Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 06, Issue: 01 (JAN-FEB, 2026)
Available online at http://www.agriarticles.com

CRISPR: Redefining the Future of Crop Improvement

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Traditional crop breeding is often too slow and inefficient to meet the rising global demand for food. Extended crop lifecycles and time-consuming processes such as crossing and selection make it difficult to develop improved varieties quickly. Moreover, decades of reliance on conventional methods have narrowed crop genetic diversity, further limiting progress. To address these challenges, plant breeders are increasingly adopting modern approaches such as speed breeding, high-throughput phenotyping, and genome editing. Among these methods, CRISPR/Cas9, a recently developed RNA-guided endonuclease, has emerged as a powerful tool for precise and efficient gene editing in plants.
The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system was first identified through unique DNA sequences in the bacterium *Streptococcus pyogenes*. These sequences consisted of short, repetitive elements interspersed with distinct non-repetitive spacers. Initially considered to be of unknown function, they were later found to be widely present in both bacteria and archaea. A breakthrough occurred with the identification of nearby genes, now known as CRISPR-associated (Cas) genes, which suggested a functional connection to the repeat sequences. Further analysis revealed that many spacer sequences matched fragments of viral DNA, indicating a possible role in protecting bacteria from viral attacks. This led to the understanding that CRISPR-Cas functions as an RNA mediated adaptive immune system in prokaryotes, enabling them to recognize, remember, and target invading genetic elements. This natural defense mechanism has since been adapted into a powerful tool for precise and efficient genome editing across a wide range of organisms.

CRISPR systems are broadly classified into two primary classes based on their associated Cas proteins and interference mechanisms. These classes are further divided into six distinct types, each defined by unique signature Cas genes. Class 1 CRISPR systems (Types I, III, and IV) utilize multi-protein complexes composed of various Cas proteins to mediate interference. In contrast, Class 2 systems (Types II, V, and VI) rely on a single, large effector protein that functions in association with CRISPR RNAs (crRNAs) to carry out interference. The genome editing technology commonly used today is derived from a Class 2 CRISPR system that uses RNA-guided mechanisms to target and cleave specific DNA sequences.

Components of CRISPR/Cas9 system

The CRISPR system is composed of two types of RNA and a protein. These include the CRISPR RNA (crRNA), which pairs with the target DNA through sequence complementarity; the trans-activating CRISPR RNA (tracrRNA), which hybridizes with the crRNA to form a functional guide complex; and the Cas9 protein, which binds this complex and introduces a precise double-stranded break in the DNA. For gene-editing purposes, the crRNA and tracrRNA have been combined into a single synthetic molecule called single guide RNA (sgRNA). This engineered CRISPR/Cas9 system typically functions with two core components: the sgRNA, which contains a 20-nucleotide sequence at its 5' end that determines the DNA target site, and the Cas9 protein, which uses the sgRNA to locate and cut the corresponding DNA near a 5'-NGG-3' protospacer adjacent motif (PAM). Although the Cas9 mRNA remains unchanged regardless of the application, the sgRNA is customized for each specific gene target to ensure accurate genome editing.

Molecular mechanism of CRISPR/Cas9 Genome editing system

The CRISPR/Cas9 genome editing process operates through a well-defined sequence of three major steps: target recognition, DNA cleavage, and cellular repair. The process begins with the recognition of a specific DNA sequence within the genome. This is facilitated by a synthetic single guide RNA (sgRNA), which is designed to contain a 20-nucleotide region complementary to the target DNA. This guide RNA is essential, as the Cas9 protein remains in an inactive state without it. Once the sgRNA binds to Cas9, it forms a ribonucleoprotein complex that actively searches the genome for sequences matching the sgRNA's target site. A critical requirement for Cas9 to bind and cleave the DNA is the presence of a protospacer adjacent motif (PAM) immediately downstream of the target sequence. The PAM is a short, conserved DNA sequence typically 2–5 base pairs in length, and its specific sequence varies depending on the bacterial species from which the Cas protein is derived. In the commonly used *Streptococcus pyogenes* Cas9 (SpCas9), the PAM sequence is 5′-NGG-3′, where "N" can be any nucleotide.

