Review Article
Microbial Biotechnology: Innovations for Crop Protection and Improvement

Abstract

Microbial biotechnology is revolutionizing crop protection and improvement by harnessing the power of beneficial microorganisms to enhance agricultural productivity and sustainability. Innovations in this field involve the use of bacteria, fungi, and viruses to combat plant pathogens, improve soil health, and promote plant growth. Techniques such as biofertilizers, biopesticides, and microbial inoculants are being developed to reduce dependency on chemical inputs, thereby mitigating environmental impact and promoting eco-friendly farming practices. Additionally, advancements in genetic engineering and microbial genomics are enabling the creation of tailor-made microbial solutions that can boost crop resilience to stresses like drought and salinity. These cutting-edge approaches not only enhance crop yield and quality but also contribute to a more sustainable and resilient agricultural system, addressing the growing global food security challenges.

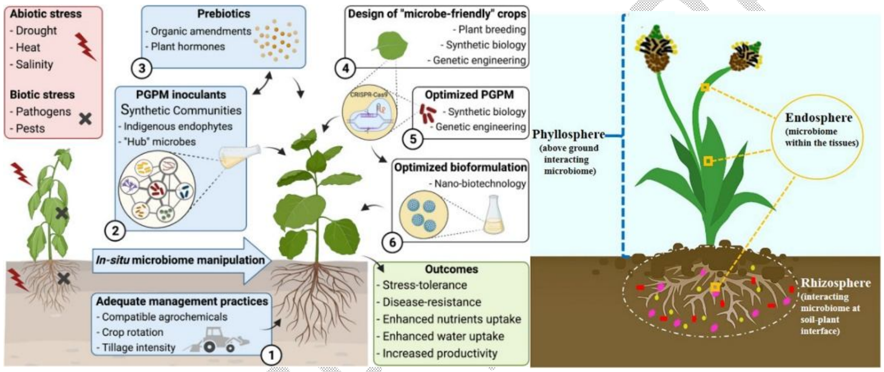
Introduction

A microbe, also known as a microorganism, is a kind of creature that is so tiny that it cannot be seen by the naked eye. These organisms include of microalgae, viruses, bacteria, and fungus, as well as protozoa. In addition to living in environments that are more commonplace, such as soil, water, food, and the intestines of animals, microbes may also be found in environments that are more severe, such as rocks, glaciers, hot springs, and deep-sea vents**[15]**. The great diversity of biochemical and metabolic characteristics that have emerged in microbial populations as a result of genetic variation and natural selection is reflected in the large range of environments that microbes may be found in. Microbial biotechnology, enabled by genome studies, will lead to breakthroughs such as improved vaccines and better disease-diagnostic tools, improved microbial agents for biological control of plant and animal pests, modifications of plant and animal pathogens for reduced virulence, development of new industrial catalysts and fermentation organisms, and development of new microbial agents for bioremediation of soil and water contaminated by agricultural runoff**[40]**. In order to make significant progress in the areas of food safety, food security, biotechnology, value-added products, human nutrition and functional foods, plant and animal protection, and the advancement of basic research in the agricultural sciences, it is essential to conduct research on microbial genomics and microbial biotechnology**[16]**.

The purpose of plant breeding programs is to enhance the qualitative attributes of plants in order to produce crops that are more productive and nutritious, as well as to boost plant resistance against biotic and abiotic stress. Plant breeders are compelled to choose genotypes that are resistant to water and temperature stressors as a result of the growing virulence of pests and diseases, legislation that restricts the use of agrochemicals, and climatic changes that widen the circumstances under which abiotic stress occurs**[41]**. Because of these negative limits, the output is inadequate, and there has been a significant decline in the qualitative characteristics. In order to acquire plants with enhanced characteristics in terms of yield and quality features, as well as increased stress resistant traits, genetically altered agricultural plants have been created for a long time via the use of crosses and mutagenesis through the process of genetic engineering**[69]**. Mutagenesis, which may be accomplished by the use of radiation or chemical agents, has been used since the 1920s, when it was not feasible to introduce desirable characteristics from the germplasm that was available. This has resulted in the development of genetically modified organisms (GMOs), which are a product of genetic engineering and biotechnologies, which have expanded the toolbox of geneticists and breeders by providing them with new procedures and equipment**[70]**. These approaches have made it possible to isolate and transfer genes to crops from sexually incompatible plant species and other creatures, which has resulted in a significant expansion in the gene pool that is theoretically accessible to plant breeders. In spite of the fact that genetically modified crops covered 191.7 million hectares in 2018, their usage continues to be connected with significant public concern due to the potential threats that they pose to human health and other aspects of the environment**[17]**.

