CRISPR/CAS9: A Potent Method for Genome Editing in Crop Improvement

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OPEN ACCESS

Keywords

Genome editing, CRISPR/CAS9, Biotic/abiotic stress, GMOs, Climate change

How to cite this article:

Mandal, A., 2024. CRISPR/CAS9: A Potent Method for Genome Editing in Crop Improvement. *Vigyan Varta* 5(11): 70-73.

ABSTRACT

Agriculture is the main source of India's population for their livelihood. Due to a growing population and a lack of arable land, the need for food is rising daily. Crop output is in significant danger from pest invasion and increased abiotic stress brought on by climatic changes. Numerous methods, including traditional breeding, mutagenesis, translocation breeding, and others, are being developed to create high-yielding cultivars that are resilient to biotic and environmental stress in order to control all of these risks. Later, transgene insertion was developed and used in genetically modified crops to achieve the desired results. However, modern advances in genome editing are the new weapon against agricultural hazards. To combat biotic and abiotic stress, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an effective technique that is both simple and precious. Agriculture could undergo a transformation by the application of CRISPR/CAS9 technology, particularly in the face of climate change.

INTRODUCTION

The world's population and food crisis are the current highlight situation along, with the severe climate change ratio. Conventional breeding methods are the way to improve a crop's qualitative and quantitative characters but it takes much time

to develop a improve variety. Whereas genetic engineering plays a crucial role in genome modification within a shorter time period. Genome editing has revolutionized biology by allowing for targeted genome modifications in many different species.

Many tools that help to solve the problem of precise genome editing of crops have been more challenging. For the first time, it was demonstrated in 1996 that "Zinc Finger Nucleases" (ZFNs) is an artificial endonuclease that function as site-specific nucleases by fusing a specified Zinc Finger Protein (ZFP) to the Fok-I restriction enzyme. In 2010, it was revealed that the basic component of TALENs consists of the nuclease FokI fused with a specific DNA binding domain, enabling the incorporation of a DSB at a specific site. Eventually, in 2012, the most successful genome editing tool, Clustered Regularly Interspaced Short Palindromic Repeats **(**CRISPR) developed, which is a simple, precise, and less expensive technique. CRISPR is first observed in the *Streptococcus pyogenes* defense system by eliminating foreign DNA. The CRISPR/Cas9 is basically 'micro scissors' that cut the DNA in a shorter and more precise manner than other technique. It's a site-specific genome editing techniques for multiple sites (Waryah *et al.*, 2018).

Mechanism and action of CRISPR/Cas9

CRISPR Arrays: The defense mechanism of archaea and bacteria is CRISPR/Cas that helps to chop foreign DNA in a sequence-specific manner. Spacers, short foreign DNA segments, provide immunity by integrating between repeats at a CRISPR locus. CRISPR RNAs (crRNAs) from the CRISPR array and trans activating CRISPR RNA (tracrRNA) jointly help to activate the Cas9 enzyme.

Cas9 Enzyme: Cas9, an RNA-guided enzyme, targets and breaks down foreign nucleic acids with complementary sequences to the loaded RNA during bacterial immunity. The recognition (REC) lobe and the nuclease (NUC) lobe are the two portions that make Cas-9. RuvC, His-Asn-His endonuclease (HNH), and protospacer adjacent motif (PAM) make up the NUC lobe, whereas the REC lobe is made up of the REC1 and REC2 domains, which bind guide RNA. Each single-stranded DNA strand is chopped by the RuvC and HNH domains, whereas the PAM interaction domain gives PAM specificity and starts the binding process to the target DNA (Asmamaw and Zawdie, 2021).

Single guide RNA (sgRNA): Custom designed targeting sequence (crRNA) and transactivating CRISPR RNA (tracrRNA), jointly known as sgRNA, that binds and guides Cas9 nuclease for site-specific DNA cut. The guide RNA (gRNA) sequence determines the targeted cleavage site. CRISPR RNAs (crRNAs) help to determine the specific DNA sequence and attached to a single strand and tracrRNA attached to another strand with the Cas9 enzyme.

CRISPR/Cas9 Effector Complex: Cas9, with guide RNA, forms a DNA surveillance complex. The spacer sequence in crRNA determines DNA target specificity. The seed sequence of RNA nucleotides within the spacer region of crRNAs is particularly important for target specificity. Close homology in the seed region may cause off-target binding.

