



Advances in Genome Editing for Major Vegetable Crop Improvement: Opportunities and Challenges

Rahul Chandrakant Kaldate¹,
Girija Choudhary¹,
Ashutosh Singh¹,
Sharwan Kumar Shukla¹,
Ashutosh Kumar¹,
Govind Vishwakarma^{*2}

¹RLB Central Agricultural
University, Jhansi, U.P., India

²SVP University of Agriculture
and Technology, Meerut,
U.P., India



*Corresponding Author
Govind Vishwakarma*

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INTRODUCTION

Tradition plant breeding systems have been implemented since last six decades for the improvement of agricultural crops for various agronomic and polygenic traits. Marker-assisted breeding come into existence in the last of 19th century and have been employed for the introgression of desirable traits in many crops in context to crop improvement. Cereal crops are more focused for improvement using molecular breeding approaches as compare to vegetable crops (Mojica, et al., 2009). Like cereal crops, vegetable crops are also grown for food, nutritional security, industrial processing and medicinal purposes. Vegetable crops also need strategic improvement for various oligogenic and polygenic traits. Vegetable crops are highly influenced by the insects, pests, diseases and abiotic threats like, drought, chilling, soil salinity, soil toxicity and high temperature (Chang, et al., 2017). So, increasing the yield along with pathogen resistance and abiotic stress tolerance of the major vegetable crops is urgent problem of the modern agriculture would need to improve. However, in last two decades transgenic approaches and molecular breeding system have been implemented in the improvement of vegetable crops for quality, yield and biological threats (Scully, et al., 2019). In last few years, the genome edition techniques come in to existence for the alternation of crops genomes for the special purposes. The genome editing tools like *ZFNs*, *TALENs* and Cas9 based *CRISPR* are enabled to precisely editing of the gene for the alteration of genome.

In vegetable crops, several attempts of the genome editing have been made for the alteration of genome for various traits in context to crop improvement.

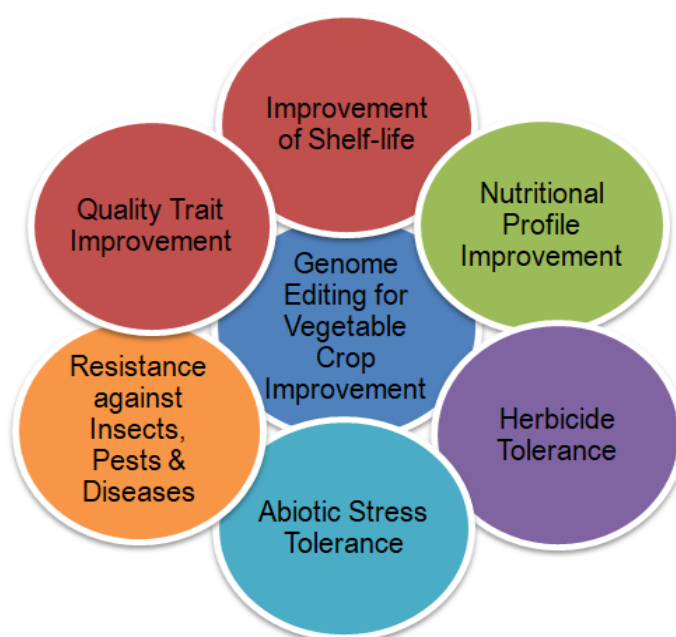
Important traits like plant disease resistance, abiotic stress tolerance, fruit berry quality improvement, flower and fruit colour modification, modification of juvenile period, flower longevity, shelf life, modification in the plant architecture have been targeted for the edition in the vegetable crops like tomato, potato, brinjal, melon, water melon, carrot, cabbage, radish, cauliflower etc.

In recent years, several studies have been published on genome editing of the vegetable and fruit crops in which important traits like disease resistance, alteration of several physiological and biochemical traits and pathways has been attempted. In context to genome editing of the vegetable crops, *CRISPR* based on Cas9 have been employed for many traits for the addition or deletion of the nucleotide sequences on the various genomic region for the alteration of genome (Malzahn, et al., 2017). Tomato is one of the main crops used for genome edition through *CRISPR* technology and several traits like fruit colour, pigmentation, locule number, hormonal response, rapid flowering, disease resistance and drought tolerance have been attempted. Other than *CRISPR*, *ZFNs* and *TALENs* have also been employed for the development of herbicide tolerance and

dwarfism in the some vegetable crops via altering the nucleotide sequences on the various genomic regions. In this literature, authors have explained in in brief about the genome editing tools and their application and achievement of the altering of genomic region of the some useful traits of the vegetable crops.

2. Improvable traits of vegetable Crops

Vegetables are the essential source of the mineral nutritions and bioactive compounds for the human health. Productivity of the vegetable crops is enhanced by several climatic factors and pathogens. Numerous aspects of the vegetable crop improvement have been attempted by researchers for development of novel vegetable cultivars. The gene editing techniques like *ZFNs*, *TALENs* and *CRISPR/Cas-9* have been employed in many vegetable crops for abiotic stress tolerance, resistance against disease and pathogens, improvement of physiological and biochemical traits and some agronomically important traits (Shujuan, et al., 2018). The major vegetable crops improved for nutritional quality trait, prolonged self-life, herbicide tolerance, abiotic stresses tolerance and resistance to diseases.



