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Precision breeding: Innovations from Future Prospects

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Abstract

Precision breeding is a modern approach to improve the genetic characteristics of plants using advanced technologies to achieve specific, targeted changes. It offers significant advantages like increased efficiency, specificity, and predictability. Key areas include enhanced disease resistance, improved abiotic stress tolerance, nutritional fortification, yield improvement, pest resistance, and quality traits. It not only accelerates the breeding process but also ensures that new varieties are better suited to address specific agricultural challenges. As the technology advances, it also contributes to global food security, sustainability, and nutritional outcomes. The expansion of base editing methodologies in rice and wheat, and the efficacy of the CRISPR/Cas9 system in facilitating high- efficiency multiplex genome editing across monocot and dicot plants (Singh and Pandey, 2024).

Keywords: Precision breeding, CRISPR-Cas9, TALENs, ZFNs.

Introduction:

As per UN, the world's population is expected to be 9.7 billion in 2050 and could peak nearly 10.4 billion in the mid-2080s. Crop production appears to declining due to climate change and the limited availability of arable land; a 60% increase in production yields would be needed to feed a global population of 10 billion people (Springmann et al., 2018). Agriculture has always relied on breeding to improve crop varieties. Traditional breeding methods which involve crossing plants with desirable traits and selecting the best offspring over several generations, this process is very time-consuming and imprecise. There is a need to utilize new technologies in the development of improved crop varieties to overcome the difficulties faced in conventional breeding programs (Fiaz *et al,* 2021a). Precision breeding, which is also known as gene editing or genome cutting, is a cutting-edge technology which revolutionizes traditional breeding methods by allowing precise and targeted modification of an organism's DNA. It allows scientists to add, remove or alter genetic material at particular location within DNA, thus enabling the development of plants. This article explores the mechanism and technology associated to CRISPR/Cas9, TALENs, ZFNs emphasizing its utility in plant improvement.

Several advanced tools in Precision breeding:

CRISPRS-CAS

By using CRISPR-Cas9 (Jinek *et al.* 2012), researchers can insert, delete, or alter genes responsible for desirable traits, such as disease resistance or improved nutritional content. This advanced gene-editing technology allows scientists to precisely alter DNA sequences within an organism's genome, facilitating the introduction, deletion, or modification of specific genes. The CRISPR-Cas9 system, the most widely used variant, employs a guide RNA to direct the Cas9 enzyme to a particular DNA sequence, where it creates a double-strand break. The cell's natural repair mechanisms then repair the break, either by non-homologous end joining, which can introduce small insertions or deletions, or by homology-directed repair, which can incorporate new genetic material provided by the researcher. Here are some examples:

- **Rice (*Oryza sativa*)**: Scientists are using CRISPR-Cas9 to enhance rice varieties by editing genes for traits like disease resistance and yield. Example the knock-out of the OsSWEET13 gene has increased resistance to bacterial blight, a devastating rice disease.
- **Tomato (*Solanum lycopersicum*)**: Researchers used CRISPR-Cas9 to develop tomatoes with improved shelf life and disease resistance. By targeting the SlMlo1 gene, they are producing tomatoes with enhanced resistance to powdery mildew, a fungal disease.
- **Wheat (*Triticum aestivum*)**: In wheat, CRISPR-Cas9 has been used to edit the TaQsd1 gene which results in varieties with better grain quality and higher yield potential. This modification addresses the challenges of feeding a growing global population.
- **Soybean (*Glycine max*)**: CRISPR-Cas9 has been utilized to improve soybean traits such as oil content and disease resistance like the editing of the FAD2-1A and FAD2-1B genes has led to soybeans with higher oleic acid content, beneficial for heart health and industrial applications.
- **Maize (*Zea mays*)**: Researchers have applied CRISPR-Cas9 to enhance drought tolerance in maize by editing the ARGOS8 gene. The resulting maize varieties show