There have been many different strategies created up to the most recent new plant breeding techniques (NPBTs, such as genome editing) in order to overcome the limitations of genetically modified crops. A significant improvement in crop genomics has been made possible by methods that use next-generation sequencing (NGS), which has also contributed to the availability of a large number of reference crop genomes to the general public**[1]**. Researchers were able to describe the allele diversity of both domesticated and wild plant populations through the use of high-throughput resequencing of hundreds of genotypes. This allowed for a deeper understanding of the history of plant domestication, as well as the identification of genes responsible for traits of agrochemical interest and gene functions**[2]**. An overview of the most important characteristics, benefits, and difficulties associated with a variety of biotechnological techniques is presented in this paper. Examples of applications for improving plant characteristics in order to better deal with biotic and abiotic pressures are also included**[71]**.

Fig .1 Applications of prebiotics for improving plant characteristics



Economics of Microbial Biotechnology in agriculture

The worldwide market for agricultural biotechnology is anticipated to reach $119.6 billion by the year 2028, with a compound annual growth rate (CAGR) of 8.4% beginning in 2023 and continuing through 2028. This increase is being driven by a number of causes, including the rising need for food as a result of the expanding population around the globe, as well as the necessity of addressing environmental problems and promoting farming techniques that are sustainable**[42]**. As a result of the ongoing evolution of the industry, new developing technologies are continually being created in order to boost agricultural yields, produce new plant types that are resistant to pests and diseases, and enhance the efficiency of farming techniques. In addition to fostering environmentally responsible farming practices, biotechnology is an essential component in the process of satisfying the ever-increasing need for food**[72]**. Companies such as Evogene, Syngenta, Corteva, BASF Plant Science, and Bayer Crop Science constitute some of the most important players in the industry. These businesses have been at the vanguard of agricultural biotechnology, producing technologies that attempt to solve issues to global food security, improve crop yield, and promote agricultural techniques that are more environmentally friendly**[88]**. The agricultural biotechnology industry is at the forefront of tackling issues related to sustainability and agricultural efficiency, as well as satisfying the growing need for food throughout the globe**[3]**. The desire for increasing agricultural yields, environmental sustainability, and climate change resistance is driving the rise of the business, which is being driven by technological innovations and genetic advancements. Leading companies such as Bayer Crop Science, Syngenta, Corteva Agriscience, BASF Plant Science, and Monsanto all play important roles in the development of the industry[73]. These companies are responsible for the creation of genetically modified seeds, innovative characteristics, and digital solutions that aim to increase crop yield and better manage the challenges that contemporary agriculture presents. Agriculture biotechnology is essential to ensuring the safety of food supplies throughout the world and promoting environmentally responsible farming methods. Its future holds the possibility for continued development, innovation, and adaptation to meet requirements that are always shifting[43].

Agricultural biotechnology and its implications

By providing farmers with technologies that may make output more controllable and less expensive, biotechnology makes it simpler for farmers to satisfy the needs of the food market while simultaneously lowering expenses[74]. Certain crops have been genetically modified to be resistant to certain herbicides, which has made the process of weed management easier and more effective. Others have been modified to be resistant to certain plant diseases and insect pests, which has resulted in an increase in the reliability and efficiency of pest management treatments[44]. Countries are able to meet the growing demand for food while also lowering their production costs because to these crop production possibilities. The United States Department of Agriculture (USDA) has deregulated a number of crops that are generated from biotechnology, and the Food and Drug Administration (FDA) and/or the Environmental Protection Agency (EPA) have evaluated that these crops are safe for consumption[45]. The research and development phases are presently underway for a great number of additional kinds of crops, which will have a wide range of applications in agriculture in the years to come. Increasing the amount of disease-fighting nutrients in foods, reducing the amount of allergens in foods, reducing the amount of saturated fats in cooking oils, and providing customers with meals that are nutritionally enhanced or that last longer are all potential outcomes of advances in biotechnology[18].

