Tomato (*Solanum lycopersicum* L.) from Solanaceae family ranks the fourth of the most important economic horticulture crop in the world [28] and the second vegetable crop next to potato. In addition to its economic importance as a global healthy diet [29], it is also considered as a dicot model plant for genetics and developmental studies [30]. Tomato plant tissues are easy to transform [31], its diploid genome comprises twelve chromosomes with a high quality of sequences [32]. All these properties make tomato as a good candidate for breeders and genetics to be used in CRISPR/CAS9 gene editing system.

Firstly, Brooks C. *et al.,* [26] tested ability of CRISPR technology in tomato by using *ARGONAUTE7 (SlAGO7*) gene that give distinguishable phenotype. CRISPR disrupted *SlAGO7* gene with 48% efficiency (Table 2). It induced tomato plant to form needle wiry leaves instead of the normal flat compound leaves. Then, several of sgRNAs have targeted many tomato genes to improve agronomic traits or even post-harvest traits. According to our search, there is a continuous increase in the tomato traits that are targeted and developed by CRISPR all over the world. Figure 2 demonstrate main fields for CRISPR applications in tomato plant and Table (2) summarized the targeted genes by this system with the produced traits.

Improvement of Tomato Physiological Features by CRISPR/CAS9

Colors and Pigments Content in Tomato Fruits: Tomato fruit color is one of the most critical agronomic traits affect its marketing value. Some consumers prefer to eat red tomato fruit as European and American, while others as in Asia countries, especially in China and Japan, they like the pink tomato fruits [33]. Using CRISPR/CAS9 to disrupt genes control pigments accumulation enable researcher to manipulate the color of tomato fruit.

Accumulation of yellow colored flavonoid compound called naringenin chalcone (NarCh) in the peel of tomato fruit is controlled by Y gene, which encodes R2R3-MYB transcription factor (*SlMYB12*) [34]. Monogenic recessive yellow (y) will prevent NarCh accumulation and result in pink fruits tomato [35]. Five null alleles have been identified for the Y gene and used in pink tomato breeding programs which need at least five years to get results with the risk of unfavorable linkage drag [34]. While by CRISPR/CAS9 system, *SlMYB12* gene was simply disrupted to get elite red-fruited inbred lines in high efficiency reach to 90.9% [36].

Moreover, depending on the carotenoid biosynthesis pathway in plant [37], we can conclude that most genes control key enzymes in the carotenoid biosynthesis pathway in tomato were targeted by CRISPR system (Figure 3). Mutations in *carotenoid isomerase (CRTISO)* and *phytoene synthase I* (*PSY1)* genes gave orange and yellow tomato fruits with mutagenesis frequencies reached to 90% and 70% respectively [38]. *PSY1* accumulates lycopene during fruit ripening [39]. In a similar way, yellow fruit instead of red from *psy1* mutants and pale yellow corolla color instead of bright yellow from *beta-carotene hydroxylase 2* (*crtr-b2*) gene mutants were achieved through CRISPR/CAS9 way [40]. The *CrtR-b2* gene converts beta-carotene to xanthophylls in flower petals [41]. Furthermore, multiplex CRISPR/CAS9 system was used to lose function to five genes at the same time:

Table 2: List of CRISPR/CAS9 applications in tomato plants that are discussed in the review with interested traits, targeted genes, functions of mutated genes and mutation efficiency in each one.

Tomato Traits (Purposes of CRISPR)	Targeted Gene (s)	Mutated Gene Function (s)	CRISPR Efficiency (%)	References
Tested ability of CRISPR technology in tomato	*SlAGO7*	Needle wiry leaves formation	48%	[26]
Fruit color	*SlMYB12*	Pink fruit color	90.9	[36]
Fruit color	*CRTISO*	Orange fruits color	90	[38]
Fruit color	*PSY1*	Yellow fruits color	50-70	[37]
Pigments content	*Psy1*	Yellow fruit color instead of red	84	[40]
Pigments contents	Multiplex to: SGR1, LCY-E, Blc, LCY-B1 and LCY-B2	Increasing lycopene content in red fruit	0- 95.83 among target sites	[49]
Pigments content	*CrtR-b2*	pale yellow corolla colour instead of bright Yellow	84	[40]
Fruit ripening	*ALC*	delay ripening	72.73	[53]
Fruit ripening	*RIN*	lower in red color with incomplete ripening	0% - 100% among plant lines	[55]
Fruit ripening	*NOR*-like1	delayed ripening fourteen days	NA	[56]
Pectin contents	*SlPL*	delay fruit firmness	NA	[45, 46]
Pectin contents	*TBG4*	Change in pericarp color	NA	[46]
Pectin contents	*PG2a*	Change in pericarp color	NA	[46]
Fruit ripening	*lncRNAs*	Delay fruit ripening	NA	[49]
Parthenocarpic fruits	*SlIAA9*	Producing parthenocarpic fruits	100	[59]
Parthenocarpic fruits	*SlAGL6*	Producing seedless fruit under heat stress	19-37	[60]
Plant size	*PROCERA*	Dwarfed plant	NA	[62]
Senescence	*SlSBPASE*	Increasing in expression of senescence-associated genes	NA	[63]
Inflorescence Architecture	*TMF*	Faster in flowering with single flowers	NA	[65]
Inflorescence Architecture	*SlBOP*	Simplification of inflorescences to single flowers	NA	[65]
Flowering Time	*SP5G*	Rapid in flowering associated with determinate and compact growth type	NA	[68]
Abscission Zone Development	*J-2*	Pedicel abscission	NA	[72]
Metabolic pathway c-aminobutyric acid (GABA) shunt	six sites in five key genes in GABA shunt	Accumulated 19 fold of GABA	61	[75]
Visual marker	*SlPDS*	Albino phenotype	61	[79]
			83.6	[30]
Drought stress	*MAPK3*	Less tolerance to drought stress	NA	[77]
Drought stress	SlNPR1	Less tolerance to drought stress	33-47	[79]
Chilling stress	*CBFs*	Sever symptoms of chilling injury	NA	[80]
TYLCV resistance	*Rep* in viral genome	Resistance against TYLCV	NA	[84]
	CP in viral genome	Resistance against TYLCV	NA	[84]
Powdery mildew resistant	*MLO1*	Full resistance to *Oidium neolycopersici*	NA	[85]
Fungi and bacteria resistance	*SlDMR61*	Resistance to *Phytophthora capsici, Pseudomonas syringae*, *Xanthomonas perforans* and *Xanthomonas gardneri*	NA	[92]
Fungi resistance	I2 receptor protein	Resistance to *Fusarium oxysporum f. sp. lycopersici* and *Phytophthora infestans*	NA	[91]
Bacterial speck disease resistance	*SlJAZ2*	Fully bacterial speck resistant variety	96. 3% gRNA score	[93]
Herbicide resistance	*ALS*	Chlorsulfuron -resistant tomato	71	[27]
Gene identification	*SlMET1*	Defects in plant development	NA	[94]

