# VEGETABLE CROP IMPROVEMENT VIA CRISPR/CAS-9 MEDIATED GENOME EDITING

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## ABSTRACT

*The rapidly growing global population leads to an increase in food demand. Vegetables are essential for meeting the daily dietary needs of humans, as they are good sources of fiber, vitamins, and macro as well as micronutrients. Deficiencies of these nutrients are a major health concern, necessitating increased production and the development of new varieties having higher yield and high nutritive value. Conventional breeding methods used in vegetable crop improvement are time-consuming and highly laborious, causing delays in the development of new varieties. To overcome the constraints of traditional breeding methods, CRISPR/Cas-9 (Clustered Regularly Interspaced Short Palindromic Repeat-CRISPR-Associated Protein-9) is an advanced genome-editing technology offering more accurate results in less time as compared with the conventional methods. This technology helps crop breeders to insert, delete, or change specific sequences of DNA for the modification of the genome. The system uses the Cas9 endonuclease in combination with a single-guide RNA (sgRNA) to recognize complementary genomic sequences and induce targeted double-strand breaks (DSBs). These double-strand breaks are repaired by different cellular mechanisms, mostly by Non-Homologous End Joining (NHEJ) or by Homology Directed Repair (HDR), they enables nucleotide insertions, deletions, or substitutions that help in targeted genetic modification. CRISPR/Cas-9 is currently used in vegetable crop improvement programmes to introduce resistance against both biotic (insects, pests, diseases, etc) as well as abiotic stresses (heat, drought, salinity, etc). For example, mutations induced by CRISPR/Cas-9 in SlMAPK3 enhance heat and drought tolerance in tomato. Resistance against powdery mildew in tomato is achieved by knocking out the S1Mlo1 gene. Despite its potential, challenges remain, including the crucial need to select the correct target gene and mutation type so as to avoid off-target editing, and difficulties associated with the transfer of the gene-editing agents into plant cells. However, its precision and speed, particularly in improving polygenic traits, CRISPR/Cas-9 holds vast future scope and is said to be a revolution in vegetable crop breeding and biotechnology.*

***Keywords: CRISPR/ Cas-9, Vegetable Breeding, Technology, Speed Breeding, Generate editing.***

# INTRODUCTION

India's population increased from 1.21 billion in 2011 to 1.46 billion in 2025, representing a 16.43% rise. Consequently, the demand for food has grown by about 54.6% over this period. To feed the huge population vegetable crops play a very crucial role as they are rich source of fibers, macro and micro nutrients, phytochemicals and vitamins which are required by the human body to meet their daily dietary requirements. A large mass of Indian population is deficient in minerals like zinc, iron, iodine etc. and vitamins like vitamin A, vitamin C etc.. in the long-run these deficiencies will lead to serious health issues, hence making it a major area of concern. To meet the food demand vegetable production needs to be increased. For increasing yield and nutrient quality of vegetable crops breeders will have to develop new varieties with high nutritive value as well as yield. Traditional breeding methods are time taking and labour intensive which may cause delay in development of the variety, however new technologies of breeding like CRISPR/Cas-9, take less time and easy to use. It shortens the breeding process by precisely editing the desired gene. We will get to know about CRISPR/Cas-9 technology, its working, application, and the article will be concluded after knowing about major challenges faced by this technology along with it’s future scope.

