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CRISPR/Cas9' applications in Vegetable Crops

Manjunathagowda M G

Abstract:-

Vegetables are an essential part of agricultural production systems, playing a crucial role in sustaining human life by providing essential nutrients, vitamins, and minerals. Their consumption is linked to numerous health benefits, including reduced risk of chronic diseases. Ensuring sustainable and increased production of fruit and vegetable crops is vital for future food security, especially given the continuous rise in global population, urbanization, and the consequent need for more food. The challenge is compounded by the impacts of climate change, which threaten crop yields and quality. Although conventional breeding techniques have significantly contributed to the development of important cultivars, new strategies are necessary to further enhance horticultural crop yields, improve resilience to environmental stresses, and ensure nutritional quality.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated protein-9 (Cas9) is an innovative breeding method with the potential to rapidly and accurately improve various traits in crops. These traits include yield, quality, disease resistance, tolerance to abiotic stress, and nutritional qualities. This technique has been employed to generate additional germplasm resources through gene-directed mutation, owing to its simplicity, high mutation efficiency, and precision. Unlike traditional breeding methods, CRISPR-Cas9 allows for the targeted modification of specific genes, making it possible to introduce desired traits without unwanted genetic baggage. The ability to precisely edit essential genes using CRISPR-Cas9 can swiftly produce new germplasm resources for developing crucial agronomic traits. This is further facilitated by the availability of whole-genome sequencing data and a deeper understanding of the roles of genes in vital traits. Additionally, CRISPR-Cas9 technology holds promise for developing crops that can adapt to changing climate conditions, combat emerging pests and diseases, and meet the nutritional needs of a growing population, thus playing a key role in sustainable agriculture and global food security.

Key words: Crispr/Cas9, Genome editing, Applications and Vegetable Crops

Applications of CRISPR/Cas9 Technology in Vegetable Crops

Vegetable crops face a multitude of abiotic and biotic challenges that can significantly impede optimal production, underscoring the importance of developing

Manjunathagowda M G

Department of Horticulture, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad, Karnataka

resistant or tolerant cultivars (Mou *et al.*, 2011). Enhancing traits such as flavor and nutritional profile, plant architecture, and shelf life are also key areas for improvement in many vegetables. To achieve these objectives and more, the CRISPR/Cas9 technique has been employed to modify the genomes of several commercially significant crops (Mounika *et al.*, 2022).

Parthenocarpy

Parthenocarpy, the fertilization-independent development of seedless fruit, is an essential agronomic trait that can help maintain steady production under variable environmental conditions. It offers energy savings by eliminating the need for seed separation in industrial production and caters to consumer preferences for seedless fruits. In tomatoes, the SlAGL6 (SlAGAMOUS-like 6) gene is crucial for parthenocarpy under high temperature stress. SlAGL6-mutant plants produce fruit that match the wild-type in weight and shape and develop properly. This gene is therefore vital for developing new parthenocarpy germplasms. Altering SlAGL6 in tomatoes results in homozygous or biallelic mutant plants that produce parthenocarpy fruits or fruits containing up to 10 seeds, respectively. CRISPR-Cas9 has also been utilized to delete the genes for SlARF7 (auxin response factor 7) and SlIAA9 (indole-3-acetic acid inducible) to produce seedless tomatoes. The T_0 generation of the biallelic and homozygous SlIAA9-mutant Micro-Tom cultivar, along with the commercial Ailsa Craig cultivar, yields seedless tomatoes as reported by Ueta *et al.*, (2017).

Furthermore, the joint in tomato plants is a weak stem area that allows the fruit to fall after ripening, aiding seed dispersal. Cultivars with jointless fruit stems, where the fruit does not fall after maturation, have been developed through years of artificial domestication. Roldan *et al.* (2017) used CRISPR-Cas to alter MBP21 (MADS-box protein 21) to create a novel jointless germplasm pool.

Improvement of Abiotic Stress Resistance

Crop production is increasingly vulnerable to abiotic stresses due to climate change. While traditional breeding methods can help ensure steady crop output, the rapid development of new types necessitates advanced techniques like CRISPR-Cas9 gene editing. This technology has significantly reduced the time needed to develop new varieties capable of responding to abiotic stress. For example, the brassinosteroid (BR)-mediated development processes involve the brassinazole-resistant 1 gene (BZR1). CRISPR-mediated mutation in BZR1 hampers the activation of RBOH1 and the generation of H2O2, though exogenous H2O2 can restore heat tolerance in the tomato bzr1 mutant. Additionally, gene-editing CBF1 (C-repeat

binding factor 1), which regulates cold tolerance, and MAPK3 (membrane protein kinase 3), which participates in the drought stress response, can produce new germplasms with enhanced cold and drought tolerance in tomatoes.

