The Journal of Phytopharmacology 2023; 12(2):127-134
Online at: www.phytopharmajournal.com

Review Article

ISSN 2320-480X
JPHYTO 2023; 12(2): 127-134
January- February127
Received: 16-01-2023
Accepted: 11-02-2023

doi: 10.31254/phyto.2023.12110

Sarankumar Chandran
Assistant Professor, Department of Plant Breeding and Genetics, Adhiparasakthi Horticultural College, G.B. Nagar, Kalavai, Tamil Nadu, India

Valarmathi Muthu
Assistant Professor, Department of Biotechnology, Adhiparasakthi Agricultural College, G.B. Nagar, Kalavai, Tamil Nadu, India

Tharshenee Umapathy
B.Sc., (Hons.) Horticultural Student, Adhiparasakthi Horticultural College, G.B.Nagar, Kalavai, Tamil Nadu, India.

Sowmiya Jayakumar
B.Sc., (Hons.) Horticultural Student, Adhiparasakthi Horticultural College, G.B.Nagar, Kalavai, Tamil Nadu, India

Sindhuja Chokkalingam
B.Sc., (Hons.) Horticultural Student, Adhiparasakthi Horticultural College, G.B.Nagar, Kalavai, Tamil Nadu, India.

Correspondence:
Dr. Sarankumar Chandran
Assistant Professor, Department of Plant Breeding and Genetics, Adhiparasakthi Horticultural College, G.B. Nagar, Kalavai, Tamil Nadu, India
Email: saran32388@gmail.com

CRISPR / Cas 9 assisted genome editing technology for the improvement of Horticultural crops

Sarankumar Chandran, Valarmathi Muthu, Tharshenee Umapathy, Sowmiya Jayakumar, Sindhuja Chokkalingam

ABSTRACT

Horticultural crops produce a wide range of useful goods for humans. There has been an increase in research focus on horticulture crop enhancement, particularly in terms of production and quality. The use of genome editing to enhance horticulture crops has seen a sharp rise in recent years due to the advancement and benefits of genome-editing technology. Here, we provide a brief overview of the various genome-editing techniques applied in horticulture research, with a particular emphasis on CRISPR/CRISPR-associated 9 (Cas9)-mediated genome editing. We also provide an overview of recent developments in the use of genome editing to enhance horticulture crops. Breeding and the rapidly growing field of genome editing will significantly boost the quantity and quality of horticulture crops.

Keywords: CRISPR / Cas 9, Genome Editing Technology, Horticultural Crops.

INTRODUCTION

Horticulture is a significant area of agriculture that dates back thousands of years and has advanced significantly throughout human history. Vegetable and fruit crops, as well as floricultural and ornamental plants, are all regarded as horticultural crops because they are grown for food, nutritional and medical purposes, as well as for aesthetic enjoyment [1]. Fruit and vegetable crops are essential for balancing our daily diet because they are low in calories but high in vitamins and minerals. The diversity and nutritional worth of horticulture goods are declining, despite an increase in availability. The low genetic variety of horticultural crops brought about by domestication and breeding, as well as reproductive barriers that prevent genetic introgression from wild relatives, can be partly blamed for these declines. As a result, the development of genetic resources with a variety of desirable traits will be extremely valuable for enhancing agricultural products [2, 3]. Humans started modifying crops by introducing new features from crossable relatives thousands of years ago. The transfer of advantageous genetic variants was the main objective. The differences were only produced naturally or spontaneously as recently as the 1930s. Breeders subsequently found how to create mutants using radiation or chemical mutagens. Crop productivity and quality have grown dramatically as a result of both natural and induced mutations. However, getting acceptable materials for crop improvement has proven to be difficult and time-consuming due to the rarity and randomness of these alterations [4, 5]. DNA sequence-specific modification has developed into a potent tool as molecular biology has advanced so quickly. In 1987, many animal scientists developed homologous recombination-based gene-targeting technologies (HR). After introducing a donor template into mouse embryonic stem cells, this ground-breaking approach allowed researchers to precisely modify (though rarely) an endogenous gene [6, 7]. Subsequently, similar advancements were reported by plant scientists, but with a much lower editing frequency of 0.5-7.2 x 10^4 [8, 9]. Later, HR frequency was increased in gene targeting by using DNA double-stranded breaks (DSBs), which frequently lead to HR in meiotic chromosomes. In addition to HR, somatic cells have another mechanism for DSB repair known as the error-prone nonhomologous end-joining (NHEJ) pathway, which can result in alterations due to minor deletions or insertions at break sites. Meganucleases (MNs), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9), and CRISPR from Prevotella and Francisella 1 (CRISPR/Cpf1) are the types of engineered endonucleases that researchers have used to introduce site-specific. In several biological systems, these designed endonucleases have made genome editing possible [10, 11, 12, 13]. Finally, the use of genome editing in horticulture crops has rapidly expanded with the development of CRISPR/Cas9.

Mechanism of genome edition

The contact between a transcription factor and a promoter, is an example of sequence-specific DNA binding. The aforementioned nucleases can target the specific sequences to create DSBs for genome editing, guided by either RNA-to-DNA base pairing (CRISPR/Cas9 and CRISPR/Cpf1) or Protein to DNA interaction (MNs, ZFNs, and TALENs) [14].

Homing endonuclease

Homing endonucleases, or MNs, were found in the genomes of microbes or organelles and represent the first class of nucleases for genome editing. These nucleases cleave both strands of DNA in a site-specific manner by identifying DNA sequence components ranging in size from 12 to 40 bp. The I-CreI protein is one of the MNs that has attracted the most research attention and is successful in maize [15]. Nevertheless, due to the rarity of recognized sites, I-CreI and other MNs are unable to edit desired target sites. Researchers have created MN variants that target the targeted DNA sequence via mutagenesis or combinatorial assembly to expand the application of MNs. However, the modified MNs' overlapping recognition and catalytic domains provide challenges and frequently impair their catalytic activity [11]. These factors have hindered plant scientists from using MNs frequently.

