Rewriting Plant Genetics: CRISPR-Cas9

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The world population is expected to reach 9.7 billion by 2050, and feeding this population is one of the biggest challenges facing humanity. Agricultural productivity needs to be increased while simultaneously minimizing the environmental impact of farming practices. One solution to this problem lies in plant breeding, which involves the selection and manipulation of plant traits to create new, improved varieties. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a third-generation gene editing technology. CRISPR/Cas9 technology has revolutionized the plant breeding field, providing scientists with a strong tool to edit plant genomes with unprecedented precision. In this article, we will explore the science behind CRISPR-Cas9 technology, its applications in plant breeding, and the ethical considerations surrounding its use.

The science behind CRISPR-Cas9 technology

CRISPR-Cas9 is a gene editing tool that uses a system of bacterial immune defence to make precise, targeted changes to DNA sequences. The system relies on a protein called Cas9, which acts like a pair of molecular scissors, and a small RNA molecule known as single guide RNA (sgRNA), which directs Cas9 to cleave the target DNA strand, leading to a double strand break (DSB). These breaks can be repaired by Non-Homologous End Joining (NHEJ) or Homology Directed Repair mechanisms (HDR). In NHEJ, the ends of DSBs are linked by the DNA ligase enzyme directly and it does not depend on the homologous DNA sequences. Thus, it is a fast repair process but is not accurate. Whereas, the homologous repair procedure is complex but accurate as It can only take place in the G2/S phase of the cell cycle and needs for a homologous DNA sequence template.

A novel target gene modifying technique has been developed based on the CRISPR/Cas system i.e., base editing technology. It utilizes a tethered deaminase domain for base conversion from A>G or C>T or C>G even in the absence if the donor DNA. Base editors are recently used to create single and multiple nucleotide alterations in the cells.

Mechanism of Cas9 protein

The Cas9 protein comprises six domains, namely

(1) Recognition lobe (REC I)

(2) REC II

(3) Arginine-rich bridge helix

(4) PAM Interacting

(5) HNH, and

(6) RuvC

The REC I domain plays a crucial role in binding with the gRNA, while the specific function of REC II has not been thoroughly investigated. The arginine-rich bridge helix triggers the cleavage activity once it binds to the targeted sequences. The interaction with PAM (Protospacer Adjacent Motif) determines the specificity towards PAM sequences, which is crucial for binding with the target sequence. The HNH and RuvC domains serve as nuclease domains, responsible for cutting/chopping the target sequence.

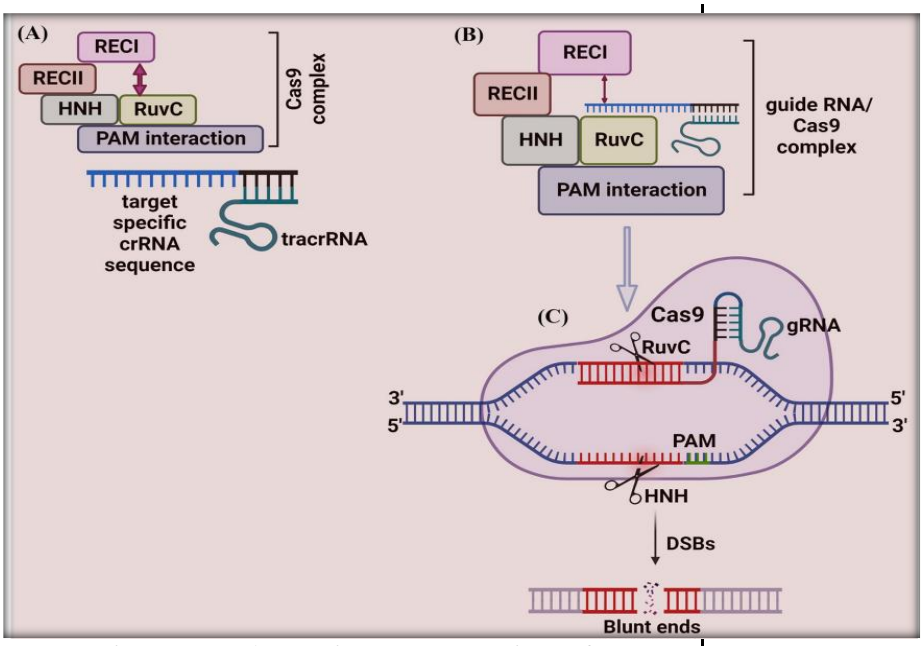

Figure 1: Schematic representation of CRISPR/Cas9 mechanism

(Source: Hillary *et al.*, 2023)

The inactivity of the Cas9 protein is attributed to the absence of guide RNA (gRNA). The engineered gRNA adopts a T-shaped structure, consisting of one tetra-loop and three stem-loops. The gRNA is designed to have a complementary 5′ end that matches the target sequence. Upon binding to Cas9, the programmed gRNA induces conformational changes in the protein, transforming the inactive Cas9 into its active form.