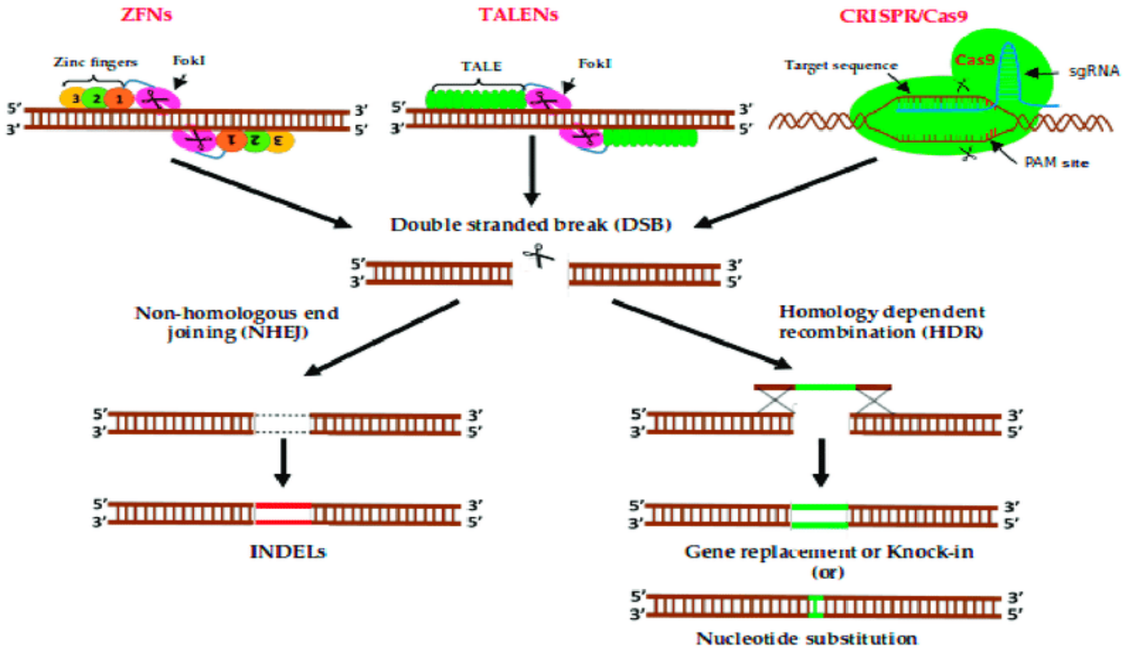
improved growth under water-limited conditions, addressing the critical challenge of climate change.

TALENs (Transcription Activator-Like Effector Nucleases)

They provide an alternative method to CRISPR-Cas for precise genome modifications (Christian *t al. 2010)* operated by exploiting the specificity of TALE proteins, which originate from the plant pathogen Xanthomonas bacteria to alter transcription of genes in host plant cells. The mechanism begins with engineering the TALEs to recognize and bind to specific DNA sequences in the target genome. Each TALE protein is composed of a series of repeat domains, each recognizing a single base pair, which can be customized to bind to any desired DNA sequence. Once the TALENs bind to their target site, the FokI nuclease domains, which are attached to the TALE proteins, dimerize and create a double-strand break in the DNA. This break prompts the cell's natural repair mechanisms: non-homologous end joining (NHEJ), which often results in small insertions or deletions, or homology-directed repair (HDR), which can introduce precise genetic changes if a repair template is provided. TALENs are known for their high specificity and efficiency, making them a valuable tool in precision breeding for developing crops with desirable traits such as enhanced disease resistance, improved yield, and better stress tolerance.

Zinc Finger Nucleases (ZFNs)

They are the type of engineered DNA-binding protein that facilitate targeted genome editing, playing a crucial role in precision breeding and also an alternative method to CRISPR-Cas (Kim *et al.* 1996). The working mechanism of ZFNs involves the combination of a DNA-binding domain, composed of zinc finger motifs, and a DNA-cleaving domain, usually derived from the FokI nuclease. Each zinc finger motif recognizes and binds to a specific three-nucleotide sequence in the DNA, and multiple zinc fingers can be linked together to target longer sequences with high specificity. When two ZFN proteins bind to their adjacent target sites on the DNA, the FokI nuclease domains dimerize, creating a double-strand break at the specified location. This break triggers the cell's natural DNA repair processes: non-homologous end joining (NHEJ), which can introduce small insertions or deletions, or homology-directed repair (HDR), which can incorporate new genetic material if a repair template is provided. ZFNs are particularly valuable in precision breeding for their ability to induce precise genetic modifications, enabling the development of crops with enhanced traits such as improved disease resistance, increased yield, and better environmental stress tolerance.



