Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 02, Issue: 06 (NOV-DEC, 2022)
Available online at http://www.agriarticles.com

CRISPR/Cas9 Technology and Its Application in Horticultural Crops

(*Ashok Kumar Mahawer, Jagdish Yadav and Bhagwaan Singh Dhaked)
ICAR-Indian Agricultural Research Institute, New Delhi
*Corresponding Author's email: ashokmahaver2012@gmail.com

Abstract

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated 9 (CRISPR/Cas9) system has recently become one popular technology due to its efficiency, precision, and simplicity compared with other genome editing tools such as Zinc Finger Nucleases (ZFNs) and Transcription Activator Like Effector Nucleases (TALENs). Horticultural crops provide energy and health-keeping nutrients to humankind. Genome-editing technology has become widely adopted in horticultural breeding with the increasing demand for high yield and better-quality horticultural crops. The application of this technology in horticultural plants for stress responses enhancement, fruit quality improvement, and cultivation traits modification. This detailed review was compiled to help establish comprehensive understanding of the CRISPR/Cas9 systems and provide a reference for further developing this technology to manipulate horticultural plant traits effectively

Introduction

Horticultural crops provide the nutritional support required for human life. Therefore, horticultural research and breeding should be constantly innovated to meet the increasing demand for high-yield and better-quality horticultural products. Traditional breeding of horticultural plants largely relies on natural variation of alleles and randomly mixed genes to obtain the desirable traits, and thus time-consuming, is less efficient, and labour intensive, so new breeding methods are urgently needed. Genetic engineering breeding is the technology that has relieved this dilemma in recent decades, by introducing the desirable recombinant DNA into the plant's genome to provide new genotypes and traits for breeding purposes (Parmar et al., 2017; Chen et al., 2020). However, the insertion of exogenous recombinant DNA into the plant genome sparked controversy over the safety of transgenic crops, and the transgenic breeding technology has been limited by the rigorous approval process for transgenic crops. Therefore, looking for transfer DNA (T-DNA) insertion-free breeding technology draw the focus on gene-editing technologies. Scientists have developed several systems to introduce site-specific gene editing by using engineered endonucleases, including zinc finger nucleases (ZFNs),transcription activator-like effector nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 (CRISPR/Cas9). CRISPR/Cas9 technology has become one popular technology because of its efficiency, precision, and simplicity. The CRISPR/Cas9 technology is considered the most desirable choice to obtain genome-edited crops because of its lower cost, more flexibility, and high reliability. However, the CRISPR/Cas9 technology still has potential for improvement when used in horticultural crops. Therefore, the evolution of gene-editing technologies, the construction of the CRISPR/Cas9 system, the optimization of the CRISPR/Cas9, and its application in horticultural crops, thus providing a reference for the technology development in horticultural crops traits improvement.

Types and mechanisms of gene-editing technologies

Gene-editing tools rely on engineered endonucleases to recognize the specific DNA sequence through their DNA binding domain (DBD) proteins or guide RNAs and cleave the DNA sequence precisely and efficiently to make double-strand breaks (DSBs) in the specific target site. The DSBs are then repaired through two kinds of cellular DNA repair mechanisms, homology-directed repair (HDR) or error-prone non-homologous end-joining breaks (NHEJ), resulting in modifications of the target sites. ZFN was the first engineered endonucleases with customized DBDs for specific gene editing. Unlike ZFNs, TALENs recognize specific DNA sequences through the DBD of transcription activator-like effectors (TALEs). ZFNs and TALENs are been gradually replaced by the new emerging genome-editing method CRISPR/Cas9. In the last decade, CRISPR/Cas9 technology has become the most widely used gene-editing technology due to its high efficiency, simplicity, and ease to use. The CRISPR/Cas system contains CRISPR RNA (crRNA), trans-activating crRNA (tracr-RNA), and Cas protein. crRNA contains a sequence homologous to the target gene and interacts with tracrRNA to form a single guide RNA (sgRNA) that directs the Cas protein to the target gene site to produce DSBs (Fig.1). Based on the structure of Cas proteins, the CRISPR/Cas systems have been divided into two classes and six types.