ZFNs and TALENs

As implied by their names, the DNA cleavage domain of the endonuclease Fokl is fused with zinc fingers (ZFs) or with transcriptional activator-like effectors to produce ZFNs or TALENs (TALEs). The FokI endonuclease domain does not recognize any sequences; instead, it mediates independent, non-specific DNA cleavage upon dimerization. To produce site-specific cleavage, a pair of ZFs or TALEs, each fused with a Fokl endonuclease domain, is created [16]. Transcription factors contain ZFs, and each finger domain of a ZF may recognize three distinct nucleotides. When a ZFN arises as a dimer, it typically displays an array of 3 or 4 finger domains that can detect 18–24 bp sequences. Although numerous experiments have been done to increase the effectiveness, and precision of ZFN, there are still issues with the small number of recognition sites and interference from nearby finger domains [17]. The DNA-binding domains of TALEs, which are proteins secreted by the common bacterial plant pathogen Xanthomonas, enable TALENs to attain sequence specificity as opposed to ZFNs [18]. TALEs trigger gene expression to aid pathogenesis by binding to a particular sequence of plant promoters. 13–28 repeat sequences make up TALEs' core binding domain. The variable di residues at the 12^{th} and 13^{th} positions allow each repeat, which encodes a 34 amino acid sequence with high conservation, to recognize and bind to a single nucleotide [19]. Targeting specific sequences is made possible by TALENs' one-to-one pairing and the minimal context-dependence on nearby repetitions. ZFNs often perform worse than TALENs in terms of accessibility and precision.

CRISPR/Cas9 and CRISPR/Cpf1

The CRISPR system depends on RNA-DNA binding to establish sequence specificity, as opposed to the ZFN and TALEN systems, which depend on protein-DNA binding specificity. Its role in bacterial resistance to viruses was experimentally proven during the functional elucidation of the CRISPR/Cas system, and numerous components, including crRNA, PAM motif, and tracrRNA, were found to be essential for this system [20]. This programmable RNA-guided CRISPR/Cas9 system may be used for genome editing in creatures other than bacteria, which is particularly intriguing given that reconstituted essential components of the CRISPR/Cas9 system can generate DSBs in a site-specific manner. In the approach, RNA-DNA pairing of a 20-nt region in the chimeric single-guide RNA (sgRNA) with the target is used to establish site-specific binding to the target. The target is also in contact with the other crRNA- and tracrRNA-derived sequences, forming an RNA: DNA heteroduplex that is identified by the contacts of numerous Cas9 domains, including PI, REC1, RuvC, and NUC. Then, 3 nucleotides upstream of the PAM motif, respectively, the RuvC and HNH domains cut the noncomplementary and complementary DNA strands [21, 22, 23]. The identifiable Cas9 PAM motif is 5′-NGG-3′ (N=A, T, C, or G), and this Grich property forbids the creation of sgRNAs in T-rich regions [24]. It has been discovered that Cpf1, another endonuclease in the class 2 Type V CRISPR system, is effective in editing the genome of plants and exhibits certain characteristics. First off, Cpf1 may create a mature crRNA without the aid of a second tracrRNA. Second, Cpf1 recognizes Trich PAM sequences in contrast to Cas9, which only recognizes G-rich PAM sequences. Finally, cutting by the Cpf1 endonuclease results in cohesive ends as opposed to blunt ends produced by cutting by the Cas9 endonuclease. Through the manipulation of the nuclease-inactivated Cas9 enzyme, CRISPR genome-editing systems can be utilized to achieve gene control in addition to creating site-specific mutations (dCas9) [25, 26]. Due to variations in its underlying mechanisms, every endonuclease employed for genome editing has a different set of characteristics. CRISPR/Cas systems have been modified for precise base editing in addition to producing indel mutations at target sequences. The majority of base editors are composed of a sgRNA-guided Cas9 nickase (nCas9) linked with a deaminase that converts C to T or A to G bases [27, 28, 29, 30]. These resources significantly broaden and are available for the precise manipulation of horticultural crops.

Genome editing technology in Vegetable crop

Vegetable crops are prone to a wide range of abiotic and biotic challenges that might complicate optimal production, which highlights the significance of creating resistant/tolerant cultivars. The quality, nutritional content, plant architecture, and shelf life of many vegetables need some improvement. To accomplish these and other objectives, the CRISPR/Cas9 technique was used to modify the genome of numerous commercially significant crops [31, 32, 33]. The CRISPR/Cas9 method has been used to cause mutations in the PDS gene in several vegetable crops, including cabbage, Chinese kale, tomato, and watermelon, mostly as a proof of concept. However, there is no financial gain, and this research can only provide information on how well the genome editing method works with a particular crop. Tomatoes are the vegetable species that have gathered the most research using the CRISPR/Cas9 system, either due to the crop's economic relevance or the simplicity of Agrobacterium-based genetic modification. Given consumer desire and processing needs, parthenocarpy in tomatoes may be a desirable characteristic [34]. The CRISPR/Cas9 system can be utilized as a breeding technique to create parthenocarpic tomato plants [35]. On the tomato variety Micro-Tom, five different sgRNAs were initially used to introduce mutations into SlIAA9, a crucial gene that regulates parthenocarpy. The commercial cultivar Ailsa Craig was altered using the sgRNA2 since it had the highest mutation efficiency. The authors also discovered bi-allelic and homozygous mutations in Micro-Tom and Ailsa Craig T0 regenerated plants. As anticipated, fruit without seeds was produced by

regenerated mutants. Similar to this, plants with a homozygous or biallelic mutation in the SlAGAMOUS-LIKE 6 (SlAGL6) gene in the tomato produced predominantly parthenocarpic fruits and a few low-seeded fruits after the SlAGL6 gene was altered (containing up to 10 seeds) [36].

