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CRISPR-Based Precision Plant Breeding: Enhancing Crop Improvement through Gene Editing and Plant-Microbe Interactions in Biotechnology

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Abstract: Precision plant breeding and Crispr/cas genome editing have revolutionized agricultural biotechnology and opened up previously unheard-of opportunities for further crop improvement. By precisely altering plant genomes through the use of cas/crispr systems, crops with beneficial traits—like increased disease resistance, better nutritional value, and increased productivity—can be produced more quickly. At the same time, improving crop resilience and productivity requires an understanding of how microbes or plants interact with one another. Plant health depends on microbial populations, which influence growth, stress tolerance, and disease resistance. Gene editing and microbial interactions have a cooperative relationship that has the potential to completely transform agriculture by enabling innovative and sustainable farming practices.

Researchers are finding new strategies to grow crops that are more resilient, resource-efficient, and high-performing by fusing the benefits of plant-microbe interactions with crispr/cas technology. This all-encompassing strategy seeks to solve the issues posed by climate change and the growing world population while also enhancing food security.

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I. INTRODUCTION

Crops have a big impact on civilization since they are crucial for human survival and provide energy, sustenance, and other resources. With 9.6 billion people on the planet by 2050, demand for crops is predicted to increase by 100–110% from 2005 levels. Crop breeding technology advancements are desperately needed to boost agricultural productivity and promote sustainable agricultural development in order to feed and nourish a rapidly expanding population in the face of climate change, declining arable land, and dwindling water supplies. In contemporary agriculture, cross-breeding, mutation breeding, and transgenic breeding are the primary techniques for crop enhancement. Increasing genetic diversity through recombination and introducing advantageous features through crossbreeding can take several generations.[1] Animals and plants in the natural environment interact with a variety of microorganisms at every stage of their lives. Our ability to live a healthy life has been improved by the diverse microbial environment we have been exposed to since the dawn of human evolution. The same is true for plants, which coexist with a variety of microorganisms known as microbiota, including bacteria, fungus, protists, and archaea. Since microbial life has existed for nearly 3.5 billion years, it is likely that interactions between microbes have developed and diversified over time, predating even the adaptation of plants to terrestrial life, or 450 million years ahead of time.

Higher plants and photosynthetic algae evolved to include cyanobacteria in their cells, which are today referred to as chloroplasts or plastids. Because of this, bacteria and plants share a similar evolutionary history and are dependent on one another for existence. Consequently, the 'plant microbiota' has drawn more interest, especially when it comes to its presence inside or close to plant components' surfaces [2]. Soil is a substrate for plant growth and development and is home to enormous microflora and fauna, ranging from mega to micro. As a result, plants would already be interacting with their own microflora (fungi and bacteria) and fauna in a very dynamic way.

Today, plants are also acknowledged as composite organisms rather than distinct entities, as proponents of the "theory of hologenome evolution" have claimed, thanks to the strong backing of microbiologists and plant scientists. A plant is thought of as a "holobiont" in conjunction with its microbial populations, which are referred to as the "plant microbiota." The terms "phyllospheric microbiome," "rhizospheric microbiome," and "endospermic microbiome" refer to the microbial populations found in a plant's phyllosphere, rhizosphere, and end sphere, respectively. Furthermore, plant microbiota is transported both horizontally and vertically. Over the course of evolution, this link between microorganisms and plants has grown increasingly complicated and sophisticated. [3]

II. PLANT-MICROBE INTERACTIONS

Microbe-plant interactions are essential to the production and health of plants. Numerous microorganisms, including bacteria, fungus, nematodes, and viruses, are encountered by plants in their surroundings and in the soil. The growth, development, and general health of plants are greatly impacted by these interactions. Microbes can cause diseases and negatively impact plant health, but some can also help plants by improving growth or providing necessary nutrients. [4]