NA: not available

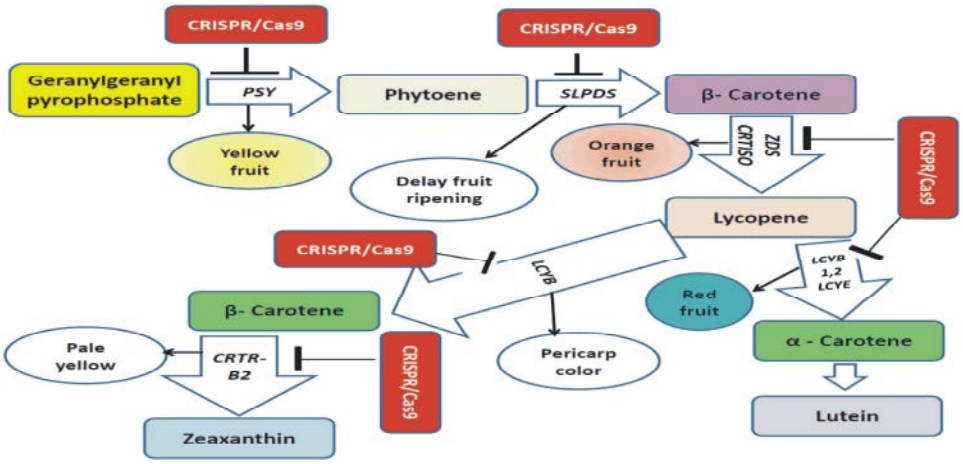

Fig. 3: Carotenoid biosynthesis pathway in tomato plant in the rectangular shapes. Targeted genes by CRISPR/CAS9 are written inside the arrows. Results for each knock out are written inside the circles.

SGR1, *lycopene cyclase (LCY E), beta-lycopene cyclase (Blc), lycopene beta cyclase 1 (LCYB1)* and *LCYB2* (Figure 3). Red fruits were resulted from mutants tomato with five folds of lycopene contents more than the wild type [42]. Otherwise, lycopene content was increased 5% and associated with deep-red colored tomato fruits by null mutation of β-cyclase 2 (*LCYB2*) gene in transgenic plants [43].

These generated mutants in the key genes of carotenoid biosynthesis pathway via CRISPR/CAS9 could be incorporated in different breeding programs. Different combinations of these mutants could be used for production different tomato traits as fruits colors, pigments contents, or biochemical properties. Also, some of edited genes could be used as selectable markers with other traits in multiplex CRISPR due to their easily visual detection.

Tomato Fruit Ripening and Shelf Life: In tomato, the climacteric species plant, there are several genes have been studied extensively at a molecular level due to their role in fruit ripening regulation. Degradation of pectin, the heteropolysaccharide found in primary cell walls is one of the important processes leads to softening and ripening in tomato fruits [44]. Several enzymes degrade pectin in tomato were mutagenized by CRISPR system as; *pectate lyase* (*PL)* [45], *β- galactanase* (*TBG4*) and *polygalacturonase 2a* (*PG2a*) [46].

Silencing of *SlPL* gene to delay fruit postharvest deterioration was achieved in tomato through RNA interference technology [47] as well as by CRISPR/CAS9 [45] which indicated that the *PL* gene fastens fruit softening by breaks down the crosslinked HG polymer in the middle lamella and tricellular junctions of the cell wall without any detrimental effect in fruit weight, plant yield, total soluble solids, ethylene biosynthesis, or fruit color.

In another trial, a comparative study to physiochemical tomato fruits traits that produced from different CRISPR mutants lines (*TBG4, PG2a* and *PL* mutants) indicated that *PL* has the major impact on fruit softening [48]. Since *PL* CRISPR mutants gave firmer fruit than *PG2a* and *TBG4 CRISPR* mutants. Whereas *TBG4* and *PG2a* mutants lines showed change in pericarp color, which is resulted from *lycopene beta-cyclase (beta-LCY*) activity in increasing β-carotene and reduction lycopene content (Figure 3). The *pl* CRISPR mutants proved again the effectiveness of CRISPR-edited alleles in delay fruit firmness.

Recently, CRISPR/CAS9 was used to identify this transcripts through loss-of-function mutants. They founded that lycopene accumulation and ethylene production were highly reduced in *lncRNA1459* mutants which significantly repress tomato ripening process [49]. Also the same technique proved that *lncRNA1459* mutants can greatly delay fruit ripening through downregulation in Lycopene synthesis genes as *phytoene desaturase* (*SLPDS*), *PSY* and *f-carotene desaturase* (*ZDS*) and upregulation to *lycopene b-cyclase* (*LCY-b*) gene [50].

In other hand, successful long shelf life verities of tomato were obtained through encompassed previous naturally mutated genes in breeding programs as *ripening inhibitor (rin)*, *non-ripening* (*nor)* and *alcobaca* (*alc)* mutations in plant breeding programs [51, 52]. *ALC* gene

was targeted by CRISPR/CAS9 system to prolong the shelf life of tomato and improve storage time for tomato breeding elites [53]. Mutations in *RIN* gene was one of the naturally mutated genes in tomato that produced green fruits never colored red and keep flesh firmness for several months with suppression to ethylene production [54]. So, *RIN* gene was a good candidate to target by CRISPR/CAS9 mutagenesis technology. Three of sgRNAs were designed for targeting *RIN* gene in three independent regions which led to less RIN protein accumulation and less red color in *rin* mutants fruits than wild type with incomplete ripening [55].