Upon activation, Cas9 surveys the DNA in search of a corresponding sequence that matches the PAM sequence (5′-NGG-3′). Following this identification, Cas9 employs its HNH and RuvC domains to cleave the double-stranded DNA, precisely three base pairs ahead of the PAM site. The HNH domain acts on the DNA strand that complements the 20-nucleotide sequence found in the gRNA's crRNA (known as the target strand). Simultaneously, the RuvC domain cleaves the non-target DNA strand, positioned opposite to the complementary strand.

In the spyCas9 system, the recognition of the target DNA involves identifying a short "seed" sequence containing the di-nucleotide 5'-NGG-3' with a PAM sequence. The tracrRNA:crRNA complex is fused together to form a single guide RNA (sgRNA), allowing the CRISPR/Cas9 system to cleave the targeted double-stranded or single-stranded DNA sequences.

Potential applications of CRISPR-Cas9 in plant breeding

The ability to edit the genome of plants using CRISPR-Cas9 technology has significant implications for plant breeding. It enables plant breeders to precisely edit specific genes associated with desired traits, leading to the development of crops with higher yields, enhanced resistance to pests and diseases, and improved tolerance to environmental stresses.

One area of focus for CRISPR-Cas9 in plant breeding is disease resistance. By targeting genes responsible for susceptibility to particular diseases, researchers can develop plants that are resistant to those diseases. This can lead to reduced use of pesticides and herbicides, as well as increased crop yield and quality.

Another area of focus is the improvement of nutritional content in crops. With the help of CRISPR-Cas9, plant breeders can target specific genes responsible for the production of essential vitamins and minerals, thus improving the nutritional value of crops. For example, scientists have used CRISPR-Cas9 to develop rice plants that

are enriched in beta-carotene, which the body converts to vitamin A.

Finally, CRISPR-Cas9 technology can also be used to develop crops with improved tolerance to environmental stresses such as drought, salinity, and extreme temperatures. By editing genes associated with stress response pathways, plant breeders can develop crops that can thrive in challenging environments, leading to improved yield and food security in regions that are prone to such stresses.

Base editing in plants allows for precise genome editing and has been used to edit the genomes of a number of plant species, such as rice, cotton, maize, oilseed rape, strawberry, tomato and watermelon. Base editors have contributed to a large extent in increasing the yields, improving herbicide resistance, and increasing stress tolerance.

Regulatory and ethical considerations

The use of CRISPR-Cas9 technology in plant breeding raises some regulatory and ethical considerations. While the technology is more precise and efficient than traditional breeding methods, it is still considered a form of genetic modification, which raises concerns about safety and potential environmental impacts.

In some countries, CRISPR-Cas9 edited plants are subject to regulation as genetically modified organisms (GMOs). This means that regulatory authorities require safety assessments before the plants can be released into the environment. The regulatory framework for CRISPR-Cas9 edited plants varies across countries, with some countries being more permissive than others.

In addition, there are ethical considerations related to the use of CRISPR-Cas9 in plant breeding. Some people argue that the technology should only be used to address pressing global challenges such as food security, while others raise concerns about the potential unintended consequences of editing the genome of organisms.

Regulation of CRISPR-Cas9 Technology: The use of CRISPR-Cas9 technology in plant breeding is currently subject to regulation in many countries. In the United States, for example, plants created using CRISPR-Cas9 technology are subject to the same regulations as conventionally bred plants. However, in the European Union, plants edited using CRISPR-Cas9 are subject to the same regulations as genetically modified organisms (GMOs), which are subject to more stringent regulations. This has created a regulatory grey area, as many argue that plants edited using CRISPR-Cas9 should not be subject to the same regulations as GMOs.

Future of CRISPR-Cas9 Technology in Plant Breeding: The future of CRISPR-Cas9 technology in plant breeding is bright. As the technology becomes more widely available and affordable, it has the potential to revolutionize the way we breed crops. It can be used to create crops that are better suited to changing climates, reducing the environmental impact of farming, and feeding a growing world population. However, it is important that the technology is used responsibly, with careful consideration of its potential impact on the environment and society.

Conclusion

CRISPR-Cas9 technology has the potential to revolutionize plant breeding by enabling precise and efficient editing of plant genomes. It offers the ability to develop crops with improved yield, disease resistance, nutritional content, and tolerance to environmental stresses.

However, the technology also raises regulatory and ethical considerations that need to be carefully addressed. As the technology continues to evolve, it is essential to strike a balance between the potential benefits and the potential risks associated with the use of CRISPR-Cas9 in plant breeding.

Overall, CRISPR-Cas9 is a promising technology that has the potential to contribute to global food security and sustainable agriculture. Its applications in plant breeding continue to expand, and scientists and policymakers must work together to ensure that its use is safe, ethical, and beneficial to society.

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