Table 1 Factors Influencing Plant-Microbe Interactions

Sr. No.	Factor	Description
1	Host factors	Plants prefer to contact with microorganisms that can function well in particular environmental settings since their phenotypic and fitness are dependent on these interactions. The species and genotype of the host are the main host characteristics that influence microbial interactions.
2	Edaphic factors	Microbes can be recruited from the soil because it is the largest reservoir of all microflora and microfauna. Therefore, the structure and function of microorganisms associated with plants are influenced by the physical, chemical, and biological properties of the soil. The bacterial and fungal community has a high correlation with soil pH, soil carbon, C:N ratio, soil water content, and nutrient status. These factors indirectly affect the composition of the microbiome. Out of all the edaphic parameters, the two that have the greatest influence are soil pH and soil carbon.
3	Environmental factors	Given that bacteria need particular circumstances to thrive and survive, environmental factors also play a significant role in the population of microorganisms linked with plants. Furthermore, a plant's phenotypic traits vary depending on its growing environment, which in turn influences the microbial assemblage.
4	Anthropogenic factors	Plant-microbe intraction is not an exception to the human desire to control processes that benefit them. We have changed the genetic composition of crops through domestication and genotype selection. The genetic diversity of most crops has decreased as a result of crop breeding efforts, which are primarily focused on improving productivity. Due to the loss of different genomic selection in agricultural plants brought about by this decrease in generic variety, there are now fewer plant-associated microorganisms that interact with those selections. Research has indicated that the diversity of related microorganisms was larger in ancient plant genotypes than in modern ones.

III. POSITIVE AND NEGATIVE PLANT-MICROBE INTERACTIONS

- *Beneficial Plant–Microbe Interactions*

Beneficial microorganisms are essential for improving crop growth and tolerance to a variety of stressors. They assist plants resist stressors like heat, salinity, and drought, improve nutrient uptake, and fortify plant immunological responses. Crops' associated microbiomes and capacity to support growth in harsh conditions have a significant impact on their prospective yield. Therefore, it is important and exciting to comprehend the mechanisms behind interactions between microbes and plants. [6]Fig-1

- *Harmful Plant–Microbe Interactions*

Negative interactions can be harmful to plants because invasive microorganisms can promote necrotrophy in the colonizing plants and be saprophytic. Thus, it is essential to comprehend plant-microbe interactions in order to identify the beneficial and detrimental effects of microbes on plants. Some bacteria are pathogenic, which is the main adverse effect of plant-microbe interactions. These microorganisms and their host plants may be saprophytic or parasitic. Some parasites feed on the nutrients of their host while living inside or on the surface of the roots. Because biotrophic phytopathogens sustain plant cells, they are able to suppress the plant's defenses and establish a close relationship with the plant. Moreover, phytopathogens create phytotoxins that damage plant cells and cause illness. [5] fig-1