Moreover, An experimental search was systematically done to *NAC* genes using both CRISPR/CAS9 and virus-induced gene silencing (VIGS) tools to identify the targets of NAC transcription factors. Silencing of *NOR*-like1 gene by VIGS caused inhibition to specific features of ripening. Notably, CRISPR/CAS9 was also produced similar phenotypes in fruits that resulted from VIGS-NOR-like1 as fourteen days delay in ripening initiation, inhibited chlorophyll and softening loss, reduced ethylene production and decreased the accumulation of lycopene [56]. CRISPR/CAS9 was clearly proved that *NOR-like1* is a positive regulator to tomato fruit ripening, in addition to its role in regulating transcription with the related network. Therefore, if a multiplex CRISPR is established by combine *NOR-like1* gene with other genes from the carotenoid biosynthesis pathway, the tomato fruit ripening process will be easily manipulated.

Production of Parthenocarpic Tomato Fruits: Production of parthenocarpic tomato fruits resulted in more tolerance plant to environmental conditions [57] with high industrial beneficial properties. Wang *et al.* [58] reported that IAA9 transcription factor was expressed in fruit as well as in leaf and parthenocarpy tomato fruits were produced when antisense RNA downregulate the *IAA9* gene. Also, using CRISPR/CAS9 system to disrupt *SlIAA9* gene in Micro-Tom cultivar and Ailsa Craig commercial cultivar was very practical way in producing parthenocarpic fruits. PCR-RFLP marker indicated that the 20 nts gRNA knocked *SlIAA9* gene by 100% efficiency at the somatic cell level with high expression level to gRNA and CAS9 in regenerated transgenic plants during callus formation [59]. Furthermore, producing parthenocarpic fruits in tomato through CRISPR/CAS9 mutagenesis was done by inducing mutation to *SlAGAMOUS-LIKE 6* (*SlAGL6*) gene, with fruits morphology in mutants plants similar to the wild type plants [60].

Generation of Dwarfed Tomato Cultivars: Latterly, production of dwarfed tomato plant through the CRISPR/CAS9 system has been considered as a desirable commercial trait obtained through reducing gibberellin response in mutant plants [61, 62]. sgRNA was used to induce loss of function in *PROCERA* gene, which encodes DELLA protein and depress tomato growth. Plant mutants with homozygotes (PRO^D/PRO^D) and heterozygotes (PRO^D/PRO) alleles were smaller in size than wild type plants (*PRO/PRO*). Although the PRO^D/PRO seedling gave intermediate phenotype, but retain dwarfed at the adult stage as PRO^D/PRO^D mutants. Comparing with wild type plants, CRISPR induced mutants were in shorter stems and internodes, darker green leaves and delayed in flowering. Notably, no significant reduction in fruits sizes, whereas fruit weight was significantly less with lower seeds number per fruit and same average number of fruits per plant. The resulted dwarfed tomato cultivars by CRISPR/CAS9 could be useful in cultivation practices. Less side-shoot management, pruning, trailing and additional mechanical support in dwarfed cultivars.

Characterization of Senescence Related Genes in Tomato: Jasmonates (JAs) hormones induce leaf senescence in tomato as well as several plant species. Role of sedoheptulose-1,7-bisphosphatase (SBPase) enzyme in methyl jasmonate (MeJA)- and dark- induced leaf senescence in tomato plants was demonstrated through site-directed mutagenesis [63]. Mutagenesis of *SlSBPASE* ended with senescence-associated characteristics; as increasing in the expression of senescence-associated genes, chlorophyll losing, photosynthesis repression and leakage of membrane ion.

Architecture of Tomato Inflorescence: Architecture of inflorescence in tomato is a marked diversity regulated by *TERMINATING FLOWER* (*TMF*) gene with a transcriptional cofactors BLADE-ON-PETIOLE (SLBOP) that inhibits maturation of meristem to maintain it in the vegetative State [64]. *mf* mutants produced by CRISPR/CAS9 accelerated flowering with single flowers instead of primary inflorescences. Also, same phenotypes resulted from the elimination of *SlBOP* function by CRISPR/CAS9 [65]. This indicated that flowering defection was enhanced by combination both *slbop* and *tmf* mutants and there is an interaction between *SlBOP-TMF* family for gradual meristem maturation to enhance inflorescence complexity. CRISPR/CAS9 was helpful in

understanding the mechanism of meristem maturation, inflorescence architecture and production of flowers in tomato plants.

Flowering Time in Tomato: Tomato belongs to day-neutral plant, even though there were some suggestions considered wild species of tomato as short-day plants, in addition to many cultivars that delay in flowering under long day conditions [66, 67]. CRISPR/CAS9 technology could be an ideal tool to recognize tomato photoperiod response through introduce mutations in the flowering repressor florigen paralog *SELF-PRUNING 5G* (*SP5G*) gene [68]. At conditions of long days, wild species expressed *SP5G* in high levels whereas cultivated plants were not. Mutants of *sp5g* were rapid in flowering associated with determinate and compact growth type, which led to early production of the yield. During short day conditions, flowering was similar in both wild-type and *sp5g* mutants. This confirm that *SP5G* is the responsible gene for loss of day length sensitivity in tomato. The resulted mutants could give light to one step breeding process in eliminating day length sensitivity and produce early harvesting varieties.

Development of Abscission Zone in Tomato Fruit: One of the advantageous postharvest traits effect on quality of tomato fruit yield and must be managed, is the formation of abscission zone in the fruit pedicel. This trait has been successfully studied and two independent non-allelic loci were determined in controlling the jointless phenotype; *jointless2* (*j2*) and *jointless* (*j*) [69, 70]. *j-2* gene was used mostly in breeding programs and successfully used in introgression to cause pedicel abscission without any drawback [71]. CRISPR/CAS9 system was applied to induce loss of function mutations in *j2* gene that prove expression of "MADS-box protein SlMBP2" reached to a conclusion that *SlMBP21* is the *J2* gene [72].

CRISPR/CAS9 and Metabolic Pathways Studies in Tomato Plants: In the field of metabolic engineering technologies, CRISPR system could be helpful in increasing plant products, improving quality of plants and changing the metabolic composition of the cell [73, 74]. Commonly there are several regulatory key genes in each metabolic pathway. One of the metabolic pathways have been targeted by CRISPR/CAS9 system was gamma-aminobutyric acid (GABA) pathway which participate in many physiological processes [75]. Robust CRISPR/CAS9 targeted six sites referred to five key genes in GABA and g Genome-edited plants were achieved with single to quadruple mutants lead to nineteen fold accumulation of GABA metabolites in the leaves and fruits of quadruple mutants that significantly affected vegetative and reproductive growth of tomato.