A major goal of many plant breeding initiatives, particularly for fleshy fruit and vegetables, has been to enhance quality features to make products more nutrient-dense and shelf-stable. Post-harvest losses are a constant risk to the production chain and have a negative impact on returns and earnings. The HDR repair pathway was used to replace the recessive 'alc' allele of Alcobaca (SlALC) to create tomatoes with a long shelf life. It was then able to create an alc homozygous mutant that was free of foreign DNA and had better storage capabilities than wild-type controls. Lycopene improvement and fruit quality features are two more traits that were effectively changed to enhance the tomato [37]. GABA (gamma-aminobutyric acid) is regarded as a functional substance that promotes health and has drawn a lot of attention in traditional tomato breeding studies. The GABA content of plants with tomato genome editing increased 19-fold as a result of editing numerous GABA pathway genes. To produce outstanding potatoes, it is essential to reduce the quantity of steroidal glycoalkaloids (SGAs), such as solanine and chaconine, in tubers. These compounds may have an unfavorable impact on human health when present in high concentrations. As a result, the potato steroid 16-hydroxylase (St16DOX) involved in SGA production was targeted using the CRISPR/Cas9 system [38]. The tomato fruit's ripening process was sped up by editing the genes for APETALA2a (AP2a), NON-RIPENING (NOR), and FRUITFUL (FUL1/TDR4 and FUL2/MBP7). Edited plants naturally ripened their fruit earlier, and this study revealed new information on how FUL1 and FUL2 function during fruit ripening. In SELF PRUNING 5G-edited CRISPR/Cas9 tomato plants, several intriguing traits including day-neutral, improved compact determinate growth, rapid flower output, and early yield were also noted [39].

Vegetable output is severely hampered by weed infestation, so controlling it using selective herbicides is a crucial management strategy. A single point mutation was created in the acetolactate synthase (ALS) gene, a crucial enzyme for the manufacture of the branched-chain amino acids valine, leucine, and isoleucine, to produce herbicide-resistant watermelon plants. The acetolactate synthase (ALS) gene was also altered to produce herbicide resistance in tomatoes and potatoes. It was done using cytidine base editing (CBE) methods, which change the base from C to T. As a result, tomato plants with accurate base editing effectiveness of up to 71% were produced that were resistant to chlorsulfuron. More significantly, in the first generation of tomatoes and potatoes, respectively, 12.9% and 10% of transgene-free altered plants were created [40]. The improvement of drought tolerance is one of the additional features that were changed. The master regulator SlNPR1, which is important in plant's defense responses to pathogens, underwent modification. In comparison to wild-type (WT) plants, slnpr1 mutants showed poorer drought tolerance with larger stomatal aperture, higher electrolytic leakage, malondialdehyde (MDA), and hydrogen peroxide (H2O2) levels, and lower antioxidant enzyme activity levels [41]. Vegetable disease resistance by genome editing is another new area of research that is starting to get attention now that the CRISPR/Cas technology has become routine for modifying the genomes of many different plant species. Two sgRNAs were used to create precise mutations (48-bp deletion in a homozygous configuration) in the MLO1 locus, the main factor influencing susceptibility to the fungus pathogen Oidium neolycopersici, resulting in a tomato plant resistant to powdery mildew disease [42]. By altering the tomato SlDMR6-1 (downy mildew resistance 6 gene) ortholog, broad-spectrum bacterial disease resistant tomato plants were also produced [43]. Additionally, cucumber plants resistant to viruses could be produced via sabotage of the recessive eIF4E gene (eukaryotic translation initiation factor 4E) [44]. It is envisaged that numerous other successful experiments on heritable disease resistance utilizing the CRISPR/Cas system will be conducted in a larger variety of vegetable crops as more and more targets are discovered.

Genome editing technology in Fruit crops

In 2013, the CRISPR/Cas9 method for genome editing in plants was first disclosed. The CRISPR/Cas9 system has been successfully employed for genome editing in several fruit crops, including apple, banana, cacao, citrus, grape, kiwifruit, and pear, in addition to the groundbreaking work in the model plants Arabidopsis and Nicotiana. To enhance the CRISPR/Cas9 technology's application inside a fruit cultivar, various strategies have been examined. The phytoene desaturase (PDS) gene, which encodes an enzyme in the carotenoid biosynthesis pathway, was the focus of the majority of these investigations. This gene's disruption affects the generation of carotenoid and chlorophyll, leading to an albino phenotype, and it is a simple target for alteration to demonstrate the effectiveness of the genome editing system [45]. For instance, full albino and variegated phenotypes among regenerated plantlets and a mutation efficiency of 59% were produced by transgenic production of Cas9 led by 19-bp sgRNA tailored to target the conserved area of two PDS genes in the banana genome [46]. Also reported in bananas was a higher editing efficiency (100%) using polycistronic gRNAs to target the same gene [47]. Induced PDS gene mutations in diploid and octoploid strawberries frequently produced a conspicuous albino phenotype [48]. Following the modification of the PDS gene in the Carrizo citrange, similar outcomes were seen. The mutation efficiency rose and in certain cases was reported to be 100% when the Cas9 gene was driven by the Arabidopsis YAO gene promoter rather than the often utilized cauliflower mosaic virus 35S promoter [13]. It has also been demonstrated that PDS gene mutations caused by CRISPR/Cas9 result in an albino phenotype in apples [49, 50], grapes [51], kiwifruit [52], pear [50, 53]. A prolonged non-flowering period is caused by the protracted juvenile phase seen in many perennial fruit crops. Depending on the fruit harvest, this may extend for three to fifteen years. The traditional breeding efforts to generate cultivars are hampered by their prolonged juvenile phase [54, 55]. High levels of the protein known as terminal flowering (TFL) are typically linked to juvenility [56]. By preventing the expression of numerous proteins that promote flowering, including the FLOWERING LOCUS T (FT), LEAFY (LFY), and APETALA1 (AP1), TFL functions as a negative regulator of flowering [56, 57]. The TFL1 gene was the target of two distinct sgRNAs. Despite there being one mismatch between the target and the sgRNA1, the same construct was also employed to modify the pear TFL1. 93% of the apple transgenic lines targeted by the MdTFL1.1 gene and 9% of the pear transgenic lines targeted by the PcTFL1.1 gene showed early blooming.This mismatch may be the cause of the reduced frequency of the mutant phenotype seen in pears. It's also likely that in pears, both TFL1 genes (PcTFL1.1 and PcTFL1.2) need to be edited to fully unwind the floral repression. A third approach involved concurrently targeting the two kiwifruit CEN-like genes AcCEN4 and AcCEN with the CRISPR/Cas9 system. A climbing woody perennial with axillary inflorescences that develops after several years of juvenility was turned into a compact plant with quick terminal flower and fruit