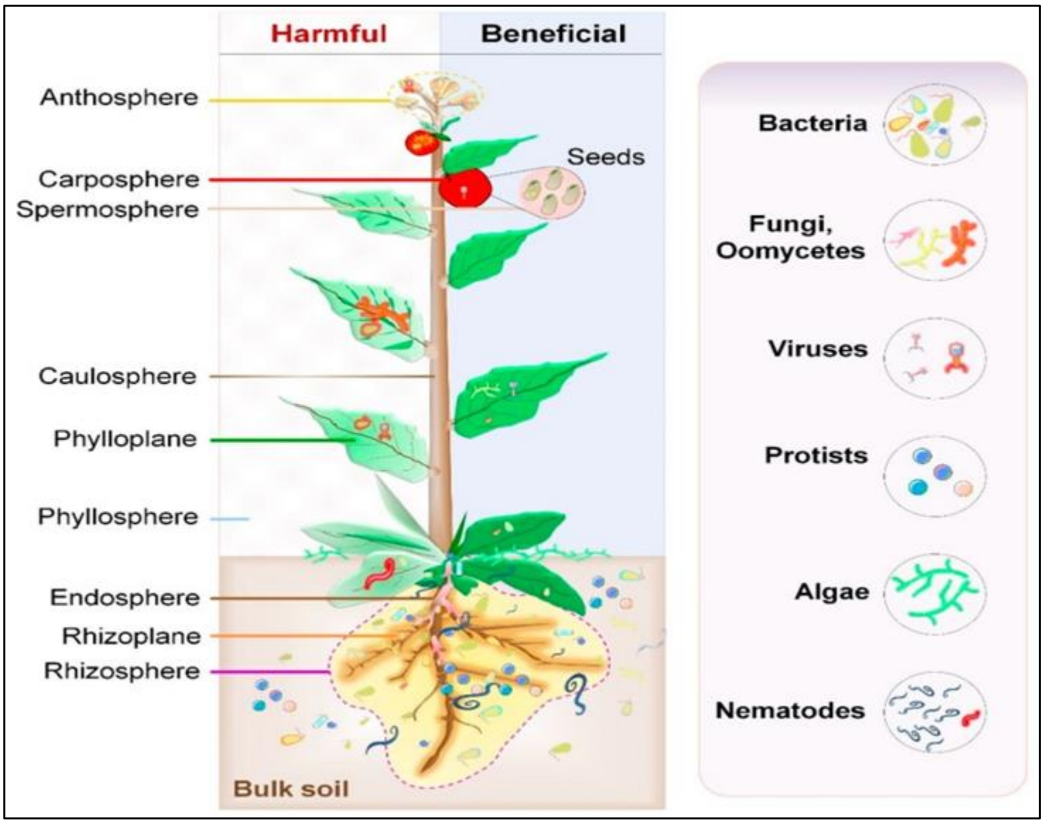

Fig-1 Positive and Negative Plant-Microbe Interactions

IV. BIOTECHNOLOGY AND GENE EDITING IN CROP IMPROVEMENT

Due to the fast population expansion and the abundance of competing dairy and meat products, there is an increasing demand for feed, food, biofuels, and cattle. By 2050, there will likely be about 9 billion people on the planet, which might lead to a 100–110% rise in demand for food production. Consequently, only a 38–67% rise in the efficient production of staple crops such as rice (Oryza sativa), maize (Zea mays), soybean (Glycine max), and wheat (Triticum aestivum) is expected. [8–9]

ZFNs revolutionized the field of genome manipulation research since they were the first protein reagents to truly target proteins. ZFNs are DNA binding domains that are able to identify three base pairs at the intended location. [**10**]. ZFNs have frequently been used to modify specific genes in a variety of plant species, including maize, Nicotiana tabacum (tobacco), and Arabidopsis thaliana (Arabidopsis). [11,12 ,**13**]. Another site-driven mutagenesis genome-editing system, TALENs, was defined first in plant pathogenic bacteria (*Xanthomonas*) and is based on a concept similar to that of ZFNs. TALENs target one nucleotide at the target site (instead of three), thus rendering TALENs precise. TALENs were successfully used for genome editing in angiosperms and bryophytes [14]. Since there were no effective genome-editing methods for eukaryotes at particular locations, these methods were first developed in prokaryotes. But with the development of eukaryotic genome editing, the CRISPR technology has transformed our capacity to produce targeted crop modifications [14]. For every DNA target site, the CRISPR system just needs to modify the guide RNA sequence. Utilizing and modifying CRISPR technology is fairly easy and effective in a variety of situations. [15]