Target Recognition: The CRISPR/Cas9 system is composed of sgRNA and Cas9 nuclease, which recognize and cleave DNA, causing gene mutation. Cas9 nuclease can target any DNA sequence with 5'N (20-22)-NGG (N is any nucleotide) by altering the 20-22 nucleotide guide sequences (Zhang et al., 2019). Cas9 contains an HNH domain and a Ruv-C like domain, which cleave the CRISPR

RNA and the opposite strand of double-stranded DNA, respectively. The sgRNA, consisting of the seed sequence and nonseed sequence adjacent to the protospacer adjacent motif (PAM) sequence, is crucial in the CRISPR/Cas9 system.

PAM Recognition: Cas9 requires a protospacer adjacent motif (PAM) that is particular to the bacterial species that encodes the Cas9 gene. The widely utilized Cas9 from S. pyogenes detects the PAM sequence NGG downstream of the target DNA. A conserved 5′-NGG-3′ PAM and a seed sequence that matches the RNA and target DNA are required for cleavage. The guide RNA with Cas9 enzyme attached and PAM sequence recognized by tracrRNA and seed sequence recognized by crRNA, formed a DNA-RNA hybrid.

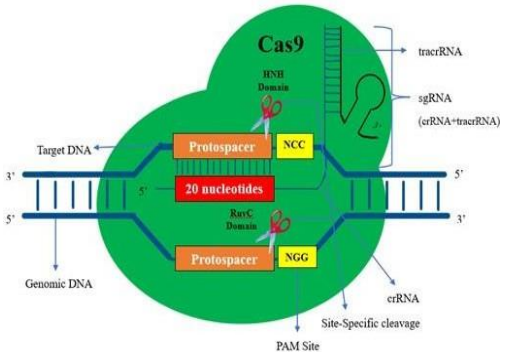

Fig. 1: Mechanism of CRISPR/Cas9

Target Cleavage: Two domains of Cas9 cut the target DNA double strands according to the compatibility of crRNA against the protospacer. The strands join together when the target DNA and the guide RNA are compatible, enabling the DNA to unzip. However, Cas9 needs the first PAM binding and +1 phosphate stabilization for efficient binding and stability. The PAM's third and fourth nucleotides were then accurately cut by the HNH and RuvC nucleases.

DNA Repair: CRISPR-Cas9 is a genome editing tool that creates breaks in DNA to activate cellular repair pathways. These breaks can lead to mutation or changes in the genome. There are two main repair mechanisms: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ joins broken DNA ends without the need for a template, but it can introduce errors. HDR, on the other hand, uses a template to make precise repairs and is most active in specific phases of the cell cycle. In CRISPR gene editing, HDR requires additional donor DNA templates for precise gene insertion or replacement at the break site (Arora and Narula, 2017).

Advantages of the CRISPR/Cas9 System

- CRISPR cuts and edits the sequence of DNA preciously, minimizing off-target cleavage compared to other gene editing techniques.
- This technique is cost-effective, efficient, and can be used in different organisms, including animals, microorganisms, plants, *etc.*
- CRISPR-Cas9 can be easily programmed by replacing the sgRNA's guide sequence (20 nucleotides in native RNA) with any DNA sequence of interest.
- CRISPR avoids the traditional GM crop production methods that include introducing exogenous or foreign DNA into the plant genome using a vector.
- The CRISPR/Cas9 system generates stable and heritable mutations, which can easily segregate from the Cas9/sgRNA construct to avoid further modification by CRISPR/Cas9.
- Regulation of gene expression without changing the DNA sequence can be done by CRISPR.

Applications in Agriculture

- CRISPR allows precise gene alteration of plants to confer pest, disease, or environmental

resistance, reducing chemical pesticide reliance and promoting sustainable farming methods.

- CRISPR can optimize plant photosynthesis pathways, enhancing agricultural productivity and efficiency by altering genes related to plant growth and development, resulting in more fruit, a higher seed set, and quicker maturity.
- CRISPR can alter genes controlling water intake, transport, and retention in plants, enabling them to survive in drought-prone areas and create crops resistant to drought, salt, and extreme temperatures.
- CRISPR can modify genes to create crops that are more adaptable to climate changes and local conditions by altering genes associated with environmental parameters.

CONCUSSION

Plant breeding is dealing with crossing between selected suitable parent lines that combine desirable characteristics of crops for improvement. This method is a long procedure and less precious. In Indian economic system the losses due to biotic and abiotic stress is a major problem. Genome editing is a game-changing approach that aims to improve crop traits by addressing biotic and abiotic stressors. In the Indian economy, crop losses due to diseases like bacterial leaf blight and thermal blast can reach 50% and 90% under epidemic situations. CRISPR/Cas9-based genome editing is a game-changing approach that can be applied to crop modification to improve yield, nutritional value, disease resistance, and other qualities. Although progress is preliminary, genome editing with CRISPR/Cas9 is expected to become more common, helping to achieve the zero-hunger goal and feed the growing human population.

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