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Review Article

Approaches of Genome Editing in Crop Improvement

Parveen Fatima

Assistant Professor cum Jr. Scientist, Nalanda College of Horticulture, Bihar Agriculture University, Sabour.

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E-mail Id:
parveenfrizvi@gmail.com
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A B S T R A C T

Modern agriculture has profited from a number of methods that aid in plant genetic improvement since the development of molecular biology, with a focus on transgenics, marker-assisted selection, and genome editing .Using designed nucleases, genome editing is a potent and rapidly developing approach that allows for precise change in the genome in many different organisms. The foundation of all genome editing techniques is the formation of double-strand breaks (DSBs) at the target locus, which are then repaired using either the non-homologous end-joining (NHEJ) or homology-directed repair (HDR) pathways. These processes can result in the desired genetic alterations. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas9 system are the primary genome editing technologies. These technologies can generate distinct phenotypes in a variety of sciences, including biology, biotechnology, and medicine, by precisely modifying genes. Since 2010, the introduction of TALENs has made it feasible to modify the genomes of model organisms. Then, in 2013, the discovery of the CRISP/Cas9 technology ushered in a new age of genome editing research—a biological revolution, if you will. Furthermore, genome editing will likely be used to treat hereditary illnesses in the near future. Furthermore, it is encouraging that genome editing may be used to create diverse crops and livestock with beneficial traits. These goods are not genetically modified organisms (GMOs); instead, they are referred to as altered crops.

Keyword: Genome, Transcription, Crops, Zinc, Genes

Introduction

In a wide range of organisms, genome editing refers to the modification of genomic DNA at a specific target site. This modification can involve the insertion, deletion, or replacement of DNA, which can lead to the inactivation of target genes, the acquisition of new genetic traits, or the correction of pathogenic gene mutations.[1, 2] With the life sciences developing at a rapid pace, genome editing technology has emerged as the most effective way to study gene function, investigate the etiology of hereditary diseases, identify new gene therapy targets, breed crop varieties, and more.[3, 4] Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and RNA-guided CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) nucleases systems are currently the three main genome editing techniques available worldwide.[8–10] CRISPR-Cas systems are the most extensively used genome editing method in molecular biology labs worldwide because of its benefits, which include a quick cycle time, strong repeatability, low cost, high efficiency, and simple design.[5, 6]

Genome Editing

The phrase "genome editing" describes methods for modifying the genome of living things at particular locations, such as targeted mutagenesis or site-directed insertion, deletion, or substitution. The process of editing a genome involves creating site-specific double-strand breaks (DSBs) and allowing the body to heal itself using either the error-free homology-directed repair (HDR) pathway or the error-prone non-homologous end-joining (NHEJ) pathway. DSBs are mostly repaired in plants by nonhomologous end joining (NHEJ), a process that joins the break ends of DSBs directly without the assistance of a homologous repair template. In higher eukaryotes, NHEJ occurs throughout the cell cycle and shows limited fidelity in the repair.[7]

Genome Editing Technologies

Gene-editing technology is linked to many approaches, and scientists and researchers can create new techniques as a result of this process. Often employed methods include: A. Zinc-Finger Nucleases (ZFNs) P3

A. Zinc-Finger Nucleases (ZFNs)

ZFNs are synthetic nucleases that are produced by combining a nonspecific DNA cleavage domain that is taken from the type II restriction endonuclease FokI with an artificial, sequence-specific zinc finger DNA-binding domain.[8] The DNA-binding domain has many linked zinc finger (ZF) motifs, each of which may identify a distinct DNA sequence that is three base pairs long . A single ZFN can identify a specific DNA sequence that is 9–18 bp long and contains three to six ZFs. To break double-stranded DNA, the FokI nuclease needs to dimerize. In order to create a DSB at the intended DNA site, two ZFNs must be designed.[9] In order for FokI dimerization to occur, a spacer sequence of 5to 7 base pairs must be inserted between the forward and reverse target sequences, and two distinct ZFNs must bind to the forward and reverse strands, respectively.

Transcriptional Activator-Like Effector Nucleases (TALENs)

The TALEN (Transcription Activator-Like Effector Nucleases) system was a further technique for improving genome editing's effectiveness, security, and accessibility that was created in 2011. The transcription activator-like effectors (TALES) generated by the phytopathogenic bacteria belonging to the Xanthomonas species gave rise to the TALEN system.[10] Like the transcription factors of eukaryotic genomes, the effector proteins are members of a family of proteins that bind to DNA and can be utilized to induce the expression of their target genes.

Clustered Regularly Interspaced Short Palindromic Repeats

(CRISPR)/CRISPR-associated protein 9 (Cas9)

Genome engineering was completely changed with the introduction of a quick, low-cost, and reasonably effective genome-editing technique with the discovery of CRISPR/Cas as a new gene-editing platform. Through spacer acquisition, synthesis, and target destruction, CRISPR/Cas is a novel technology that functions in prokaryotes (Bacteria and Archaea) as an adaptive immune system to defend them against phage invasion.[11]

CRISPR/Cas12a System

The CRISPR/Cas12a system has been able to overcome several of the CRISPR/Cas9 system's limitations due to some variations between Cas12a and Cas9. For instance, Cas12a requires a single RNA molecule (crRNA) to cut DNA, whereas the Cas9 enzyme requires two molecules—tracrRNA and crRNA. While Cas12a only contains the RuvC nuclease domain, which cuts only one strand to produce two sticky ends at the target sites, Cas9 contains both the HNH and RuvC nuclease domains and cleaves both DNA strands at the same site, resulting in blunt ends.[14] When choosing a site for editing, the Cas12a protein offers more alternatives and cuts DNA at many locations. In Cas9, the cleavage and detection sites are adjacent, whereas in Cas12a, they are not. Cas9's PAM sequence is abundant.

Advantages of Gene Editing

A potent tool for agriculture, effective genome editing methods enable very specific (non-random) modification of plant genomes in their native chromosomal setting. Using genome editing techniques for crop breeding is one of the greatest advantages that allows the simultaneous improvement of multiple traits directly in elite lines in a very short period of time.

Conclusion

Over the past three decades, the development of genome editing tools like CRISPR/Cas, ZFNs, and TALENs has made remarkable contributions to modern agriculture. Numerous organisms and tissues can now have precise and targeted genome modification because to the development of various genome editing techniques as TALENs, ZFNs, and CRISPR systems. By enhancing crops, genome editing technology can improve food safety and nutrition in agriculture in addition to medical and pharmaceutical research. The technique was made more accurate and efficient with the discovery of the novel protein Cpf1, which has more editing activity than Cas9. The growing ethical

questions raised by the application of CRISPR technology present another difficulty. As a result, guidelines must be established before doing genome editing.

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