Importance of Precision Breeding:

The importance of precision breeding lies to address several critical challenges in agriculture, including food security, climate change, and sustainability. By precisely editing genes, scientists can develop crops that are more resilient to environmental stresses such as drought, heat, and pests, thereby ensuring stable yields under fluctuating climatic conditions. CRISPRS/ Cas has been adopted rapidly in manipulation of crop genomes to develop abiotic stress tolerant and high-yielding mutants (Bhat *et al.* 2021). It can also enhance nutritional quality and increase the efficiency of resource use, such as water and fertilizers, promoting more sustainable farming practices. This technology also reduces the dependency on chemical inputs, leading to healthier ecosystems and reduced environmental pollution. In the context of waterlogging resistance in soybeans, for instance, precision breeding can be instrumental in identifying and incorporating genes that enhance tolerance to excess moisture, thus safeguarding crop productivity in flood-prone areas. Moreover, precision breeding aligns with the goals of precision agriculture, where data-driven insights and advanced technologies are utilized to optimize crop management and production. As the global population continues to rise, the demand for food will inevitably increase, making it imperative to adopt innovative approaches like precision breeding to ensure food security and agricultural sustainability. This method not only accelerates the breeding process but also opens up new possibilities for developing crops with complex traits that would be challenging to achieve through conventional methods. Overall, precision breeding represents a significant leap forward in the field of agricultural science, offering a robust tool to meet the growing challenges of feeding an expanding world population while mitigating the impacts of climate change and preserving natural resources.

Applications in Crop improvement:

Precision breeding is an advanced agricultural technique which has revolutionized crop and animal improvement by integrating cutting-edge technologies to enhance productivity, resilience, and sustainability. CRISPR-Cas9 technology enables precise modifications in plant genomes, leading to the development of crops that can withstand extreme weather conditions, pests, and diseases. This is particularly valuable in the context of climate change, where crops need to adapt to fluctuating environmental conditions. Furthermore, precision breeding facilitates the enhancement of nutritional content, improving food quality and addressing malnutrition. Golden rice, enriched with beta-carotene, is a notable example of biofortification through precision breeding, aimed at combating vitamin A deficiency in developing countries. Its application extends to crops like potato, mustard, watermelon, soybean, maize, which highlights the potential of CRISPR/Cas in editing genes associated with herbicides to enhance crop yield and quality (Chen et al., 2019; Pramanik et al., 2021). ZFNs have been used to make targeted indels in soybean (Curtin *et al.* 2011; Sander *et al.* 2011) and to introduce specific mutations and transgene insertions that confer herbicide resistance in tobacco (Townsend *et al.* 2009) and corn (Shukla *et al.* 2009). TALENs have been used to make knockout mutations in *Arabidopsis (Cermak et al. 2011)* and to introduce resistance to infection by *Xanthomonas* bacteria in rice by disrupting the target sites of naturally occurring TALEs that contribute to pathogenicity (Li *et al.* 2012).

Conclusion:

Precision breeding stands at the forefront of agricultural innovation, offering transformative solutions for crop improvement. This approach is not only confined to laboratory but to environment sustainable practices. By using advanced genomic tools and techniques, it enhances productivity, resilience, and sustainability, addressing the challenges of modern agriculture and facilitating the way for a more food-secure future. By improving the efficiency of resource use, such as water and nutrients, precision-bred crops can thrive with minimal environmental impact which reduces the reliance on chemical inputs like fertilizers and pesticides, fostering eco-friendly farming practices. It also aids in conserving biodiversity by enabling the development of diverse crop varieties that can adapt to various environmental conditions. As a result, farmers are better equipped to manage the challenges posed by climate change, pests, and diseases, ensuring food security and agricultural sustainability.

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