CRISPR/CAS9 Decreases the Impact of Environmental Stress on Tomato: Environmental conditions have a large impact on crop production [76]. Recently, CRISPR/CAS9 technology have great potential in increase plant tolerance to environmental stresses. In tomato, numerous genes related to environmental stresses were identified and characterize their function against abiotic stress (Table 2).

CRISPR/CAS9 complex was used to mutate drought related gene *Mitogen-activated protein kinase3*(*MAPK3*) [77]. Plants with *slmapk3* mutants under drought conditions were more wilting, more membrane damage, higher rate of hydrogen peroxide production and less activity in antioxidant enzymes than the wild type plants. So *SlMAPK3* modulates stress related genes expression and protects plasma membrane from oxidative damage. Thus in order to get a high tolerant tomato plant to drought stress, genetic engineering must be applied to upregulate expression of *SlMAPK3* gene.

Nonexpressor of pathogenesis-related 1(NPR1) is a receptor protein for salicylic acid defense hormone [78]. Mutant tomato (*SlNPR)* that resulted by CRISPR method had higher levels of MDA and H_2O_2 and low levels of APX, CAT, POD and SOD antioxidant enzymes. In addition to that other drought-related key genes as *SlGST*, *SlDHN* and *SlDREB* were suppressed [79]. These observations proved that *SlNPR1* gene could be a good candidate in tomato breeding to tolerate drought conditions.

Moreover, the resulted *C-repeat binding factors* (*SlCBFs)*mutants by CRISPR system were negatively affected by chilling effect, it showed higher MDA, H_2O_2, electrolyte leakage and antioxidant enzymes than tomato wild type [80]. A clear reduction in chilling tolerance in *slcbf1* mutants investigated gene mediation in tomato chilling resistance. This lead to understanding the regulatory mechanism associated with *SlCBFs* -mediated chilling tolerance in tomato plants.

Role of CRISPR/CAS9 in Tomato Resistance to Biotic Stress: Using CRISPR/CAS9 technology in controlling plant diseases has a high impact on

reducing crop losses resulted from biotic stresses. It has been applied against several diseases caused by viruses, fungi and bacteria.

Borrelli *et al.* [81] listed three important reasons make CRISPR technique as a promising technique in pathogen control: the first reason is the availability and efficient knowledge of molecular mechanisms for numerous pathosystems that permit determination target genes and gain resistance plant. The second reason is single gene modification in a plant can be enough for disease resistance. And the last reason that CRISPR/CAS9 mutagenesis is an easy method to apply in plant diseases protection through loss-of-function mutation in susceptible genes.

The viral resistance could be achieved through integration of CRISPR- encoding sequences in the host plant to interfere viral genome. Different frequencies of virus inference were achieved by different gRNAs in the model plants *Arabidopsis* and *Nicotiana benthamiana* [82]. Mutated *Nicotiana benthamiana* that was infected by Tomato Yellow Leaf Curl Virus (TYLCV) showed stable expression of CAS9 with less viral DNA accumulation and reduction in disease symptoms [83]. Likewise, CRISPR/CAS9 system gave efficient interference to *Replicase* (*Rep*) and *coat protein* (*CP*) genes of TYLCV in the tomato plant. It decreased viral genome accumulation in *cp* mutants more than *rep* mutants [84].

According to the fungal diseases which cause annually high economic losses in crop production susceptible genes ere targeted and mutagenized by CRISPR/CAS9. "Tomelo", a full resistant powdery mildew variety to *Oidium neolycopersici,* was produced just within few months through targeting two sites at *MILDEW RESISTANT LOCUS O* (*SlMlo1*) by CRISPR/CAS9 system [85]. Notably, from the 16 *SlMlo* identified genes in tomato (*SlMlo1* to *SlMlo16)*, *SlMlo1* is the most susceptible gene to powdery mildew [86]. Meanwhile, mutants of *MLO* genes are well-defined resistant genes to powdery mildew in several plants as grape (*VvMLO7*) [87] and wheat (*TaMLO-A1*) [24]. In tomato, whole genome sequencing of Tomelo mutant variety confirmed that it is a free-transgenic plant carry indistinguishable deletion mutation from that occur naturally. There were no any negative effects on the weight or morphology of Tomelo fruits compared with wild type tomato [85].

Likewise, depending on the published transcriptomic data to *SlDMR61(*Solyc03g080190) tomato gene, variation in *SlDMR6-1* regulation was clear when tomato infected by *Phytophthora capsici* and *Pseudomonas syringae* pv. *tomato* (DC3000) [88, 89]. There was a strong suggestion that *SlDMR6*-1 may has function in tomato like that function of its ortholog *DMR6* in *Arabidopsis thaliana* [90]. Moreover, responsiveness *to Fusarium oxysporum f. sp. lycopersici* and *Phytophthora infestans* was increased in tomato when a mutation was done by gene editing technology to single-residue in N-terminal CC domain of tomato I2 receptor protein [91].

Surprisingly, this tomato mutant was resistant at the same time to *Pseudomonas syringae*, *Xanthomonas perforans* and *Xanthomonas gardneri* bacteria [92].

Virtually, CRISPR technology would be an excellent way for producing a bacterial resistant plant. It implicates plant-pathogen interaction and edited susceptible genes in crop species. CRISPR/CAS9 generated resistant tomato variety to bacterial speck disease with a stable transmission, which is caused by *Pseudomonas syringae* pv. *tomato* through editing *SlJAZ2* gene, which is a co-receptor to coronatine (COR) [93]. Similarly, the ortholog gene in *Arabidopsi (AtJAZ2*) was truncated to generate *Atjaz2Δjas* mutant which prevented stomatal re-opening after the bacterial invasion [91].

A novel resistance gene could be engineered by gene editing that gives multipathogen resistant plants. In other words, results obtained through CRISPR/CAS9 engineering system confirmed the ability of this technique to be a powerful tool in producing broad-spectrum pathogens resistant plants.