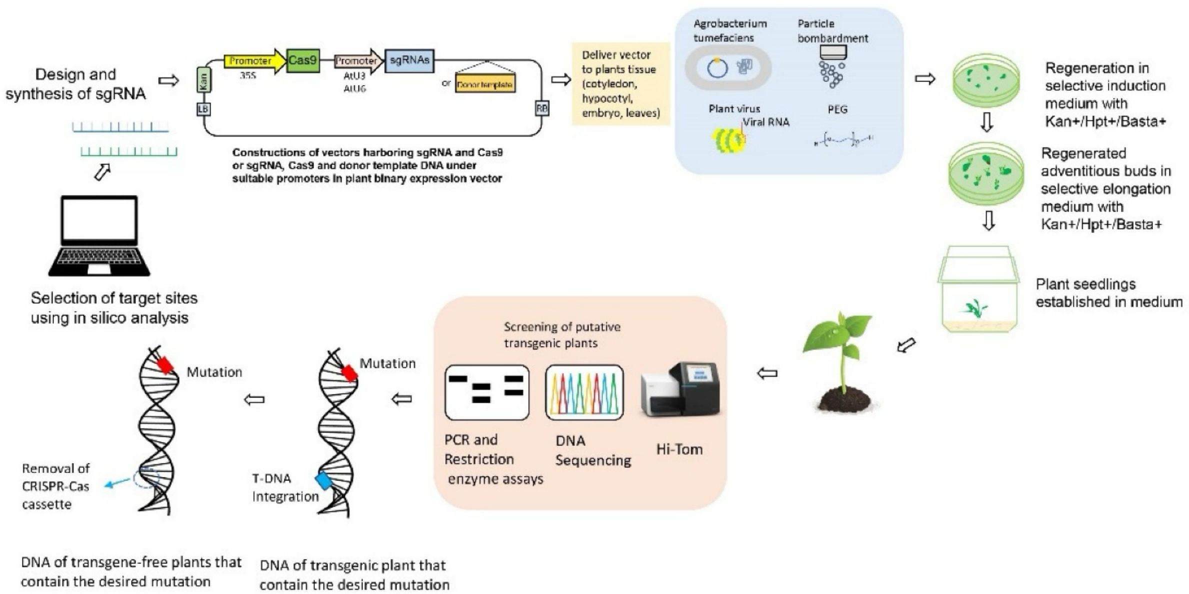
## CRISPR/Cas-9 Technology

CRISPR/Cas-9 stands for Clustered Regularly Interspaced Short Palindromic Repeat-CRISPR-Associated Protein-9. It is one of the advanced gene editing technique which enables breeders/scientist to add, remove or change specific sequences of DNA so as to modify a specific part of genome. There are mainly three types of CRISPR/Cas-9 systems usually utilised utilized for interfering targets (Rouillon et al., 2013). Type-II Cas-9 utilizes its distinctive nuclear domains, that are RuvC and HNH, so as to interfere with a basic effort-module design (Gasiunas et al., 2012). The type-II Cas-9 is isolated from bacteria *Streptococcus pyogenes* (spCas9) is one of the popular CRISPR nuclease used in CRISPR/Cas-9 Technology (Doudna and Charpentier, 2014). The Cas-9 cleaves DNA at the protospacer adjacent motif (PAM) identified by sgRNA-Cas Complex and creates forking DNA or separates strands which eventually activates cellular DNA repairing process. CRISPR-Cas-9 originates from a natural defence system in bacteria, used to fight against viruses. It was discovered by E. Coli in 1987, where as, its function in bacterial immunity was later identified. Nowadays its application in vegetable breeding has enhanced, opening a lot of opportunities for breeders to develop new vegetable varieties with desired characters comparatively in shorter duration when compared to conventional breeding.

## Working of CRISPR/Cas-9 Technology

CRISPR/Cas-9 system is divided into two classes, based on their structure and function. Class-1 includes type-1, type-3 and type-4, where as, class-2 includes type-2, type-5 and type-6. Cas-9 system contains, Cas-9 protein and single stranded guide RNA known as sgRNA. The sgRNA targets at a specific DNA sequence, single or multiple target sites, located 3bp upstream of a PAM (Protospacer Adjacent Motif) sequence. A Complex was formed by Cas-9 with sgRNA molecule so as to cleave the target DNA double strands by RuvC and HNH domain, responsible for non-complementary and complementary strand break (Gasiunas et al., 2012). Cas-9 protein is then evolved artificially so as to create new variety with high fidelity or relaxed PAM.

The nuclear Cas-9 invokes double stranded breaks in the specific area of the genome. Once DNA is cleaved the resulting DNA DSB(Double Stranded Break) are repaired mostly by two Pathways, namely HDR (Homology Directed Repair) and NHEJ (Non-Homologous End Joining), for repairing different DNA segments NHEJ required certain enzyme (Liu et al., 2019; Shuman and Glickman, 2007). This cell repair mechanism is mostly exploited due to often occurrence of errors causing minor insertions or deletions(Yang et al., 2020). Whereas HDR is a bit precise in gene insertion or replacement of the DNA segments at the specific DSB site, it requires large quantity of homologous DNA templates(Liu et al., 2019; Yang et al., 2020). In this way, CRISPER technology is used for genome editing in crop improvement program.




(Wan et al., 2021)

## Implementation of CRISPR/Cas-9 Technology for vegetable crop improvement

In most of the vegetable crops production and quality of the produce is influenced by biotic and abiotic stress factors like water light temperature, insect, pest etc.. We can mitigate them by the use of resistance genes or tolerant cultivars.

CRISPR/Cas-9 technology is a recent introduction in vegetable crop improvement program. It was introduced in the year 2017.

## For Abiotic stresses

Stress conditions created through drought, salinity, light etc.. causes great loss to vegetable production, by traditional methods of plant breeding we can reduce stress effect to some extent but with the new technology of CRISPR/Cas-9 , breeders can develop more resistant varieties against these kind of stresses, also by trading methods breeders have to breed a single plant for generations to develop resistance (Haque et al., 2018). High temperature conditions inhibit the growth of the crop, which ultimately leads to overproduction of ROS (Reactive Oxygen Species) causing oxidative damage, impairing the normal functioning of the cells. MAPKs (Mitogen Activated Protein Kinase) are involved so as to tolerate heat stress (Sharma et al., 2020). Removal of BRASSINAZOLE RESISTAT1 (BZR1) impairs the induction of RESPIRATORY BURST OXIDASE HOMOLOG1 (RBOH1) resulting in induction of $H_2O_2$ and heat tolerance in bzr1 tomato mutant (Yin et al., 2018). Mutation induced via CRISPR/Cas-9 in sLmapk3 imparts higher heat stress tolerance in the tomato crop. This mutant also shows less wilting, mild membrane damage, low production of ROS and improved antioxidant enzymatic activity under heat stress (Yu et al. 2019). For drought stress sLmapk3 shows higher tolerance to drought stress by stimulating transcription of stress related genes. For salt tolerance, editing in HKT-1 and 2 alleles should be done and are then inserted. In tomato Hong Kong cultivars by CRISPR/Cpf-1 mediated homology directed repair mechanism showing stable inheritance against saline conditions (Vu et al., 2020).