development by mutations in these genes [58]. Fruit crops are vulnerable to a variety of worms, viruses, bacteria, fungi, and bacterial diseases. These elements limit the development and growth of plants, which negatively impacts crop productivity. Each has different economic significance depending on the time and place, but generally raises production costs and may result in substantial losses. One of the most efficient and cost-effective solutions to these issues is the production of resistant/tolerant cultivars. Thus, the production of these biotic stressors can be significantly impacted by CRISPR/Cas9-mediated resistance to them. Citrus canker is a disease brought on by the bacterial pathogen *Xanthomonas citri* subspecies citri (Xcc), and *Citrus sinensis* LATERAL ORGAN BOUNDARIES (CsLOB1) is a susceptibility gene for it [59, 60]. The Xcc pathogenicity factor PthA4 interacts with specific regions in the promoter region of this gene, inducing CsLOB1. The CRISPR/Cas9 method was used to edit the PthA4 effector cis-elements in the promoter of CsLOB1, and it was shown that the mutant plants had less Xcc infection [61]. However, these findings suggested that only plants with mutations in both of the CsLOB1 promoters were resistant to citrus canker, indicating that activation of just one allele of the CsLOB1 gene via the PthA4 binding site is sufficient to cause disease onset. To alter the PthA4 binding region in the CsLOB1 promoter of "Wanjincheng" orange, created five CRISPR/Cas9 constructs [62]. The resistance to *Botrytis cinerea* was improved in grapes by CRISPR/Cas9-mediated deletion of WRKY52, encoding a transcription factor associated with biotic stress responses [52]. To direct the Cas9 nuclease, four gRNAs were created for various locations in the first exon of WRKY52. Biallelic transgenic mutant lines were found to be more resistant than monoallelic mutant lines. The defensive response repressor gene NPR3 (NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES3) was edited in cacao using CRISPR/Cas9 [63]. The CRISPR/Cas9 cassettes were temporarily expressed in cacao leaf tissues as the first test of gene editing. This resulted in the deletion of 27% of the NPR3 copies in the treated tissues and the development of *Phytophthora tropicalis* resistance in the edited tissues. The scientists postulated that cells with mutant NPR3 triggered their defense mechanisms. To verify the efficiency of CRISPR/Cas9 at a whole plant level in cacao, they subsequently produced stably transformed and genome-edited somatic embryos [64]. The output of bananas is severely constrained by the banana streak virus (BSV). Under stressful circumstances, the dsDNA virus integrates into the host's genome and releases infectious viral particles [65]. The plantain cultivar Gonja manjaya's host genome contains BSV sequences that were modified using the CRISPR/Cas9 technology. Under water stress, 75% (6 out of 8) of the assessed regenerated genome-edited events persisted asymptomatically in contrast to the non-edited plants [66]. Recently, MaGA20ox2 gene mutations were also induced using CRISPR/Cas9. Banana dwarfism is regulated by this gene [67]. Following genome editing, seven mutant lines with a semi-dwarf phenotype were found. The endogenous GA levels in the various organs were measured in this investigation, and the findings were in line with the phenotype. In comparison to the non-transformed control, there was a substantial difference in the amounts of GAs in the mutants in both the leaves and the roots [68]. This approach can then be used to create the desperately required semi-dwarf or dwarf banana varieties.