CRISPR/CAS9 Can Produce Herbicide Resistant Tomato Plant: One of the recent approaches in CRISPR/CAS9 applications on tomato plant is the production of herbicide resistant tomato. Chlorsulfuron-resistant tomato and potato were produced through knocked *acetolactate synthase* (*ALS*) gene precisely with cytidine base editors (CBEs) tool in an efficient way reach to 71%. *Agrobacterium*-mediated transformation was applied to deliver CBE components. In tomato, 289 edited plantlets from 378 (76%) were strongly resistant to chlorsulfuron [27].

CRISPR/CAS9 Can Identify Genes Functions in Tomato Plant: Another important application of CRISPR/CAS9 in plant and particularly in tomato is the genes identification. Gene editing was helpful in understanding regulation mechanism and function of DNA methylation in tomato plants. Function of *Methyltransferase 1* (*SlMET1*) gene in tomato was identified through created *slmet1* mutants via CRISPR technology [94].

CONCLUSION

Succeeding of CRISPR system in inducing targeted knockout lines in a precise manner gave a direct and positive impact on tomato plant yield as well as on the quality of tomato fruits. It is considered as a scientific breakthrough in genome editing techniques that acts like a star among other tools. It gave the ability to determine the favored tomato fruit colors, performed desirable post-harvest traits and increased tomato shelf life. Meanwhile this method has produced tomato lines with specific attractive characters like parthenocarpic plants, dwarfed varieties, plant with abscission pedicle zone, or even altered flowering time. All these make it a powerful tool in determining different metabolic pathways and enable researchers to identify and modify genes that could lead to crop improvement.

In another hand, through using CRISPR system, many applications were done to reduce the effect of abiotic stress in tomato production through increasing tomato tolerance to the hard environmental conditions. In the same way, resistant tomato plants have been regenerated against fungal, bacterial and viral diseases. A strategy for producing broad spectrum resistant tomato against several pathogens is attractive that need many efforts to apply.

List of CRISPR applications could be endless. Every day a new idea with new use can be added with limitless purposes. A public agricultural agency should be formed in order to organize and support CRISPR research all over the world in every crop plant. Apply this technique for commercial tomato plant production or incorporate the resulted lines from CRISPR in breeding pipelines should be carried out. These approaches will be real solutions to the increasing global demand.

REFERENCES

1. Tilman, D., C. Balzer, J. Hill and B.L. Befort, 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences, 108: 20260-20264.
2. Tscharntke, T., Y. Clough, T.C. Wanger, L. Jackson, I. Motzke, I. Perfecto, J. Vandermeer and A. Whitbread, 2012. Global food security, biodiversity conservation and the future of agricultural intensification. Biological Conservation, 151: 53-59.
3. Kim, Y.G., J. Cha and S. Chandrasegaran, 1996. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proceedings of the National Academy of Sciences, 93: 1156-1160.
4. Christian, M., T. Cermak, E.L. Doyle, C. Schmidt, F. Zhang, A. Hummel, A.J. Bogdanove and D.F. Voytas, 2010. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics, 186: 757-761.
5. McCallum, C.M., L. Comai, E.A. Greene and S. Henikoff, 2000. Targeting induced locallesions in genomes (TILLING) for plant functional genomics. Plant physiology, 123(2): 439-442.
6. Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna and E. Charpentier, 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 337: 816-821.
7. Forster, B.P., B.J. Till, A.M.A. Ghanim, H.O.A. Huynh, H. Burstmayr and P.D.S. Caligari, 2014. Accelerated plant breeding. Cab Rev, 9: 1-16.
8. Ming, M., Q. Ren, C. Pan, Y. He, Y. Zhang, S. Liu, Z. Zhong, J. Wang, A.A. Malzahn, J. Wu and X. Zheng, 2020. CRISPR-Cas12b enables efficient plant genome engineering. Nature Plants, 6: 202-208.
9. Ishino, Y., H. Shinagawa, K. Makino, M. Amemura and A. Nakata, 1987. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli and identification of the gene product. Journal of Bacteriology, 169: 5429-5433.
10. Mojica, F.J.M., C. Ferrer, G. Juez and F. Rodriguez-Valera, 1995. Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning. Molecular Microbiology, 17: 85-93.
11. Jansen R., J.D. Embden, W. Gaastra and L.M. Schouls, 2002. Identification of genes that are associated with DNA repeats in prokaryotes. Molecular Microbiology, 43: 1565-1575.
12. Bolotin, A., B. Quinquis, A. Sorokin and S.D. Ehrlich, 2005. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology, 151: 2551-2561.
13. Hidalgo-Cantabrana, C., R. Sanozky-Dawes and R. Barrangou, 2018. Insights into the human virome using CRISPR spacers from microbiomes. Viruses, 10: 479.