**For biotic stress**

Plants have both resistant as well as acceptable genes for the particular disease. Knocking the susceptible gene from genome makes plant resistance to that specific disease. By knocking S1Mlo1 gene confers susceptibility to *Oidium neolycopersici* which causes powdery mildew disease, making plant resistant to fight against disease (Nekrasov et al.,2017). By targeting at two position of the gene by double sgRNA creates transgene-free form powdery mildew hence developing resistance in tomato. Similarly, resistance for powdery mildew is developed in wheat plants (Wang et al., 2014). eIF4E gene is a major host factor required by RNA viruses for their multiplication in the host, resulting in viral infection in the host plant. Silencing the eIF4E gene in tomato and melon plants makes them resistant to the RNA viruses. By knocking out the eIF4E gene with the help of CRISPR/Cas-9 Technology, with the help of two gRNAs at two different sites of eIF4E gene, from the cucumber, induces resistance in plant against viruses belonging to the potyviridae family (Chandrasekaran et al., 2016)

**Traits modified by CRISPR/CAS-9 technology in vegetable crops**

| Crop | Target gene | Traits modified | Reference |
|---|---|---|---|
| | GBSS | Starch and tuber quality | Andersson et al. (2017) |
| | St16DOX | Glycoalkaloids metabolism | Nakayasu et al. (2018) |
| Carrot | Coilin gene | Biotic (PVY) and abiotic stress resistance | Makhotenko et al. (2019) |
| | F3H | Change in the anthocyanin biosynthesis pathway | Klimek-Chodacka et al. (2018) |
| | DcMYB113-like | Anthocyanin biosynthesis | Xu et al. (2019b) |
| Watermelon | ALS | Enhanced herbicide resistance | Tian et al. (2018) |
| Pumpkin | GRF12, AHA1, and HAK5 | Salt sensitivity | Huang et al. (2019) |
| Lettuce | LsNCED4 | Seed germination inhibition | Bertier et al. (2018) |
| Chinese cabbage | BraFLCs | The early-flowering phenotype that did not depend on vernalization | Jeong et al. (2019) |

## CHALLENGES

CRISPR/Cas-9 gene editing technology is a technology of recent time, but it has a lot of challenges which has to be overcome. Firstly, the gene which needs to be targeted, for mutation and the type of mutation to avoid off target gene editing, needs to be selected. Without knowing about the gene sequence of the organism we cannot perform gene editing. For adding desirable mutant alleles, breeder needs to apply effective CRISPR/Cas mediated target site specific insertion, deletion and chromosomal recombination procedures (Zhu et al., 2020). Editing a single gene does not result in desirable phenotype changes , because of significant agronomic factors are quantitative. Both the processes, transfer of CRISPR/Cas-9 gene agents into plant cells and the regeneration of the putative added plant, are challenging

## FUTURE SCOPE

Vegetable crops are highly susceptible to biotic and abiotic stresses, necessitating the development of resistant varieties to overcome these constraints and achieve high yield. Conventional breeding is a lengthy process, and it is also difficult to efficiently improve polygenic traits. With CRISPR/Cas9 technology, breeders can mutate as well as insert multiple genes through Homology-Directed Repair (HDR). The high efficiency of targeted mutagenesis using the CRISPR/Cas9 tool will also aid in identifying gene function in crops, which, in turn, helps design more effective genome editing strategies. Significant effort is currently being dedicated to the standardization of this technology, which further enhances its future scope. Given the precision and time-saving nature of CRISPR/Cas9, it can lead to a revolution in the breeding of vegetable and other crops. The increasing exploitation of Biotechnology combined with CRISPR/Cas9 technology will undoubtedly bring about a great transformation in the field of vegetable crop breeding.

## CONCLUSION

Vegetables are eaten as a staple food around the globe due to their nutritive value. However, these vegetable crops are susceptible to biotic and abiotic stresses, which cause a loss in their yield. To overcome these challenges, scientists have undertaken breeding procedures to generate resistant varieties. By traditional breeding methods, the process of crop improvement is time-consuming and laborious, often concentrating only on monogenic inheritances. CRISPR/Cas9 technology is a precise tool that requires less time for gene editing and for developing resistance against a specific disease. This speed breeding technology helps breeders develop new varieties with both high nutritional content and high yield. This technology has wide future scope in the field of crop breeding and biotechnology. CRISPR/Cas9 enables breeders to edit the plant's genome at a specific location to achieve a desirable result. It is a revolution in the field of vegetable crop breeding, and the future of crop breeding lies in this technology.

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