Genome editing technology in other horticultural crops

To meet the ever-changing needs of consumers, significant attempts have been made to raise yield, biotic and abiotic resistance, or improve quality. Historically, conventional breeding has produced the majority of good results. However, the majority of horticultural crops either have a combination of high levels of self- and cross-incompatibility, extended juvenile periods, complex genomes (triploid or polyploid species), high levels of heterozygosity, and long juvenile periods [69, 70]. Although it has its drawbacks, genetic engineering has emerged during the past 20 years as a useful tool to augment crop breeding for those species [71, 72]. "Foreign DNA," which includes selectable marker genes, is inserted into transgenic plants. Additionally, the random incorporation of the foreign DNA may disrupt or change the expression of endogenous genes. The ability to produce non-transgenic plants is one of the main advantages of CRISPR/Cas9 over transgenic methods. Due to the diverse genomic locations of the CRISPR/Cas9 expression cassettes and their target sites, segregation and removal of the CRISPR/Cas9 cassettes are feasible through selfing or crossing in the following generations. It was possible to create tomato plants with the necessary mutations and phenotype (resistance to powdery mildew disease), but without transgenes [35]. Two sgRNAs were added to the tomato to introduce mutations; these sgRNAs were then eliminated through gene segregation in the following generations. However, because most fruit trees have a protracted juvenile period and need several years to reach the reproductive stage, segregation, and removal of the CRISPR/Cas9 cassettes through selfing or crossing is not practical. Additionally, many species are often vegetatively propagated and have complex genomes with high levels of polyploidy and heterozygosity. In these circumstances, transiently expressing the CRISPR/Cas9 components in the nucleus can be used to create transgene-free altered plants. This is possible because the CRISPR/Cas9 cassettes that have not integrated into the genome can still be produced and function for the brief period required to introduce precise alterations. The creation of T-DNA-free modified apple lines on apples showed the viability of this technology [50]. Although this temporary system's overall effectiveness was very low (0.4% of modified lines), it won't be long until improvements in the genetic transformation process lead to improved efficiency, making this editing procedure commonplace. Genome editing without transgene integration can also be accomplished in plant cells by delivering preassembled Cas9 protein gRNA ribonucleoproteins (RNPs). Following delivery in cells, the RNPs immediately modify the target sites before being destroyed by endogenous proteases. As a result, the genome has no DNA integration changed lettuce protoplasts using purified Cas9 ribonucleoproteins while converted grapevine and apple protoplasts to adopt a DNA-free delivery methodology [73, 74]. Using this technique, mutagenesis efficiencies in grapevine and apple ranged from 0.1% to 0.5-6.9%. In addition, created a step-by-step approach for the precise and effective construction and transfer of CRISPR-Cas9 components in apple and grapevine protoplasts [75]. The full methodology using CRISPR-Cas9 RNPs delivered directly has the benefit of producing exogenous DNA-free plants in as little as 2-3 weeks, whereas the plasmid-mediated method requires more than 3 months to regenerate plants and analyze the mutations. Given the strict and expensive rules on genetic alteration currently in place, the development of transgene-free plants using the CRISPR/Cas9 technology is crucial. Plants that are free of transgenes may not be subject to the current restrictions on genetic modification, potentially saving time and money even if the regulation of gene-edited crops is still up for debate [76, 77]. Private businesses, for instance, have made significant financial gains from their investments in the production of transgenic crops like soybeans and maize. The development of transgenic fruit crops, which are primarily perennial and vegetatively propagated, is undoubtedly hampered by this investment. Thus, the potential for facilitating the commercial release of CRISPR-plants devoid of transgenes could be

advantageous for both private businesses and public research institutions, incentivizing them to invest in the creation of fruit cultivars with gene-edited fruit that is free of transgenes. Since it is a plant that is not seen as transgenic, it can also help with public acceptance. All of this suggests that transgene-free genome editing techniques could be an effective tool for the genetic enhancement of numerous fruit crops.

Future challenges

Through targeted, regulated modifications to the genome, the CRISPR/Cas9 system is a new, revolutionary technique for biological research and crop breeding. Most CRISPR/Cas9 investigations in horticultural crops to date use NHEJ-mediated gene repair to produce precise mutations that either knock out or change the function of the target gene. Many times, the phenotype that resulted from the technique either confirmed its efficacy or revealed a particular gene function, while in other instances, it enhanced desirable agronomic traits like disease resistance. These findings demonstrate the significant value of the CRISPR/Cas9 system for horticulture crop-specific applications. There are still some challenges to be resolved, though. First, the target organism's genome needs to be sequenced to create precise sgRNA and prevent off-target gene editing. In organisms without a whole genome sequence, gene editing work is challenging to perform. While the genome sequences of other widely consumed species like the olive and avocado, as well as tropical crops, are not yet available or the quality can be improved, high-quality genome assemblies have been developed for species like the banana, peach, raspberry, cocoa, papaya, clementine mandarin, coffee, and grape. A more thorough understanding of the genome structure, gene networks, and gene functions of all commercially significant horticultural species may be possible thanks to the development of newer sequencing technology. This will make CRISPR/Cas9 more beneficial for breeding those crops. Many horticultural crop species, as well as other plant genomes, exhibit polyploidy. For instance, the polyploid nature of commercial varieties of kiwifruit, strawberry, and banana makes breeding and genome editing more difficult. Two or more copies of the target gene must be mutated to produce the desired phenotype. Therefore, for the future development of this and other crops, a very effective editing platform for generating biallelic or multiallelic mutations within the same generation is crucial. To ensure a higher frequency of induced mutations, it is imperative to take into consideration aspects such as target site selection and sgRNA design, application of several gRNAs for the same target, and an appropriate promoter to produce both the gRNA and Cas9. Many horticultural crop species, as well as other plant genomes, exhibit polyploidy. Another essential prerequisite is an effective way of introducing or transiently expressing the CRISPR/Cas9 components into plant cells and afterwards in vitro production of whole plants from these transformed cells, as in commercial varieties of kiwifruit. Over the past 30 years, plant transformation technologies have been established in the majority of commercially significant horticultural crops. This suggests that many crops have uniform transformation and regeneration procedures. However, genetic transformation techniques, such as those mediated by Agrobacterium or those utilizing purified ribonucleoproteins in protoplasts, are frequently genotype-specific and necessitate the adjustment of numerous parameters for their application within a species to attain high efficiency. Therefore, it would be crucial to create effective and repeatable delivery mechanisms as well as selection and regeneration protocols to make CRISPR/Cas9 a common tool in horticulture crops. Compared to earlier developed standard genetic engineering tools, the new gene editing techniques are more accurate. Some people are worried about how mutations on non-target genes (also known as "off-target") can have unintended effects. This is especially true for species with big and complex genomes. Even when mutations arise "on-target," gene editing can nevertheless have unforeseen consequences. Small DNA insertions or deletions typically affect the gene's reading code, limiting the creation of proteins or even leading to the generation of proteins that are altered and have unclear effects. Before using a cultivar, these unwanted changes should be recognized, however, the methods for doing so (bioinformatics and next-generation sequencing) may still have limitations. It is crucial to remember that CRISPR/Cas9 is a very new approach, and there is currently insufficient knowledge about its safety, even if there are concerns about the risk of genome editing in plants.