Phytoremediation, which includes the detoxification of contaminants in the soil or the absorption and accumulation of polluting chemicals out of the soil for the purpose of safe harvesting and disposal, is another use of genetically altered plants that are now being under development[90]. It is also possible to employ biotechnology to help preserve natural resources, make it possible for animals to make better use of the nutrients in their feed, reduce the amount of nutrients that run off into rivers and bays, and assist in meeting the growing demand for food and land throughout the globe[46]. The goal of the research being conducted is to develop more resilient crops that can flourish in even the most severe settings. These crops will demand less fuel, labour, fertilizer, and water, which will contribute to a reduction in the amount of strain placed on land and animal habitats.Not only has biotechnology enabled the manufacturing of genetically modified crops, but it has also made the synthesis of antibiotics more effective via the use of microbial fermentation[89]. Additionally, biotechnology has enabled the creation of novel animal vaccinations through genetic engineering for illnesses such as rabies and foot and mouth disease[75]. Biotechnology has been used in agriculture, which has led in a number of advantages for farmers, producers, and consumers. These benefits include safer insect pest control and weed management, enhanced weed control, and higher crop quality. Biotech crops have the potential to increase crop quality and yields, simplify labour for farmers, and improve safety for them, all of which may contribute to increased profitability in farming. It is possible that they will produce higher quality features, such as greater amounts of beta-carotene in rice, superior oil compositions in canola, soybean, and maize, and crops that are able to thrive in saline soils or better survive drought conditions[19].

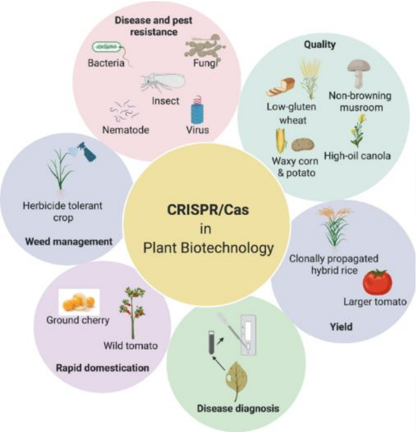
Researchers have found the instruments of agricultural biotechnology to be very helpful in gaining a knowledge of the fundamental biology of living species[20]. These technologies have also made it possible for us to increase the safety of our food supply and generate superior animal and plant kinds. With the United States Department of Agriculture (USDA), the Environmental Protection Agency

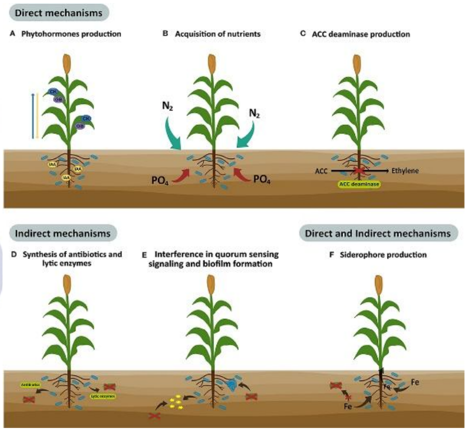
(EPA), and the Food and Drug Administration (FDA) working to ensure that crops produced through genetic engineering for commercial use are properly tested and studied to minimize significant risks to consumers and the environment, agricultural biotechnology has been the subject of extensive evaluation by breeders for centuries[47]. Breeders have been conducting this evaluation for centuries. Crops that have been genetically modified are the only ones that have been subjected to official reviews in order to evaluate the possibility of unique features being transferred to wild cousins. Before the two plants are allowed to be released, it is necessary to conduct an analysis of the possibility that they would exchange characteristics via the use of pollen[21]. Before the approval of field testing and commercial release, the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA) examine the environmental implications of pest-resistant biotechnology-derived crops. Honeybees, beneficial insects, earthworms, and fish are some of the animals that are subjected to testing in order to guarantee that these crops do not have any unexpected repercussions linked with them[48]. Food safety is another problem that arises when the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) investigate new characteristics that have been introduced to plants that have been produced from biotechnology[91]. In order to determine whether or whether these features create proteins that are potentially harmful and have the ability to trigger an allergic reaction, research is being conducted. Before these proteins are introduced into the food or feed supply, they are subjected to a series of tests that investigate their thermal and digestive stability, as well as their resemblance to proteins that are known to cause allergic reactions[4].

The use of microorganisms such as bacteria, fungus, and algae in a variety of scientific and industrial activities is what is known as microbial biotechnology. The creation of antibiotics such as penicillin, as well as the synthesis of therapeutic proteins, vaccines, and biopharmaceuticals, are all examples of the tremendous contributions that these bacteria have made to the field of medicine[92]. Because of their ability to increase crop output, improve soil fertility, and reduce the amount of chemical fertilizers and pesticides used in agriculture, microorganisms are a crucial component of agricultural production. The manufacturing of biopesticides and biofertilizers, which are ecologically benign alternatives to traditional agricultural inputs, is another way in which they contribute to the production of these products[49].