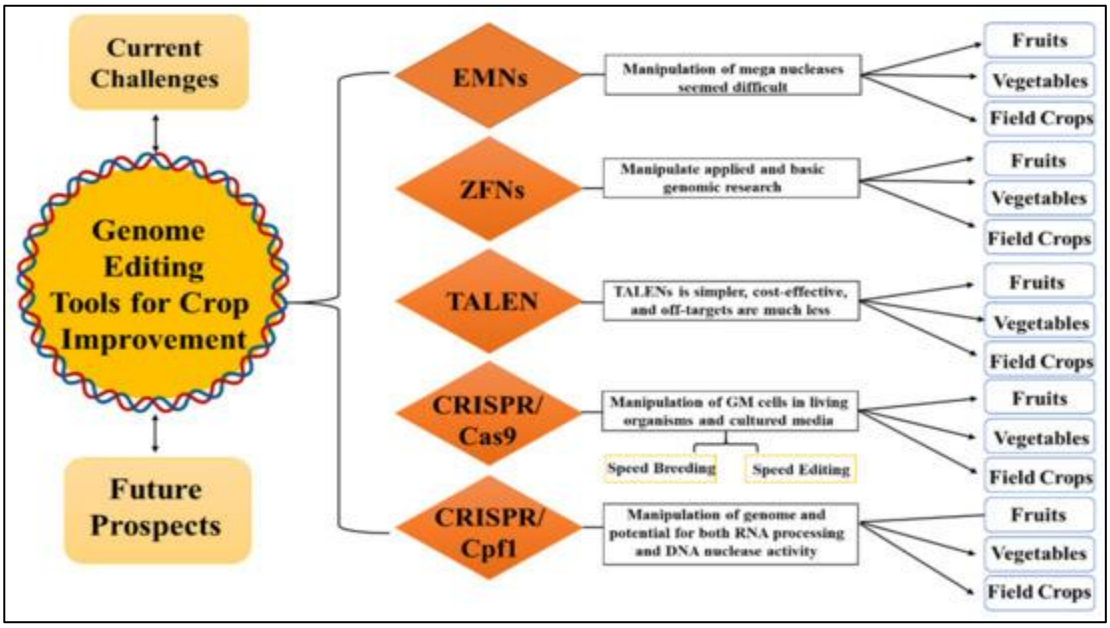

Fig -2 Overview of the Different Aspects Covered in the Present Review Related to Genome Editing in Plants, Such as its Applications, Challenges, and Advantages. [16]

- *Speed Breeding and MAS Using Genome-Editing Tools*

Another magic technology that has garnered significant attention recently is speed breeding (SB). SB speeds up agricultural research by accelerating generation progress, in addition to shortening the breeding cycle [17–16]. This method speeds up crossbreeding and inbreeding between different types by using artificial light and warmth. A recent study found that offering a 22-hour photoperiod and regulated temperature greatly shortens the generation time in a number of crops, including spring bread wheat (Triticum aestivum), durum wheat (T. durum), barley (Hordeum vulgare), chickpea (Cicer arietinum), pea (Pisum sativum), canola (Brassica napus), the model grass Brachypodium distachyon, and the model legume Medicago truncatula, when compared to the field or a greenhouse without additional light [16].

By combining SB with other contemporary agricultural technologies like genome editing, high-throughput genotyping, and genomic selection, researchers think SB has a lot of potential to accelerate crop improvement. These potent genome-editing tools, when combined with SB, will be used in the next ten years to accelerate and improve plant genotypes for farmers and consumers throughout the globe. [16]

- *Speed Editing Strategy for Gene-Family Members*

Humans have chosen plants with the best qualities for increased productivity or improved environmental adaption throughout the history of agriculture. By crossing types with desired qualities, examining the phenotypic variance among their offspring, and choosing individuals with better traits, conventional breeding uses genetic variety to its advantage. Nevertheless, this procedure is sluggish, necessitating several crossing generations and frequently producing only slight gains. Furthermore, a large number of yield-related features are regulated by either different genes in the same pathway or by numerous genes with redundant roles in plant development, each of which has a minor impact on the final phenotype. This implies that in order to make major advancements, a number of chromosomal regions must be joined. On the other hand, the speed and accuracy of plant breeding could be significantly improved by modern genomics technologies. [19&18], however further advancements are required to completely reap their advantages. [19]. Christian Damian Lorenzo and associates [20] present BREEDIT, a novel tool that expedites plant breeding by speeding up the identification of genetic determinants that govern agronomically significant traits (such as yield and stress tolerance) in maize. This tool is published in this edition of The Plant Cell. Based on 48 candidate genes with established and conserved roles in controlling plant growth in maize, rice, and Arabidopsis, the authors established a pipeline employing CRISPR/Cas9 to produce a varied collection of multiplex gene-edited plants (i.e., with editing of several distinct genes or particular DNA loci). [21, 23]