CONCLUSION

Through targeted, regulated modifications to the genome, the CRISPR/Cas9 system is a new, revolutionary technique for biological research and crop breeding. Numerous significant horticultural crops have already seen several successful examples. The development of horticultural crops with improved agronomic traits will result from the growing knowledge of CRISPR/Cas9-based tools, particularly techniques that allow the development of a non-transgenic plant, delivery methods, and genomic information. This will result in creative solutions for sustainable and competitive food production.

Conflict of interest

The authors declared that there is no conflict of interest.

Financial Support

None declared.

ORCID ID

Sarankumar Chandran: https://orcid.org/0000-0001-7153-6046

REFERENCE

1. Ravichandra NG, Ravichandra NG. Horticulture and its role in the national economies. Horticultural Nematology. 2014:1-3.
2. Janick J. Horticultural plant breeding: past accomplishments, future directions. InInternational Symposium on Harnessing the Potential of Horticulture in the Asian-Pacific Region 694 2004 Sep 1 (pp. 61-65).
3. Khoury CK, Bjorkman AD, Dempewolf H, Ramirez-Villegas J, Guarino L, Jarvis A, Rieseberg LH, Struik PC. Increasing homogeneity in global food supplies and the implications for food security. Proceedings of the national Academy of Sciences. 2014 Mar 18;111(11):4001-6.
4. Oladosu Y, Rafii MY, Abdullah N, Hussin G, Ramli A, Rahim HA, Miah G, Usman M. Principle and application of plant mutagenesis in crop improvement: a review. Biotechnology & Biotechnological Equipment. 2016 Jan 2;30(1):1-6.
5. Ahloowalia BS, Maluszynski M. Induced mutations–A new paradigm in plant breeding. Euphytica. 2001 Mar;118:167-73.

6. Thomas KR, Capecchi MR. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell. 1987 Nov 6;51(3):503-12.
7. Doetschman T, Gregg RG, Maeda N, Hooper ML, Melton DW, Thompson S, Smithies O. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature. 1987 Dec 10;330(6148):576-8.
8. Paszkowski J, Baur M, Bogucki A, Potrykus I. Gene targeting in plants. The EMBO journal. 1988 Dec;7(13):4021-6.
9. Hanin M, Volrath S, Bogucki A, Briker M, Ward E, Paszkowski J. Gene targeting in Arabidopsis. The Plant Journal. 2001 Dec;28(6):671-7.
10. Puchta H, Dujon B, Hohn B. Homologous recombination in plant cells is enhanced by in vivo induction of double strand breaks into DNA by a site-specific endonuclease. Nucleic acids research. 1993 Nov 11;21(22):5034-40.
11. Voytas DF. Plant genome engineering with sequence-specific nucleases. Annual review of plant biology. 2013 Apr 29;64:327-50.
12. Zhu C, Bortesi L, Baysal C, Twyman RM, Fischer R, Capell T, Schillberg S, Christou P. Characteristics of genome editing mutations in cereal crops. Trends in Plant Science. 2017 Jan 1;22(1):38-52.
13. Zhang H, Zhang J, Lang Z, Botella JR, Zhu JK. Genome editing—principles and applications for functional genomics research and crop improvement. Critical Reviews in Plant Sciences. 2017 Jul 4;36(4):291-309.
14. Rocha-Martins M, Cavalheiro GR, Matos-Rodrigues GE, Martins RA. From gene targeting to genome editing: transgenic animals applications and beyond. Anais da Academia Brasileira de Ciências. 2015;87:1323-48.
15. Gao H, Smith J, Yang M, Jones S, Djukanovic V, Nicholson MG, West A, Bidney D, Falco SC, Jantz D, Lyznik LA. Heritable targeted mutagenesis in maize using a designed endonuclease. The Plant Journal. 2010 Jan;61(1):176-87.
16. Bibikova M, Beumer K, Trautman JK, Carroll D. Enhancing gene targeting with designed zinc finger nucleases. Science. 2003 May 2;300(5620):764-.
17. Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, Jiang T, Foley JE, Winfrey RJ, Townsend JA, Unger-Wallace E. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Molecular cell. 2008 Jul 25;31(2):294-301.
18. Kay S, Hahn S, Marois E, Hause G, Bonas U. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. Science. 2007 Oct 26;318(5850):648-51.
19. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009 Dec 11;326(5959):1501-.
20. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007 Mar 23;315(5819):1709-12.
21. Deveau H, Barrangou R, Garneau JE, Labonté J, Fremaux C, Boyaval P, Romero DA, Horvath P, Moineau S. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. Journal of bacteriology. 2008 Feb 15;190(4):1390-400.
22. Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, Van Der Oost J. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science. 2008 Aug 15;321(5891):960-4.
23. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature. 2011 Mar 31;471(7340):602-7.
24. Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, Ishitani R, Zhang F, Nureki O. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell. 2014 Feb 27;156(5):935-49.
25. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013 Feb 28;152(5):1173-83.
26. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013 Jul 18;154(2):442-51.
27. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016 May 19;533(7603):420-4.
28. Kim YB, Komor AC, Levy JM, Packer MS, Zhao KT, Liu DR. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nature biotechnology. 2017 Apr;35(4):371-6.
29. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR. Programmable base editing of A• T to G• C in genomic DNA without DNA cleavage. Nature. 2017 Nov 23;551(7681):464-71.
30. Hua K, Tao X, Yuan F, Wang D, Zhu JK. Precise A· T to G· C base editing in the rice genome. Molecular plant. 2018 Apr 2;11(4):627-30.
31. Lintas C. Nutritional aspects of fruits and vegetables consumption. Options Mediterraennes. 1992;19:79-87.
32. Unnevehr LJ. Food safety issues and fresh food product exports from LDCs. Agricultural Economics. 2000 Sep;23(3):231-40.
33. León JS, Jaykus LA, Moe CL. Food safety issues and the microbiology of fruits and vegetables. Microbiologically safe foods. 2009 Apr 1:255-81.
34. Tian S, Jiang L, Gao Q, Zhang J, Zong M, Zhang H, Ren Y, Guo S, Gong G, Liu F, Xu Y. Efficient CRISPR/Cas9-based gene knockout in watermelon. Plant Cell Reports. 2017 Mar;36:399-406.
35. Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S. Targeted mutagenesis in the model plant Nicotiana benthamiana using Cas9 RNA-guided endonuclease. Nature biotechnology. 2013 Aug;31(8):691-3.
36. Xiong X, Liu W, Jiang J, Xu L, Huang L, Cao J. Efficient genome editing of Brassica campestris based on the CRISPR/Cas9 system. Molecular Genetics and Genomics. 2019 Oct;294:1251-61.
37. Xu ZS, Feng K, Xiong AS. CRISPR/Cas9-mediated multiply targeted mutagenesis in orange and purple carrot plants. Molecular biotechnology. 2019 Mar 15;61:191-9.
38. Soyk S, Müller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jiménez-Gómez JM, Lippman ZB. Variation in the flowering gene SELF