3. Improvement for nutritional and quality traits

The primary goal of the gene editing in major vegetable crops is to improve the intrinsic quality of fruits nutrient and bioactive compound content. Several genes are potentially involved in the synthesis and metabolism of bioactive compounds, carotenoids and beneficial secondary metabolites. In tomato, malate content was improved by regulating aluminium-activated malate transporter (ALMT9) and butylamine content was increased by gene editing in the GABA synthesis pathway. Texture and colour are then important trait in most of the vegetable crops to attract consumers (Deng, et al., 2018). In tomato, CRISPR/Cas-9 was used to modify phytoene synthase 1 (PSY1), MYB transcription factor 12 (MYB12), and anthocyanin 2 (ANT2) to obtain yellow, pink, and purple tomatoes (Nonaka, et al., 2017).

4. Improvement for prolonged self-life

Self-life is another important trait in vegetable crops has been improved through genome editing tools. *CRISPR/Cas-9* has been employed to knockout the fruit ripening inhibitors like ripening inhibitor (*RIN*) and DNA demethylase (DNA demethylase 2, *DML2*) for the development of slow ripening traits by increasing prolonged self-life in major vegetable crops. It has been observed that in some crops improved for prolonged self-life, the nutritional value and aroma were found to be reduced. In tomato, pectate lyase (*PL*) and alcohylcholine (*ALC*) genes have been inhibited using gene editing technology for the prolonged self-life without influencing nutritional and other quality attributing traits (Wang, et al., 2019).

5. Improvement for herbicide resistance

Weeds and unwanted plants are the major stress for the proper growth and development of vegetable crop that affects both yield and quality. To obtain herbicide resistance vegetable crops, *Cas-9* based *CRISPR* technology of genome editing was used to target acetolactate synthase (*ALS*) associated with herbicide tolerance through

site-directed mutagenesis. Herbicide resistant watermelon cultivars have been developed by targeting *ALS* genes through site-directed mutagenesis. In potato and tomato, Cytidinebase editing (CBE) was used for cytidine editing for mutagenesis in amino acid biosynthesis pathway in context to development of herbicide resistance (Butler, et al., 2015).

6. Tolerance development against abiotic stress

Genes like *BZR1* associated with brassinosterol and brassinosteroids were targeted for alteration of their biosynthesis in context to development of abiotic stress tolerance. In addition, *BZR1* gene has been mutated using *CRISPR/Cas-9* for the induction of *respiratory burst oxidase homolog1* (*BROH1*) for strategic production of hydrogen peroxide. Exogenous production of H_2O_2 provide tolerant to vegetable crops against high temperature and water deficit condition in term of injury recovery (Li, et al., 2018).

7. Resistance for pathogens and diseases

Vegetable crops are highly influences by viral, fungal and bacterial pathogen at various crop stages. Numerous attempts of conventional and molecular breeding have been used for the improvement of vegetable crops for various biotic threats. Recent advancement in genomic research and existence of the genome editing tools may provide strength to the vegetable crop improvement.

In response to development of virus resistance in major vegetable crops, virus genome has been targeted with virus genome linked proteins (VGp) in the plant protein associated with eukaryotic translation initiation factor 4E (eTIF4E) (Bastet, et al., 2019). *CRISPR/Cas-9* based mutation of the key site of eTIF4E affects plant-virus interaction and mediated resistance against viral pathogen and their strains. Cucumber vein yellow virus is the devastating threats of the cucurbitaceous vegetables have been targeted for the development of virus

resistance using such type of genome editing strategy using *CRISPR/Cas-9* tools.

In tomato, potato, brinjal and other solanaceous vegetables, serious economic losses have been observed by downy and powdery mildew disease. Genome edited plants using DMR6; causes over expression of DMR6 can reduce the susceptibility of Downey mildew disease by increasing resistance. In Tomato, DMR6 mutant have been targeted to knockout homologous genes for the development of resistance against the 2-oxoglutarate oxygenase Fe (II)-dependent super family and is involved in salicylic acid homeostasis (Paula de Toledo Thomazella, et al., 2016).

Leaf spot diseases in vegetable crops cause by bacterial pathogen *Pseudomonas syringae* have also improved via genome editing tools. In context to development of leaf spot resistance in major vegetable crops, C-terminal jasmonate domain (*JAZ2*) and jasmonate *ZIM* domain protein2 of *Arabidopsis thaliana* causing over expression of *JAZ2* repressor have been incorporated (Andres, et al., 2018).

CONCLUSION

Vegetable crops are major source of energy, mineral nutrients and beneficial bioactive compounds. Conventional and molecular breeding approaches have been employed for the improvement of the major vegetable crops for agronomic and stress resistance traits. Genome-wide association studies, genomic-assisted breeding have also been implemented in the development of resistance in some vegetables. Tomato is one of the models vegetable fruit have been engineered for several quality parameters and nutritional traits but not all vegetable crops. Most of the vegetable crops need accelerated improvement for biological stresses, herbicide tolerance and mineral nutrition. However, the rapid advancement in the genome editing techniques like *ZFNs*, *TALENs* and *CRISPR/Cas-9* would make grateful vegetable crops for the emerging population of the world.

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