Bioremediation, which makes use of microorganisms to breakdown and detoxify contaminants in soil, water, and air, is another way that microbial biotechnology helps tosolve environmental concerns. Cleaning up oil spills, purifying wastewater, and managing industrial waste are all examples of situations that call for the use of this natural remediation technology[50]. Additionally, microbial biotechnology plays a vital part in the manufacture of biofuels, which involves the transformation of renewable resources such as algae and biomass into sources of energy that are sustainable. Industrial applications of microbial biotechnology include the production of industrial enzymes, which are used in the processing of food, detergents, and textiles[93]. Additionally, microbial fermentation is utilized for the production of biofuels, bioplastics, and specialized chemicals throughout the manufacturing process. The adaptability of microorganisms in industrial settings continues to open up new avenues of possibility and lessen the negative effect that conventional production methods have on the environment[22].

Fig.2 Agricultural biotechnology and its implications





How microbial biotechnology help to fight hunger

Despite the fact that the world's population is projected to reach almost 10 billion by the year 2050, hunger continues to be a major worldwide concern. There are a number of factors that contribute to this, including the annual loss of arable land for agricultural production, the emergence of new infections, and the impact of climate change on the production and security of food throughout the world**[51]**. The existence of food delivery channels that are not in sync with the requirements of the people and are not aware of the issue might make hunger and malnutrition even worse. Food waste is another factor that adds to this problem; it is estimated that one third of all food is either thrown away or wasted**[76]**. The United Nations has established a number of objectives to promote sustainability, one of which is Sustainable Development Goal 2, which aims to eradicate hunger by the year 2030. However, if governments and other stakeholders do not make major efforts, it will be difficult to accomplish this goal**[94]**. Putting an emphasis on agricultural output while simultaneously investigating sustainable resources for crop production is of the utmost importance. Technologies such as precision agriculture, genetic engineering of crops, and the use of bioinoculants and biopesticides have the potential to significantly improve crop productivity**[23]**.

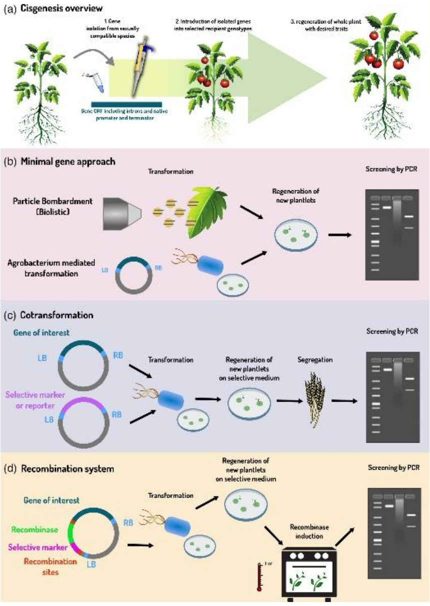
It is vital for sustainable agriculture that we minimize our dependence on inorganic fertilizers. Synthetic fertilizers have been responsible for a considerable rise in world agricultural output as well as a reduction in poverty and hunger. However, they have also been responsible for pollution, the loss of biodiversity, and the degradation of land**[77]**. Using fertilizers that have been rationally developed may help reduce losses and enhance fertilizer efficiency; nevertheless, it is essential that their performance remains constant across a variety of soil types and climates. In order to maintain adequate and sustainable agricultural output while also reducing the effect on the environment, it is essential to find solutions that are both innovative and ecologically friendly**[52]**. The presence of soil microorganisms that play an important part in the bioavailability of nutrients is crucial for sustainable agriculture. Healthy soil is essential for agriculture because it provides plants with the essential nutrients they need**[78]**. It is generally agreed that the plant microbiome, which refers to the microbial population that is linked with all of the organs and tissues of plants, is a functional extension of plants. Biogeochemical cycling, trash recycling, and water purification are some of the critical ecosystem services that are being jeopardized as a result of the unprecedented soil degradation, desertification, and erosive loss that is being caused by human activities, severe weather events, and global warming**[24]**.