PRUNING 5G promotes day-neutrality and early yield in tomato. Nature Genetics. 2017 Jan;49(1):162-8.
39. Murovec J, Guček K, Bohanec B, Avbelj M, Jerala R. DNA-free genome editing of Brassica oleracea and B. rapa protoplasts using CRISPR-Cas9 ribonucleoprotein complexes. Frontiers in Plant Science. 2018 Nov 5;9:1594.
40. Tuncel A, Corbin KR, Ahn-Jarvis J, Harris S, Hawkins E, Smedley MA, Harwood W, Warren FJ, Patron NJ, Smith AM. Cas9-mediated mutagenesis of potato starch-branching enzymes generates a range of tuber starch phenotypes. Plant biotechnology journal. 2019 Dec;17(12):2259-71.
41. Wang S, Zhang S, Wang W, Xiong X, Meng F, Cui X. Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system. Plant cell reports. 2015 Sep;34:1473-6.
42. Klimek-Chodacka M, Oleszkiewicz T, Lowder LG, Qi Y, Baranski R. Efficient CRISPR/Cas9-based genome editing in carrot cells. Plant Cell Reports. 2018 Apr;37:575-86.
43. Wang D, Samsulrizal NH, Yan C, Allcock NS, Craigon J, Blanco-Ulate B, Ortega-Salazar I, Marcus SE, Bagheri HM, Perez Fons L, Fraser PD. Characterization of CRISPR mutants targeting genes modulating pectin degradation in ripening tomato. Plant Physiology. 2019 Feb;179(2):544-57.
44. Yu QH, Wang B, Li N, Tang Y, Yang S, Yang T, Xu J, Guo C, Yan P, Wang Q, Asmutola P. CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. Scientific reports. 2017 Sep 19;7(1):11874.
45. Qin G, Gu H, Ma L, Peng Y, Deng XW, Chen Z, Qu LJ. Disruption of phytoene desaturase gene results in albino and dwarf phenotypes in Arabidopsis by impairing chlorophyll, carotenoid, and gibberellin biosynthesis. Cell research. 2007 May;17(5):471-82.
46. Kaur N, Alok A, Kaur N, Pandey P, Awasthi P, Tiwari S. CRISPR/Cas9-mediated efficient editing in phytoene desaturase (PDS) demonstrates precise manipulation in banana cv. Rasthali genome. Functional & integrative genomics. 2018 Jan;18:89-99.
47. Naim F, Dugdale B, Kleidon J, Brinin A, Shand K, Waterhouse P, Dale J. Gene editing the phytoene desaturase alleles of Cavendish banana using CRISPR/Cas9. Transgenic research. 2018 Oct;27:451-60.
48. Wilson FM, Harrison K, Armitage AD, Simkin AJ, Harrison RJ. CRISPR/Cas9-mediated mutagenesis of phytoene desaturase in diploid and octoploid strawberry. Plant Methods. 2019 Dec;15:1-3.
49. Nishitani C, Hirai N, Komori S, Wada M, Okada K, Osakabe K, Yamamoto T, Osakabe Y. Efficient genome editing in apple using a CRISPR/Cas9 system. Scientific reports. 2016 Aug 17;6(1):31481.
50. Charrier A, Vergne E, Dousset N, Richer A, Petiteau A, Chevreau E. Efficient targeted mutagenesis in apple and first time edition of pear using the CRISPR-Cas9 system. Frontiers in Plant Science. 2019 Feb 6;10:40.
51. Nakajima I, Ban Y, Azuma A, Onoue N, Moriguchi T, Yamamoto T, Toki S, Endo M. CRISPR/Cas9-mediated targeted mutagenesis in grape. PLoS One. 2017 May 18;12(5):e0177966.
52. Wang X, Tu M, Wang D, Liu J, Li Y, Li Z, Wang Y, Wang X. CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. Plant biotechnology journal. 2018 Apr;16(4):844-55.
53. Zhu C, Zheng X, Huang Y, Ye J, Chen P, Zhang C, Zhao F, Xie Z, Zhang S, Wang N, Li H. Genome sequencing and CRISPR/Cas9 gene editing of an early flowering Mini-Citrus (Fortunella hindsii). Plant biotechnology journal. 2019 Nov;17(11):2199-210.
54. Furr JR, Cooper WC, Reece PC. An investigation of flower formation in adult and juvenile citrus trees. American Journal of Botany. 1947 Jan 1:1-8.
55. Nishikawa F. Regulation of floral induction in citrus. Journal of the Japanese Society for Horticultural Science. 2013;82(4):283-92.
56. Pillitteri LJ, Lovatt CJ, Walling LL. Isolation and characterization of a TERMINAL FLOWER homolog and its correlation with juvenility in citrus. Plant Physiology. 2004 Jul;135(3):1540-51.
57. Liljegren SJ, Gustafson-Brown C, Pinyopich A, Ditta GS, Yanofsky MF. Interactions among APETALA1, LEAFY, and TERMINAL FLOWER1 specify meristem fate. The Plant Cell. 1999 Jun;11(6):1007-18.
58. Varkonyi-Gasic E, Wang T, Voogd C, Jeon S, Drummond RS, Gleave AP, Allan AC. Mutagenesis of kiwifruit CENTRORADIALIS-like genes transforms a climbing woody perennial with long juvenility and axillary flowering into a compact plant with rapid terminal flowering. Plant Biotechnology Journal. 2019 May;17(5):869-80.
59. Gottwald TR, Graham JH, Schubert TS. Citrus canker: the pathogen and its impact. Plant health progress. 2002;3(1):15.
60. Hu Y, Zhang J, Jia H, Sosso D, Li T, Frommer WB, Yang B, White FF, Wang N, Jones JB. Lateral organ boundaries 1 is a disease susceptibility gene for citrus bacterial canker disease. Proceedings of the National Academy of Sciences. 2014 Jan 28;111(4):E521-9.
61. Jia H, Orbovic V, Jones JB, Wang N. Modification of the PthA4 effector binding elements in Type I Cs LOB 1 promoter using Cas9/sg RNA to produce transgenic Duncan grapefruit alleviating XccΔpthA4: dCs LOB 1.3 infection. Plant biotechnology journal. 2016 May;14(5):1291-301.
62. Peng A, Chen S, Lei T, Xu L, He Y, Wu L, Yao L, Zou X. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene Cs LOB 1 promoter in citrus. Plant biotechnology journal. 2017 Dec;15(12):1509-19.
63. Dorantes-Acosta AE, Sánchez-Hernández CV, Arteaga-Vazquez MA. Biotic stress in plants: life lessons from your parents and grandparents. Frontiers in Genetics. 2012 Dec 6;3:256.
64. Fister AS, Landherr L, Maximova SN, Guiltinan MJ. Transient expression of CRISPR/Cas9 machinery targeting TcNPR3 enhances defense response in Theobroma cacao. Frontiers in plant science. 2018 Mar 2;9:268.
65. Lockhart BE. Banana streak badnavirus infection in Musa: epidemiology, diagnosis and control. Taipei, Taiwan: ASPAC Food & Fertilizer Technology Center; 1995 Dec 11.
66. Tripathi JN, Ntui VO, Ron M, Muiruri SK, Britt A, Tripathi L. CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of Musa spp. overcomes a major challenge in banana breeding. Communications Biology. 2019 Jan 31;2(1):46.
67. Chen J, Xie J, Duan Y, Hu H, Hu Y, Li W. Genome-wide identification and expression profiling reveal tissue-specific