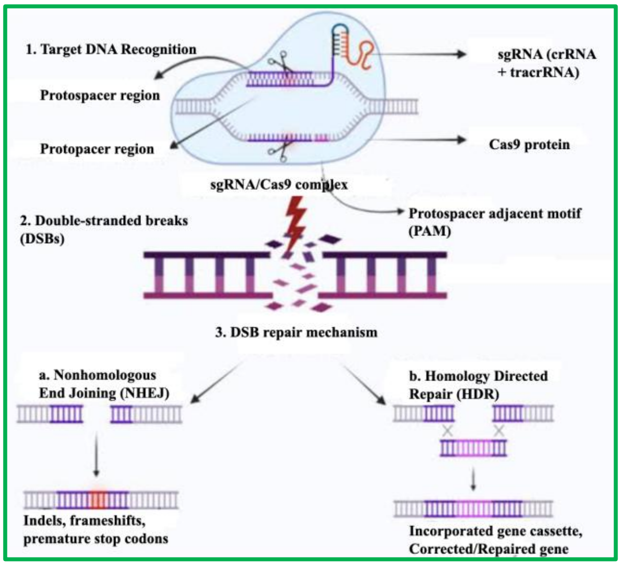

Figure.1. Mechanism of CRISPR Cas9 gene editing

Recognition of this PAM is essential; without it, Cas9 cannot engage with the target DNA. Upon a matching DNA sequence with an adjacent PAM is located, Cas9 initiates local DNA unwinding to allow the sgRNA to form complementary base pairing with the target strand, creating an RNA-DNA hybrid. Although this DNA melting step is crucial for target binding, the precise molecular mechanism by which Cas9 disrupts the DNA helix is still not fully understood. Following hybrid formation, Cas9 undergoes a conformational change that activates its nuclease domains for DNA cleavage. The enzyme contains two distinct endonuclease domains: the HNH domain, which cleaves the DNA strand complementary to the sgRNA, and the RuvC domain, which cleaves the non-complementary strand. These coordinated cuts result in a double-stranded break (DSB) at a position approximately three base pairs upstream of the PAM site. The DSBs created by Cas9 are typically blunt ended, though small overhangs can sometimes occur. Once the DNA is cleaved, the cell's intrinsic repair machinery is recruited to fix the break.

Double Stranded Breaks Repair Mechanism

In the CRISPR/Cas9 genome editing system, the double-stranded breaks (DSBs) introduced by the Cas9 nuclease are primarily repaired by two cellular mechanisms: non-homologous end joining (NHEJ) and homology-directed repair (HDR). These two pathways differ in terms of accuracy, timing, and molecular requirements.

i. Nonhomologous End Joining

NHEJ is the most predominant DNA repair mechanism in eukaryotic cells and is active throughout the entire cell cycle. This pathway repairs DSBs by directly ligating the broken

DNA ends without the need for a homologous template. The repair process involves a set of enzymes that recognize and process the broken ends, followed by ligation to restore DNA continuity. Although NHEJ is a fast and efficient repair method, it is inherently error prone. During the rejoining process, the enzymatic machinery may inadvertently introduce small insertions or deletions (Indels) at the site of repair which can alter the reading frame of the gene, resulting in frameshift mutations or premature stop codons. Such mutations often disrupt the function of the gene, making NHEJ particularly advantageous for gene knock-out applications in genome editing.

ii. Homology- Directed Repair

HDR is a high-fidelity repair mechanism that uses a homologous DNA template to accurately repair DSBs. Unlike NHEJ, HDR is confined to the late S and G2 phases of the cell cycle when a sister chromatid or homologous DNA sequence is available as a repair template. In the context of CRISPR/Cas9-mediated editing, researchers can introduce an exogenous donor DNA template containing the desired genetic modification flanked by sequences homologous to the regions adjacent to the break site. The cell's HDR machinery uses this template to guide accurate insertion, replacement, or correction of DNA sequences at the DSB location. This makes HDR the preferred pathway for applications requiring precise gene editing, such as gene knock-in, correction of point mutations, or replacement of defective gene segments. However, HDR is inherently less efficient than NHEJ, particularly in non-dividing cells, due to its dependence on specific cell cycle phases and the need for an external DNA template. To enhance HDR efficiency, various strategies such as cell cycle synchronization, chemical inhibitors of NHEJ, or the use of modified donor templates are often employed in experimental setups.

Prospects

The future of CRISPR-Cas9 in agriculture is highly promising, offering solutions to key challenges such as low productivity, pest and disease susceptibility, and climate stress. By enabling precise genetic modifications, CRISPR can enhance yield, improve nutritional content, and develop crops with better tolerance to drought, heat, and salinity. It also aids in reducing dependence on chemicals by building innate resistance in plants. Rapid breeding techniques using CRISPR can shorten crop development cycles. Furthermore, gene drive systems could help control invasive species and improve pest management. Despite its potential, widespread adoption requires addressing regulatory, technical, and funding challenges.

Conclusion

CRISPR/Cas9 has revolutionized the field of plant genome editing by offering a precise, efficient, and versatile tool to improve crop traits. From enhancing yield and nutritional quality to developing resistance against biotic and abiotic stresses, this technology addresses many critical challenges in agriculture. As research advances and integration with other modern breeding techniques continues, CRISPR/Cas9 holds immense potential to accelerate crop improvement and contribute significantly to global food security. However, careful consideration of regulatory frameworks, ethical concerns, and equitable access will be essential for its sustainable and responsible deployment.

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