A set of target genes with minor impacts on complex phenotypes were subjected to multiplex CRISPR/Cas9 genome editing to produce genetic diversity (12 guide RNAs in four vectors dubbed SCRIPTs). A library of edited plants with every possible combination of gene knockouts was created by crossing super-transformed plants (carrying the SCRIPT and Cas9 EDITOR vectors; Figure) and genotyping them using Highly multiplex (HiPlex) amplicon sequencing. The improved crossing scheme of gene-edited plants included inter-script crosses (i.e., mutations in genes of different families generated by different SCRIPT) and intra-script crosses (i.e., complementary mutations in the same gene family generated by the same SCRIPT) in addition to back-crosses and self-crosses).

High-throughput phenotyping was subsequently used to screen the segregating progenies for agronomically significant features, including as drought tolerance and yield potential. This accelerated the identification of certain combinations of gene variants linked to better features by allowing for the testing and acquisition of an incredible amount of genetic diversity in target attributes in just two generations. [22, 23]

V. FUTURE OUTCOME

Precision plant breeding using CRISPR and knowledge of plant-microbe interactions have enormous promise to revolutionize agricultural biotechnology in the future. The following are some significant results we can expect:

- Improved Crop features: Accurate genetic alterations made possible by CRISPR/Cas technology result in crops with improved features like greater nutritional value, superior disease resistance, and higher yields.
- Sustainable Agriculture: By combining plant-microbe interactions with CRISPR, we can create crops that are more resistant to environmental challenges including heat, salinity, and drought. As a result, fewer chemical inputs are required and sustainable farming methods are encouraged.
- Food Security: By 2050, there will likely be more than 9 billion people on the planet, and CRISPR-based breeding can greatly increase crop yields to satisfy the rising need for food. By creating crops that are more productive and resource-efficient, this technology can contribute to ensuring food security.
- Reduced Environmental Impact: By lowering the demand for fertilizers and pesticides, precision breeding can lessen agriculture's environmental impact. Crops that are designed to work well with microorganisms can increase soil health and nutrient uptake.
- Quick Development: New crop types can be created more quickly thanks to CRISPR than with conventional breeding techniques. Responding to the quickly shifting climate and new agricultural concerns requires this quickness.
- Integration with Emerging Technologies: CRISPR is probably going to be combined with other technologies in the future, including as machine learning, nanotechnology, and synthetic biology. The accuracy and effectiveness of crop improvement techniques will be significantly improved by this combination.

Overall, the combination of CRISPR-based gene editing and plant-microbe interactions promises a more robust, sustainable, and efficient approach to crop improvement, addressing the challenges of food security, climate change, and a growing global population [24&25]

VI. CONCLUSION

In summary, a breakthrough approach to agricultural biotechnology is provided by the combination of precision plant breeding, CRISPR/Cas genome editing, and knowledge of plant-microbe interaction. Precision genetic alterations are made possible by CRISPR/Cas technology, which advances the creation of crops with improved characteristics like increased nutritional value, larger yields, and better disease resistance. In addition to improving plant resilience and health, this strategy supports sustainable agricultural methods when paired with research on beneficial plant microbe intraction. Microbial associations and gene editing have the potential to work in concert to produce more resilient, resource-efficient, and ecologically friendly crops. In order to address the issues of food security, climate change, and an expanding global population, this integrated approach will be essential as research advances. It will also pave the way for the development of crop modification techniques for a more sustainable future.

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