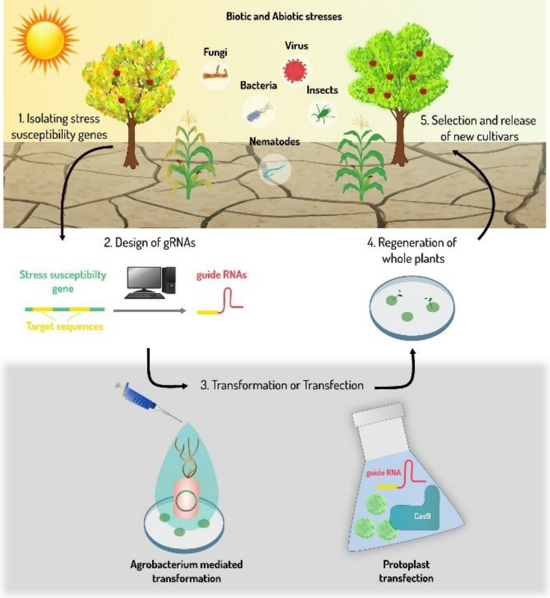
In order to address these insults and solve the soil issue, Timmis and Ramos (2021) presented the idea of treating soils as patients that need medical attention. It is necessary to coordinate the implementation of such systems on a worldwide scale in order to halt and eventually reverse the loss of soil**[79]**. The protection of the health of soils and rivers is a significant problem in the face of climate change, and it is equally important for the achievement of the Sustainable Development Goals set out by the United Nations. There has been a considerable rise in the amount of nitrogen and phosphate in soils as a result of the Green Revolution, which has had a severe influence on the health of the ecosystem and the capacity to maintain it**[25]**. As a result of this imbalance, unfavourable long-term impacts have been brought about on plant-soil-microbe networks, as well as on the metabolism and functioning of microbes. Inorganic nitrogen has been demonstrated to lessen rhizosphere dependency on root-derived carbon and weaken plant-microbe networks, according to a monitoring research that was conducted at Rothampsted Experimental Station in the United Kingdom over a period of 150 years**[53]**. When it comes to achieving sustainable agriculture, it is absolutely necessary to cut down on both direct and indirect emissions of greenhouse gases, which are closely connected to applications of fertilizer. The transition to organic fertilizers, such as manure, which have slower nutrient release patterns and are dependent on the activities of microorganisms, is an extremely important undertaking**[80]**. Because of the eutrophic effects of phosphate chemical fertilizer runoff and the diminishing phosphorous stores all over the globe, the transition to phosphate that is released by microbes is of utmost significance**[5]**.

For growth and development, plants are dependent on soil bacteria that are found inside their rhizosphere. These microbes are referred to as plant growth promoting microbes (PGPM). The presence of these essential soil components, which include bacteria and fungus, not only increases plant output but also serves as a biofertilizer. Additionally, PGPM make a contribution to and modify the levels of plant hormones, which results in the development of systemic resistance in plants. The development of microbial consortia for use as bioinoculants, such as synthetic communities (SynComs), which take into consideration the many chemical and molecular communication networks that are at play between plants and microbes, is becoming an increasingly popular approach. Soils that are effective in preventing disease are dependent on the delicate equilibrium that exists between the many plant-associated microbial communities**[54]**. These communities are regulated by both the pathogen and the plant. Biofertilizers have been shown in recent research to have the ability to rearrange the bacterial community that is found in the rhizosphere. This results in the enhancement of beneficial microbial consortia that are capable of controlling plant diseases such as banana Panama disease. Recognizing the microbiome of the soil is essential in order to strengthen defences against plant diseases that have invaded the soil**[26]**.

Applications of microbiome-based technologies that depend on the introduction of helpful bacteria include biofertilizers and biofungicides, both of which have shown to be very effective. It is possible to develop these techniques in any geographical place, and they may be adapted according to the soil and climate conditions of the area. We must investigate, integrate, and perfect a variety of tactics in order to achieve the Sustainable Development Goals. At the same time, we must make certain that these strategies are scalable and can be adapted to a wide range of environments and circumstances**[55]**.