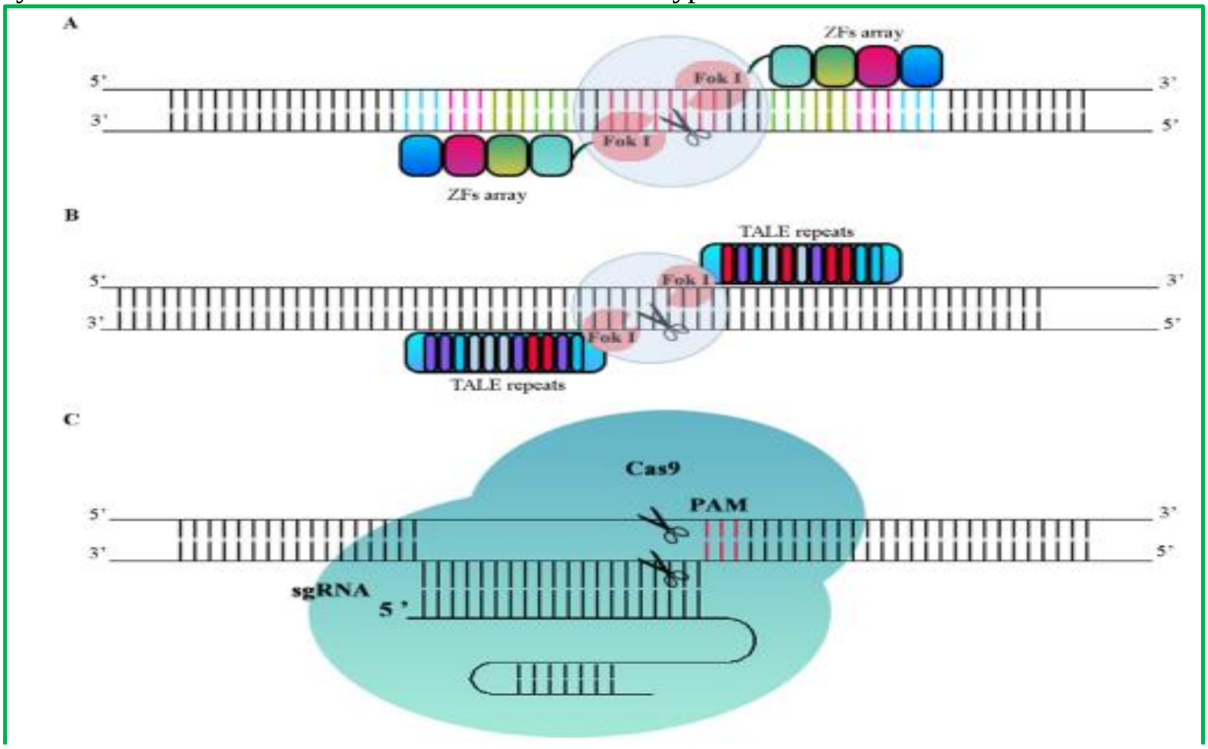

Fig. 1 Schematic description of the mechanisms of different gene-editing technologies Customized ZFs arrays (in different colors) bind to the upstream and downstream of the target site and direct the FokI restriction nuclease to make a DSB on DNA. B. FokI cleaves the target sequence mediated by the designed TALE repeats (in different colors). C. sgRNA containing 20-nt target DNA sequences directs the Cas9 to the PAM sequences (indicated by red lines) and cleaves the target DNA to achieve a DSB. Scissors indicate the cleavage sites of the engineered endonucleases.

The procedure of constructing CRISPR/Cas9 vector in horticultural plants

Since CRISPR/Cas9 editing has been successfully applied in model species, such as *Arabidopsis thaliana* and *Nicotiana benthamiana*, researchers have established this technology in horticultural crops recently. CRISPR/Cas9 system has been successfully applied in tomato, orange, apple, grape, cucumber, watermelon, strawberry, banana, kiwifruit, carrot cacao, pear, and yam. The CRISPR/Cas9 system construction and optimization in horticultural plants are summarized.

The applications of CRISPR/Cas9 in horticultural plants

The high efficiency and precision of CRISPR/Cas9 technology in genome editing motivated researchers to design this system to study the horticultural crop (Ahmar *et al*., 2020; Biswas *et al*., 2021). This technology has been applied in enhancing stress resistance, improving fruit quality, and modifying cultivation traits. We summarize the diverse applications of CRISPR/Cas9 in horticultural plants.