expression and differentially-regulated genes involved in gibberellin metabolism between Williams banana and its dwarf mutant. BMC Plant Biology. 2016 Dec;16:1-8.
68. Shao X, Wu S, Dou T, Zhu H, Hu C, Huo H, He W, Deng G, Sheng O, Bi F, Gao H. Using CRISPR/Cas9 genome editing system to create MaGA20ox2 gene-modified semi-dwarf banana. Plant Biotechnology Journal. 2020 Jan;18(1):17.
69. Anderson NO. Flower Breeding and Genetics: Issues. Challenges and Opportunities for the 21st Century, SSBM. 2006.
70. Bisognin DA. Breeding vegetatively propagated horticultural crops. Crop Breeding and Applied Biotechnology. 2011;11:35-43.
71. Jain SM. Mutagenesis in crop improvement under the climate change. Romanian biotechnological letters. 2010 Mar 1;15(2):88-106.
72. Singh RP, Singh PK, Gupta R, Singh RL. Biotechnological Tools to Enhance Sustainable Production. InBiotechnology for sustainable agriculture 2018 Jan 1 (pp. 19-66). Woodhead Publishing.
73. Woo JW, Kim J, Kwon SI, Corvalán C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nature biotechnology. 2015 Nov;33(11):1162-4.
74. Malnoy M, Viola R, Jung MH, Koo OJ, Kim S, Kim JS, Velasco R, Nagamangala Kanchiswamy C. DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. Frontiers in plant science. 2016 Dec 20;7:1904.
75. Osakabe Y, Liang Z, Ren C, Nishitani C, Osakabe K, Wada M, Komori S, Malnoy M, Velasco R, Poli M, Jung MH. CRISPR–Cas9-mediated genome editing in apple and grapevine. Nature Protocols. 2018 Dec;13(12):2844-63.
76. Jansson S. Gene-edited plants on the plate: the 'CRISPR cabbage story'. Physiologia plantarum. 2018 Dec;164(4):396-405.
77. Schaart JG, van de Wiel CC, Lotz LA, Smulders MJ. Opportunities for products of new plant breeding techniques. Trends in Plant Science. 2016 May 1;21(5):438-49.

HOW TO CITE THIS ARTICLE
Chandran S, Muthu V, Umapathy T, Jayakumar S, Chokkalingam S. CRISPR / Cas 9 assisted genome editing technology for the improvement of Horticultural crops. J Phytopharmacol 2023; 12(2):127-134. doi: 10.31254/phyto.2023.12110