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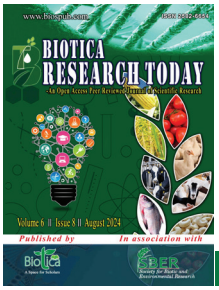

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Plant Gene Editing Approaches for Enhancing Pest and Disease Resistance

B.G. Sangeetha*, T. Makeshkumar and E.R. Harish

Division of Crop Protection, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala (605 017), India

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Corresponding Author

B.G. Sangeetha

✉: Sangeetha.G@icar.gov.in

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Abstract

The crop plants have been domesticated from 10000 years ago and conventional breeding methods were used for developing new crop varieties. With the development of new sequencing technologies about 300 of reference plant genome is available in various nucleotide database and diversity of crop wild relatives (CWRs) genome can be utilized for developing new cultivars. The CRISPR/Cas9 technology has been successfully applied across a range of crops, including rice, wheat, tomato, potato, tobacco, and maize. The gene edited plants can be developed by inserting mutations at target sites in plant genome without inclusion of foreign gene. A list of genome editing techniques have been adapted for use in plant genomes, such as homologous recombination (HR), ZFNs, TALENs, PPRs, the CRISPR/Cas9 system, RNAi, as well as cisgenesis and intragenesis.

Keywords: Pest, Gene editing, Transcriptomics, Disease

Introduction

Invasive pest and diseases emergence is one of the major threats for agriculture. About 30% of crop loss in India is due to pests attack and disease. Developing new crop varieties with resistance to pest and disease is one of the most important strategies for the management of pest and disease. Recently with the application of various omics tools the identification of genes and the genetic pathway related to the various agronomic traits is possible. The advent of gene editing technologies has enabled precise modifications in gene function within organisms. The study of molecular interaction between insects, pathogen and plants can be studied precisely using this technique. Genome editing involves altering genomic DNA at precise target sites through insertion, deletion, or replacement of DNA, resulting in gene suppression or genome modification. In plant gene editing, the CRISPR-Cas system is the most prevalent technique. This technology has been highly effective in enhancing several key agronomic characters such as resistance to biotic and abiotic stress.

CRISPR-Cas System for Plant Gene Editing

The Clustered regularly interspaced short palindromic repeats (CRISPR) system function as defense mechanism in many prokaryotes against viruses and plasmids. Among the various CRISPR systems, CRISPR/Cas9 is the most widely utilized for editing plant genomes. This system comprises two key components: (i) Cas9 protein functions as an RNA-guided DNA endonuclease, interacting directly with the guide RNA (gRNA); (ii) gRNA, a 20-nucleotide RNA segment, pairs with the target DNA sequence, directing the Cas9 protein to the precise location. Upon entering the cell, the nuclease, attached with a recognition site and nuclease domain, locates and attaches to the target DNA, inducing double-strand breaks (DSBs). The repair of these DSBs is facilitated by either the non-homologous end-joining (NHEJ) or homology-directed repair (HDR) pathways. NHEJ, a process prone to errors, frequently results in mutations like insertions or deletions, while HDR offers precise repair by utilizing a DNA template. Gene editing utilizes four main types of sequence-specific nucleases: engineered homing endonucleases (meganucleases), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR-associated proteins (Cas). The CRISPR/Cas system has significantly enhanced plant stress tolerance by modifying various metabolic pathways. Genome-edited

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plants developed using site-directed nucleases (SDNs) fall into three categories: SDN-1, SDN-2 and SDN-3.

Application of CRISPR-Cas Technology using Crop Wild Relatives (CWR)

The crop wild relatives always serve as a source of resistance genes against pest and diseases. The resistance mechanism of CWR is mainly due to antibiosis and antixenosis. Usually two methods are used to for developing pest resistant crop plants by genome editing. One approach is to identify the resistant genes and genetic pathway in CWR to modify the genes causing susceptibility to the insect pests. Another approach is to alter the genome of cultivated crops according to the resistant genes identified in CWR. The alteration or variation in the genes in cultivable and resistant wild can be studied precisely using the modern genomics tools. *S. pimpinellifolium* wild tomato was reported as resistant to pests. The six *loci* of wild *S. pimpinellifolium* was successfully utilized for genome editing and *de novo* domestication was made possible for yield (Zsogon *et al.*, 2018). The prime editors and base editors of Cas system are proved to be efficient tools for genetic manipulation of CWR as they can precisely modify amino acid sequences of proteins which are associated with disease resistance. They can also alter microRNA (miRNA) target sites, interference function of enhancer or motif sequence of transcription factor. Still the constraints for gene editing in wild plant species are lack of efficient transformation methods with rapid *in vitro* regeneration methods. To overcome the constraints for wild and recalcitrant crops standardization of plant transformation protocols is essential. Another approach is to deliver the gene construct to somatic cells by Fast-Treated Agrobacterium Co-Culture (Fast-TrACC) by meristem induction. It was reported in *Nicotiana benthamiana* which requires few plants to develop modified shoots. The meristem developed from gene edited somatic cells will form shoots and time period required for developing the entire edited plants is reduced. Once these methods are established in other economically important crops it will be helpful to establish useful traits in their respective CWR.

CRISPR-Cas Systems to Enhance Disease and Pest Resistance in Host Plants

Blanvillain-Baufum *et al.* (2017) have reported resistance against blight disease in rice by genome editing techniques. Earlier studies reported that the resistance to rice blast disease and leaf blight can be developed through Cytosine base editors (CBE) method (Ren *et al.*, 2018). The target gene used for leaf blight resistance was OsSWEET14 (Wang *et al.*, 2020). The virus disease resistance was obtained by site-specific mutation of *eIF4G* loci in rice genome (Wang *et al.*, 2021). CRISPR interference (CRISPRi) technique blocks transcription of target genes by effectively silencing them; whereas, CRISPR activation (CRISPRa or CRISPR-Act) upregulate beneficial genes, enhancing plant defense mechanisms. CRISPR-Cas technology was applied in rice to develop resistant cultivars with pest resistance by deletion of gene CYP71A1 encoding Tryptamine 5-hydroxylase. Gene mutation in phytoene dehydrogenase (PDS) of *Populus tomentosa* by CRISPR-Cas9 technique leads to development of insect resistant plants. Resistance against aphid *Rhopalosip humpadi* was developed in barley by alteration in two beta 1-3 glucanase genes using CRISPRCas9. *Spodoptera litura* and *Helicoverpa armigera* resistant soyabean plants were developed by CRISPR-Cas technology creating deletion in GmUGT and GmCDPK38 genes.

Conclusion

The expression of genes is fundamentally responsible for the different traits observed across organisms. By utilizing gene editing techniques, it is possible to modify gene expression to achieve desirable phenotypes in plants. In maize and tomato variation in the regulatory regions of the genes leads to many agronomically important traits in wild species. In many plants variation in sequences occur at distant regions of genes and some other plants the information regarding the promoter sequence and function is unknown. Gene manipulation can be achieved using synthetic transcriptional activators or repressors. CRISPR-based techniques like CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) allow for the precise regulation of multiple genes in plant systems. For plants developed using SDN-1 and SDN-2 techniques, GMO regulations are often not enforced in many countries, including India, except in the European Union (EU). The technology is prevalent in almost all areas of research worldwide due to the advantages while comparing with other technologies. Genome-edited crops can be produced by suppressing genes that make plants susceptible, thereby enhancing their resistance to insects, or by increasing the expression of resistance genes to combat pests or pathogens. The improvement of traits related to biotic stress is important for sustainable management of pest and disease.

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