14. Barrangou, R., C. Fremaux, H. Deveau, M.Richards, P. Boyaval, S. Moineau, D.A. Romero and P. Horvath, 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315: 1709-1712.
15. Doudna, J.A. and S.H. Sternberg, 2017. A crack in creation. In Gene editing and the unthinkable power to control evolution. Houghton Mifflin Harcourt.
16. Cho, S.W., S. Kim, J.M. Kim and J.S. Kim, 2013. Targeted genome engineering in human cells with the CAS9 RNA-guided endonuclease. Nature Biotechnology, 31: 230.
17. Cong, L., F.A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P.D. Hsu, X. Wu, W. Jiang, L.A. Marraffini and F. Zhang, 2013. Multiplex genome engineering using CRISPR/CAS systems. Science, 339: 819-823.
18. Hwang, W.Y., Y. Fu, D. Reyon, M.L. Maeder, S.Q. Tsai, J.D. Sander, R.T. Peterson, J.J. Yeh and J.K. Joung, 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature Biotechnology, 31: 227.
19. Jinek, M., A. East, A. Cheng, S. Lin, E. Ma and J. Doudna, 2013. RNA-programmed genome editing in human cells. elife, 2: e00471.
20. Mali, P., L. Yang, K.M. Esvelt, J. Aach, M. Guell, J.E. DiCarlo, J.E. Norville and G.M. Church, 2013. RNA-guided human genome engineering via Cas 9. Science, 339: 823-826.
21. Li, J.F., J.E. Norville, J. Aach, M. McCormack, D. Zhang, J. Bush, G.M. Church and J. Sheen, 2013. Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and CAS9. Nature Biotechnology, 31: 688.
22. Nekrasov, V., B. Staskawicz, D. Weigel, J.D. Jones and S. Kamoun, 2013. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using CAS9 RNA-guided endonuclease. Nature Biotechnology, 31: 691.
23. Miao, J., D. Guo, J. Zhang, Q. Huang, G. Qin, X. Zhang, J. Wan, H. Gu and L.J. Qu, 2013. Targeted mutagenesis in rice using CRISPR-Cas system. Cell research, 23: 1233.
24. Wang, Y., X. Cheng, Q. Shan, Y. Zhang, J. Liu, C. Gao and J.L. Qiu, 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology, 32: 947.
25. Liang, Z., K. Zhang, K. Chen and C. Gao, 2014. Targeted mutagenesis in Zea mays using TALENs and the CRISPR/CAS system. Journal of Genetics and Genomics, 41: 63-68.
26. Brooks, C., V. Nekrasov, Z.B. Lippman and J. Van Eck, 2014. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiology, 166: 1292-1297.
27. Veillet, F., L. Perrot, L. Chauvin, M.P. Kermarrec, A. Guyon-Debast, J.E. Chauvin, F. Nogué and M. Mazier, 2019. Transgene-Free Genome Editing in Tomato and Potato Plants Using Agrobacterium-Mediated Delivery of a CRISPR/CAS9 Cytidine Base Editor. International Journal of Molecular Sciences, 20: 402.
28. Vincent, H., J. Wiersema, S. Kell, H. Fielder, S. Dobbie, N.P. Castañeda-Álvarez, L. Guarino, R. Eastwood, B. Leün and N. Maxted, 2013. A prioritized crop wild relative inventory to help underpin global food security. Biological Conservation, 167: 265-275.
29. Du, M., J. Zhao, D.T. Tzeng, Y. Liu, L. Deng, T. Yang, Q. Zhai, F. Wu, Z. Huang, M. Zhou and Q. Wang, 2017. MYC2 orchestrates a hierarchical transcriptional cascade that regulates jasmonate-mediated plant immunity in tomato. The Plant Cell, 29: 1883-1906.
30. Pan, C., L. Ye, L. Qin, X. Liu, Y. He, J. Wang, L. Chen and G. Lu, 2016. CRISPR/CAS9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations. Scientific Reports, 6: 24765.
31. Van, J.E., D.D. Kirk and A.M. Walmsley, 2006. Tomato (*Lycopersicum esculentum*). Methods in molecular biology (Clifton, NJ), 343: 459-473.
32. Tomato Genome Consortium, 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature, 485: 635.
33. Lin, T., G. Zhu, J. Zhang, X. Xu, Q. Yu, Z. Zheng, Z. Zhang, Y. Lun, S. Li, X. Wang and Z. Huang, 2014. Genomic analyses provide insights into the history of tomato breeding. Nature Genetics, 46: 1220.
34. Fernandez-Moreno, J.P., O. Tzfadia, J. Forment, S. Presa, I. Rogachev, S. Meir, D. Orzaez, A. Aharoni and A. Granell, 2016. Characterization of a new pink-fruited tomato mutant results in the identification of a null allele of the SlMYB12 transcription factor. Plant Physiology, 17: 1821-1836.
35. Ballester, A.R., J. Molthoff, R. de Vos, B. te Lintel Hekkert, D. Orzaez, J.P. Fernández-Moreno, P. Tripodi, S. Grandillo, C. Martin, J. Heldens and M. Ykema, 2010. Biochemical and molecular analysis of pink tomatoes: deregulated expression of the gene encoding transcription factor SlMYB12 leads to pink tomato fruit color. Plant physiology, 152: 71-84”

36. Deng, L., H. Wang, C. Sun, Q. Li, H. Jiang, M. Du, C.B. Li and C. Li, 2018. Efficient generation of pink-fruited tomatoes using CRISPR/CAS9 system. Journal of genetics and genomics= Yi Chuan Xue Bao, 45: 51.
37. Ampomah-Dwamena, C., T. McGhie, R. Wibisono, M. Montefiori, R.P. Hellens and A.C. Allan, 2009. The kiwifruit lycopene beta-cyclase plays a significant role in carotenoid accumulation in fruit. Journal of Experimental Botany, 60: 3765-3779.
38. McCormick, S., 2018. Red fruit, orange fruit, orange fruit, red fruit: genome editing in tomato. The Plant Journal: for Cell and Molecular Biology, 95: 3-4.
39. Giorio, G., A.L. Stigliani and C. D'Ambrosio, 2008. Phytoene synthase genes in tomato (*Solanum lycopersicum* L.)-new data on the structures, the deduced amino acid sequences and the expression patterns. The FEBS Journal, 275: 527-535.
40. D'Ambrosio, C., A.L. Stigliani and G. Giorio, 2018. CRISPR/CAS9 editing of carotenoid genes in tomato. Transgenic Research, 27: 367-378.
41. Stigliani, A.L., G. Giorio and C. D'ambrosio, 2011. Characterization of P450 carotenoid β and ε-hydroxylases of tomato and transcriptional regulation of xanthophyll biosynthesis in root, leaf, petal and fruit. Plant and cell Physiology, 52: 851-865.
42. Li, C., Y. Zong, Y. Wang, S. Jin, D. Zhang, Q. Song, R. Zhang and C. Gao, 2018. Expanded base editing in rice and wheat using a CAS9-adenosine deaminase fusion. Genome Biology, 19: 59.
43. Ronen, G., L. Carmel-Goren, D. Zamir and J. Hirschberg, 2000. An alternative pathway to β-carotene formation in plant chromoplasts discovered by map-based cloning of Beta and old-gold color mutations in tomato. Proceedings of the National Academy of Sciences, 97: 11102-11107.
44. Brummell, D.A., 2006. Cell wall disassembly in ripening fruit. Functional Plant Biology, 33: 103-119.
45. Uluisik, S., N.H. Chapman, R. Smith, M. Poole, G. Adams, R.B. Gillis, T.M. Besong, J. Sheldon, S. Stiegelmeyer, L. Perez and N. Samsulrizal, 2016. Genetic improvement of tomato by targeted control of fruit softening. Nature Biotechnology, 34: 950.
46. Wang, D., T.H. Yeats, S. Uluisik, J.K. Rose and G.B. Seymour, 2018. Fruit softening: Revisiting the role of pectin. Trends in Plant Science, 23: 302-310.
47. Yang, L., W. Huang, F. Xiong, Z. Xian, D. Su, M. Ren and Z. Li, 2017. Silencing of Sl PL, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life and reduced susceptibility to grey mould. Plant Biotechnology Journal, 15: 1544-1555.
48. Wang, W., Q. Pan, F. He, A. Akhunova, S. Chao, H. Trick and E. Akhunov, 2018. Transgenerational CRISPR-CAS9 activity facilitates multiplex gene editing in allopolyploid wheat. The CRISPR Journal, 1: 65-74.
49. Li, X., Y. Wang, S. Chen, H. Tian, D. Fu, B. Zhu, Y. Luo and H. Zhu, 2018. Lycopene is enriched in tomato fruit by CRISPR/CAS9-mediated multiplex genome editing. Frontiers in Plant Science, 9: 559.
50. Nelson, B.R., C.A. Makarewich, D.M. Anderson, B.R. Winders, C.D. Troupes, F. Wu, A.L. Reese, J.R. McAnally, X. Chen, E.T. Kavalali and S.C. Cannon, 2016. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. Science, 351: 271-275.
51. Paran, I. and E. Van Der Knaap, 2007. Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. Journal of Experimental Botany, 58: 3841-3852.
52. Garg, N. and D.S. Cheema, 2008. Genotype× Environment Interactions for Shelf Life and Yield Attributes in Tomato Hybrids Heterozygous at rin, nor, or alc Loci. Journal of Crop Improvement, 22: 17-30.
53. Yu, Q.H., B. Wang, N. Li, Y. Tang, S. Yang, T. Yang, J. Xu, C. Guo, P. Yan, Q. Wang and P. Asmutola, 2017. CRISPR/CAS9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. Scientific Reports, 7: 11874.
54. Tigchelaar, E.C., 1978. Genetic regulation of tomato fruit ripening. Hort-Science, 13: 508-513.
55. Ito, Y., A. Nishizawa-Yokoi, M. Endo, M. Mikami and S. Toki, 2015. CRISPR/CAS9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. Biochemical and biophysical research Communications, 467: 76-82.
56. Gao, Y., W. Wei, X. Zhao, X. Tan, Z. Fan, Y. Zhang, Y. Jing, L. Meng, B. Zhu, H. Zhu and J. Chen, 2018. A NAC transcription factor, NOR-like1, is a new positive regulator of tomato fruit ripening. Horticulture Research, 5: 1-18.
57. Ariizumi, T., K. Murase, T.P. Sun and C.M. Steber, 2008. Proteolysis-independent downregulation of DELLA repression in Arabidopsis by the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1. The Plant Cell, 20: 2447-2459.
58. Wang, H., B. Jones, Z. Li, P. Frasse, C. Delalande, F. Regad, S. Chaabouni, A. Latche, J.C. Pech and M. Bouzayen, 2005. The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. The Plant Cell, 17: 2676-2692.