- **Enhance resistance to stresses:** Biotic and abiotic stresses cause a great threat to the production and security of foods, and gene-editing technology brings new opportunities to resolve these issues. Translation and replication interference against invasive viruses is an effective way for plants to resist viral diseases. The designed CRISPR/Cas9 vector targeting virus genes conferred interference against tomato yellow leaf curl virus (TYLCV) in tomato and the endogenous banana streak virus (eBSV) in banana (Fig 2).

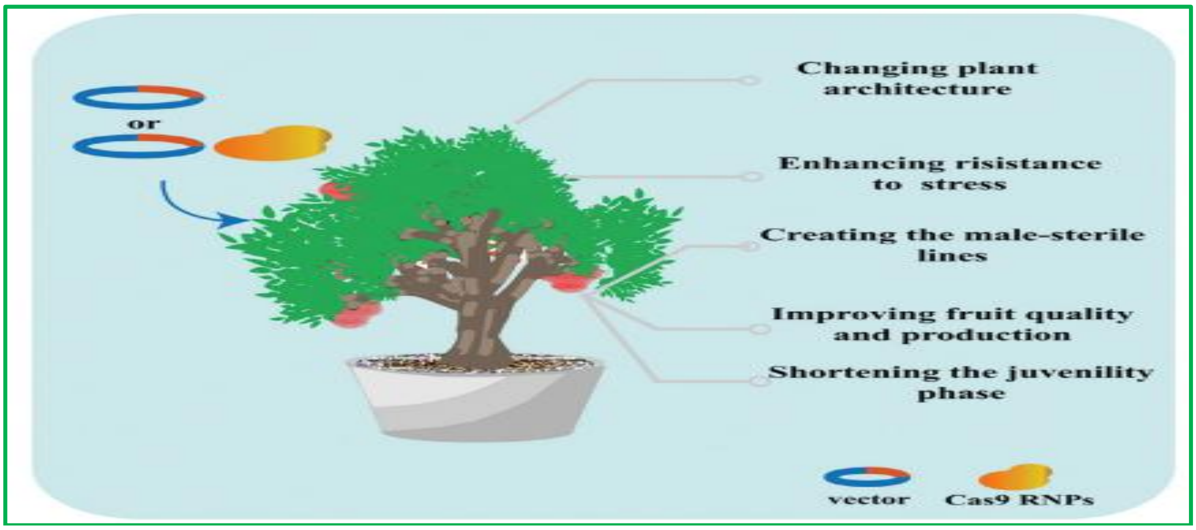

Fig. 2 Potential application of CRISPR/Cas9 systems in horticultural crops Horticultural crops with improved stress resistance, fruit quality and yield, and architecture traits are obtained by delivering CRISPR/Cas9 vectors or Cas9 RNPs/vector components in crops.

- **Improve fruit quality :** Fruit quality is important for horticultural production and sale, usually divided into intrinsic quality (nutrition, flavor) and extrinsic quality (size, color, shelf life). Tomato is a model plant for studying fruit quality, and CRISPR/Cas9 technology has been used often to explore tomato fruit quality.
- **Modify cultivation traits:** Semi dwarfism is a valuable horticultural trait when breeding for wind and rain damage resistance and for convenient management. Many QTLs and genes causing dwarf phenotypes have been described in plants. Several gibberellins (GA)-insensitive and GA-deficient mutants exhibit dwarfish phenotype. GA 20-oxidases (GA20ox) are critical in GA biosynthesis and mutated MaGA20ox2 genes by CRISPR/Cas9 system in banana leads to a lower active GA content and a semi-dwarf phenotype. Hybrid vigour improves crop yield and quality, and CRISPR/Cas9 technology provides a rapid method to establish male sterile lines. For example, knock downing Mitogen-activated Protein Kinase 20 (MAPK20) by CRISPR/Cas9 in tomato resulted in male sterility and normal maternal fertility and vegetative growth. Shortening the juvenility phase accelerates the breeding of woody horticultural crops. The Terminal Flower 1 (TFL1) gene is a class of flowering repressors, and the knockout of the TFL1 gene in apples and pears using CRISPR/Cas9 technology caused early flowering.

Conclusion and future perspectives

The CRISPR/Cas9 systems are widely applied in horticultural crops for breeding and trait improvement. The CRISPR/Cas system optimization accelerates its application in more crops. Selection of the most active interspecies U6 or U3 promoters for driving sgRNAs expression and the tissue-specific and strong promoters for driving Cas9 expression should improve editing efficiency. This methodology likely represents a promising method for transgene-free breeding.

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