Improved Herbicide Resistance

Weeds are a significant stress factor that affects vegetable yield and quality, and selective herbicides are commonly used to suppress their growth. Using CRISPR-Cas9 gene editing, the herbicide target gene acetolactate synthase (ALS) in watermelon has been site-directed mutated to produce a herbicide-resistant watermelon germplasm. Key ALS sites in tomato and potato have also been edited using cytidine base editing (CBE), resulting in amino acid changes that confer herbicide resistance.

Improvement of Biotic Stress Resistance

To enhance plant resistance to viruses, two primary methods have been utilized: creating sgRNAs to target the virus genome or altering the fruit crop genes involved in antiviral processes. The binding of the virus genome-associated protein (VPg) to the plant protein "eukaryotic translation initiation factor 4E" (eIF4E) is crucial for plant infection by the Y virus. By altering a critical location on eIF4E, the interaction between the virus and plant can be modified, leading to increased plant resistance to the virus.

Enhancing Flavour and Nutritional Profile

Beyond resistance to stresses, CRISPR/Cas9 technology can also improve the flavor and nutritional profile of vegetables. By targeting specific genes responsible for taste and nutritional content, researchers can enhance the sweetness, vitamin content, and overall palatability of vegetable crops. This not only improves consumer satisfaction but also contributes to better dietary health.

Plant Architecture and Yield Improvement

CRISPR/Cas9 can also be employed to modify plant architecture, leading to better yield and harvest efficiency. By altering genes related to plant height, branch number, and fruit placement, crops can be optimized for both mechanized and manual harvesting. This can significantly boost production efficiency and reduce labour costs.

Shelf-Life Extension

Extending the shelf life of vegetables is another critical area where CRISPR/Cas9 can make a significant impact. By modifying genes related to ethylene production and other ripening processes, the post-harvest longevity of vegetables can be increased, reducing food waste and improving supply chain efficiency.

In conclusion, the applications of CRISPR/Cas9 technology in vegetable crops are vast and varied, offering solutions to some of the most pressing challenges in agriculture. From enhancing resistance to abiotic and

biotic stresses to improving yield, flavor, nutritional content, and shelf life, this technology holds immense promise for the future of sustainable agriculture.

Vegetable crops are prone to a variety of abiotic and biotic challenges that might hinder optimal production, which highlights the significance of developing resistant/tolerant cultivars (Mou *et al.*, 2011). Flavour and nutritional profile, plant architecture, and shelf life are other possibilities for development in many vegetables. To accomplish these and other objectives, the CRISPR/Cas9 technique has been used to modify the genome of several commercially significant crops.

FUTURE CHALLENGES

Whole genome sequencing and functional genomics have facilitated CRISPR-Cas9 genome editing in vegetable crops,

Examples			
Crop Species	**Target Gene**	**Phenotypic Changes**	**References**
Solanum lycopersicum	ANTHOCHYANIN 1 (ANT1)	Increased anthocyanin content	Cermak *et al.*, (2015)
	SLMYB12	Pink tomato fruit color	Deng *et al.*, (2018)
	PHYTOENE DESATURASE (PDS)	Albino phenotype	Pan *et al.*, (2016)
	PDS, GABA-TP1, GABA-TP2, GABA-TP3, CAT9, SSADH	Increased γ-aminobutyric acid content	Li *et al.*, (2017)
	Psy 1, CrtR-b2	Carotenoid metabolism	D'Ambrosio *et al.*, (2018)
	SGR1, Blc, LCY-E, LCY-BI, LCY-B2	Increased lycopene content	Li *et al.*, (2018b)
	SlMBP21	Jointless fruit stem	Roldan *et al.*, (2017)
	St16DOX	Steroidal glycoalkaloids metabolism	Nakayasu *et al.*, (2018)
	RIPENING INHIBITOR (RIN)	Fruits never turn red, altered firmness	Ito *et al.*, (2015)
	Pectate lyase (Solyc03g111690)	Altered firmness	
	ALC	Shelf life	Yu *et al.*, (2017)
	Granule-bound starch synthase (GBSS)	Amylose free starch tubers	Andersson *et al.*, (2017)
Solanum tuberosum L	StPPO2	Reduced enzymatic browning	Nakayasu *et al.*, (2018)
	St16DOX	Reduced bitterness	Nakayasu *et al.*, (2018)
***Solanum melongena* L.**	SmelPPO4, SmelPPO5, SmelPPO6	Reduced enzymatic browning	Maioli *et al.*, (2020)
Citrullus lanatus	CIPDS	Albino phenotype	Tian *et al.*, (2017)

creating diverse germplasm resources. However, challenges remain in selecting relevant mutations for complex traits and introducing the CRISPR-Cas system into plant cells for regeneration. Techniques like Agrobacterium, gene guns, PEG, and electroporation have seen success in model crops. Effective genetic transformation and regeneration systems in vegetables are hindered by CRISPR-Cas cassette efficiency and tissue regeneration capacity. Using plant RNA and DNA viruses as vectors can streamline the transformation process and create transgenic.

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