59. Ueta, R., C. Abe, T. Watanabe, S.S. Sugano, R. Ishihara, H. Ezura, Y. Osakabe and K. Osakabe, 2017. Rapid breeding of parthenocarpic tomato plants using CRISPR/CAS9. Scientific Reports, 7: 1-8.
60. Klap, C., E. Yeshayahou, A.M. Bolger, T. Arazi, S.K. Gupta, S. Shabtai, B. Usadel, Y. Salts and R. Barg, 2017. Tomato facultative parthenocarpy results from Sl AGAMOUS-LIKE 6 loss of function. Plant Biotechnology Journal, 15: 634-647.
61. Willige, B.C., S. Ghosh, C. Nill, M. Zourelidou, E.M. Dohmann, A. Maier and C. Schwechheimer, 2007. The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. The Plant Cell, 19: 1209-1220.
62. Tomlinson, L., Y. Yang, R. Emenecker, M. Smoker, J. Taylor, S. Perkins, J. Smith, D. MacLean, N.E. Olszewski and J.D. Jones, 2019. Using CRISPR/CAS9 genome editing in tomato to create a gibberellin-responsive dominant dwarf DELLA allele. Plant Biotechnology Journal, 17: 132-140.
63. Ding, F., M. Wang and S. Zhang, 2018. Sedoheptulose-1, 7-bisphosphatase is involved in methyl jasmonate-and dark-induced leaf senescence in tomato plants. International Journal of Molecular Sciences, 19: 3673.
64. MacAlister, C.A., S.J. Park, K. Jiang, F. Marcel, A. Bendahmane, Y. Izkovich, Y. Eshed and Z.B. Lippman, 2012. Synchronization of the flowering transition by the tomato TERMINATING FLOWER gene. Nature Genetics, 44: 1393.
65. Xu, C., S.J. Park, J. Van Eck and Z.B. Lippman, 2016. Control of inflorescence architecture in tomato by BTB/POZ transcriptional regulators. Genes and Development, 30: 2048-2061.
66. Wittwer, S.H., 1963. Photoperiod and flowering in the tomato (*Lycopersicon esculentum* Mill.). Proceedings of the American Society for Horticultural Science, 83: 688-694.
67. Hurd, R.G., 1973. Long-day effects on growth and flower initiation of tomato plants in low light. Annals of Applied Biology, 73: 221-228.
68. Soyk, S., N.A. Müller, S.J. Park, I. Schmalenbach, K. Jiang, R. Hayama, L. Zhang, J. Van Eck, J.M. Jiménez-Gómez and Z.B. Lippman, 2017. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nature Genetics, 49: 162.
69. Mao, L., D. Begum, S.A. Goff and R.A. Wing, 2001. Sequence and Analysis of the Tomato JOINTLESSLocus. Plant physiology, 126: 1331-1340.
70. Zhang, H.B., M.A. Budiman and R.A. Wing, 2000. Genetic mapping of jointless-2 to tomato chromosome 12 using RFLP and RAPD markers. Theoretical and Applied Genetics, 100: 1183-1189.
71. Szymkowiak, E.J. and E.E. Irish, 1999. Interactions between jointless and wild-type tomato tissues during development of the pedicel abscission zone and the inflorescence meristem. The Plant Cell, 11: 159-175.
72. Roldan, M.V.G., C. Périlleux, H. Morin, S. Huerga-Fernandez, D. Latrasse, M. Benhamed and A. Bendahmane, 2017. Natural and induced loss of function mutations in SlMBP21 MADS-box gene led to jointless-2 phenotype in tomato. Scientific Reports, 7: 4402.
73. Alagoz, Y., T. Gurkok, B. Zhang and T. Unver, 2016. Manipulating the biosynthesis of bioactive compound alkaloids for next-generation metabolic engineering in opium poppy using CRISPR-Cas 9 genome editing technology. Scientific Reports, 6: 30910.
74. Schaart, J.G., C.C. van de Wiel, L.A. Lotz and M.J. Smulders, 2016. Opportunities for products of new plant breeding techniques. Trends in Plant Science, 21: 438-449.
75. Li, R., R. Li, X. Li, D. Fu, B. Zhu, H. Tian, Y. Luo and H. Zhu, 2018. Multiplexed CRISPR/CAS9?mediated metabolic engineering of ã?aminobutyric acid levels in *Solanum lycopersicum*. Plant Biotechnology Journal, 16: 415-427.
76. Foley, J.A., N. Ramankutty, K.A. Brauman, E.S. Cassidy, J.S. Gerber, M. Johnston, N.D. Mueller, C. O'Connell, D.K. Ray, P.C. West and C. Balzer, 2011. Solutions for a cultivated planet. Nature, 478: 337.
77. Wang, L., L. Chen, R. Li, R. Zhao, M. Yang, J. Sheng and L. Shen, 2017. Reduced drought tolerance by CRISPR/CAS9-mediated SlMAPK3 mutagenesis in tomato plants. Journal of agricultural and food Chemistry, 65: 8674-8682.
78. Wu, Y., D. Zhang, J.Y. Chu, P. Boyle, Y. Wang, I.D. Brindle, V. De Luca and C. Després, 2012. The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Reports, 1: 639-647.
79. Li, X., L. Huang, Y. Zhang, Z. Ouyang, Y. Hong, H. Zhang, D. Li and F. Song, 2014. Tomato SR/CAMTA transcription factors SlSR1 and SlSR3L negatively regulate disease resistance response and SlSR1L positively modulates drought stress tolerance. BMC Plant Biology, 14: 286.

80. Li, R., L. Zhang, L. Wang, L. Chen, R. Zhao, J. Sheng and L. Shen, 2018. Reduction of tomato-plant chilling tolerance by CRISPR-CAS9-mediated SlCBF1 mutagenesis. Journal of Agricultural and Food Chemistry, 66: 9042-9051.
81. Borrelli, V.M., V. Brambilla, P. Rogowsky, A. Marocco and A. Lanubile, 2018. The enhancement of plant disease resistance using CRISPR/CAS9 Technology. Frontiers in Plant Science, 9: 1245.
82. Aman, R., Z. Ali, H. Butt, A. Mahas, F. Aljedaani, M.Z. Khan, S. Ding and M. Mahfouz, 2018. RNA virus interference via CRISPR/CAS13a system in plants. Genome Biology, 19: 1.
83. Ali, Z., S. Ali, M. Tashkandi, S.S.E.A. Zaidi and M.M. Mahfouz, 2016. CRISPR/CAS9-mediated immunity to geminiviruses: differential interference and evasion. Scientific Reports, 6: 26912.
84. Thkandi, M., Z. Ali, F. Aljedaani, A. Shami and M.M. Mahfouz, 2018. Engineering resistance against tomato yellow leaf curl virus via the CRISPR/CAS9 system in tomato. Plant Signaling and Behavior, 13: e1525996.
85. Nekrasov, V., C. Wang, J. Win, C. Lanz, D. Weigel and S. Kamoun, 2017. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Scientific Reports, 7: 482.
86. Zheng, Z., M. Appiano, S. Pavan, V. Bracuto, L. Ricciardi, R.G. Visser, A.M.A. Wolters and Y. Bai, 2016. Genome-wide study of the tomato SlMLO gene family and its functional characterization in response to the powdery mildew fungus *Oidium neolycopersici*. Frontiers in Plant Science, 7: 380.
87. Malnoy, M., R. Viola, M.H. Jung, O.J. Koo, S. Kim, J.S. Kim, R. Velasco and C.N. Kanchiswamy, 2016. DNA-free genetically edited grapevine and apple protoplast using CRISPR/CAS9 ribonucleoproteins. Frontiers in Plant Science, 7: 1904.
88. Jupe, J., R. Stam, A.J. Howden, J.A. Morris, R. Zhang, P.E. Hedley and E. Huitema, 2013. Phytophthora capsici-tomato interaction features dramatic shifts in gene expression associated with a hemi-biotrophic lifestyle. Genome Biology, 14: R63.
89. Yang, Y.X., M.M. Wang, Y.L. Yin, E. Onac, G.F. Zhou, S. Peng, X.J. Xia, K. Shi, J.Q. Yu and Y.H. Zhou, 2015. RNA-seq analysis reveals the role of red light in resistance against Pseudomonas syringae pv. tomato DC3000 in tomato plants. Bmc Genomics, 16: 120.
90. Zeilmaker, T., N.R. Ludwig, J. Elberse, M.F. Seidl, L. Berke, A. Van Doorn, R.C. Schuurink, B. Snel and G. Van den Ackerveken, 2015. DOWNY MILDEW RESISTANT 6 and DMR 6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis. The Plant Journal, 81: 210-222.
91. Gimenez-Ibanez, S., M. Boter, A. Ortigosa, G. García-Casado, A. Chini, M.G. Lewsey, J.R. Ecker, V. Ntoukakis and R. Solano, 2017. JAZ2 controls stomata dynamics during bacterial invasion. New Phytologist, 213: 1378-1392.
92. de Toledo Thomazella, D.P., Q. Brail, D. Dahlbeck and B.J. Staskawicz, 2016. CRISPR-CAS9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. Bio. Rxiv, 064824.
93. Ortigosa, A., S. Gimenez-Ibanez, N. Leonhardt and R. Solano, 2019. Design of a bacterial speck resistant tomato by CRISPR/CAS9-mediated editing of Sl JAZ 2. Plant Biotechnology Journal, 17: 665-673.
94. Yang, Y., K. Tang, T.U. Datsenka, W. Liu, S. Lv, Z. Lang, X. Wang, J. Gao, W. Wang, W. Nie and Z. Chu, 2019. Critical function of DNA methyltransferase 1 in tomato development and regulation of the DNA methylome and transcriptome. Journal of Integrative Plant Biology, 61: 1224-1242.