Fig .3 microbial biotechnology





Approaches and possibilities in the field of plant protection against cisgenesis

Cisgenesis is a term that was first coined by Shouten in the year 2006. It describes the process of modifying crops by introducing genes that have been extracted only from sexually compatible plants. The genes of interest are separated from a species that may be used for conventional crosses and transferred, therefore retaining the species' 'original' form. As a result, these plants share the same genetic pool as traditional breeding plants**[6]**. One of the most significant drawbacks of gene introgression in a crop genome through the use of classical crosses is that a large number of undesirable associated genes are passed down to the subsequent generation along with the gene(s) of interest. This frequently has a negative impact on a variety of agronomic traits that are associated with product quality and yield. This effect, which is referred to as linkage drag, is often seen in introgression breeding, and marker-assisted selection (MAS) is frequently used in order to alleviate the quantity of genes that are not wanted**[27]**. The problem of linkage drag may be solved by the process of cisgenesis, which involves the transfer of just the appropriate gene or genes in a single step. This ensures that all of the quality qualities that were chosen in the elite cultivars are maintained. Nevertheless, the limitation of cisgenesis is that it is only applicable to monogenic characteristics, despite the fact that it might also be used for oligogenic characteristics. Over the course of the last ten years, a number of writers have made a complete assessment of the many approaches and tactics that have been used in the production of cisgenic plants. These methods and strategies have an intriguing success rate**[7]**.

Since its first use, cisgenesis has been the subject of a number of different methods. These strategies have been developed in consideration of the fact that the duration of the breeding cycle and the effectiveness of transformation and regeneration are both dependent on the type of plant that is being used**[56]**. Using vectors, in which just the gene of interest is cloned in the T-DNA region, then transferring it to plants using Agrobacterium-mediated transformation, and finally selecting it through PCR analysis, is the most straightforward method. Another method involves the use of minimum gene cassettes, which are composed just of a promoter, coding sequence, and terminator, and are introduced into the plant genome by biolistic transformation. This method helps to prevent the incorporation of partial or whole backbone modules**[28]**. When it comes to species that are resistant to transformation, the use of cisgenic reporter genes or co-transformation with selectable marker genes has the potential to significantly ease the process of recovering transformed plants. It is feasible to apply a co-transformation technique in seed-propagated crops. This involves crossing the plants with the parental or original variety and taking advantage of segregation of the selectable marker in the offspring**[57]**. This results in the plants having the cisgenebut not the selectable marker. An rise in disease resistance in a variety of crops, including potatoes, apples, grapevines, melons, wheat, barley, poplar, rice, and strawberries, has been achieved by the use of cisgenic techniques at different times. These techniques have been used in a variety of species with the intention of enhancing quality characteristics. As an example, a marker-free method was used in order to create four cisgenic late blight (Phytophthora infestans)-resistant potato varieties**[81]**. On the other hand, cisgenic apple types were created by inserting the apple scab resistance gene Rvi6 into the susceptible cultivar 'Gala'. Another organism that was utilized to test the recombinase system was the banana, which was used to induce the deletion of the green fluorescent protein that was employed as a reporter gene. Several genes that provide resistance to pathogens have been extracted from species that are sexually compatible with Vitis vinifera and then overexpressed in transgenic lines**[82]**. It has also been claimed that selected marker excision may be accomplished by the use of a heat shock controlled FLP/frt recombination system using methods. In order to impart resistance to Pseudoperonosporacubensis, the fungus that causes downy mildew in cucurbits, transgenic lines of melon have been produced. These lines overexpress the glyoxylate aminotransferase At1 and At2 genes**[8]**.

In durum wheat, cisgenic lines expressing the 1Dy10 HMW glutenin gene were developed by the application of biolistic co-transformation with minimum gene cassettes. These lines were related with increased baking quality. During the fourth generation, homozygous cisgenic lines were acquired by the process of segregation. Wheat lines that were cisgenic and had a class I chitinase gene exhibited a degree of tolerance to fungal infections. In order to evaluate the viability of cisgenic evolution in barley, Holme et al. (2012b) used a barley phytase gene known as HvPAPhy_a in conjunction with the co-transformation technique**[29]**. As a result, they obtained lines that exhibited higher phytase activity. Cisgenesis has also been used in the field of rice in order to combat Magnaporthe grisea, which is one of the most widespread and destructive infections. This was accomplished by using a co-transformation technique in order to transfer the rice blast disease resistance gene Pi9 into peak rice cultivars. Cisgenesis is an effective method for changing various crop features, as evidenced by the poplar plant, in addition to its ability to protect against stress**[58]**. The only genetic material that intragenic plants contain is that which originates from species that are sexually compatible with one another. However, the inserted gene is the consequence of a genetic element that was extracted from various species. An intriguing example of this strategy is the overexpression of cisgenicpolygalacturonase inhibitor protein (FaPGIP) in strawberry, which resulted in the development of resistance to grey mould**[9]**.

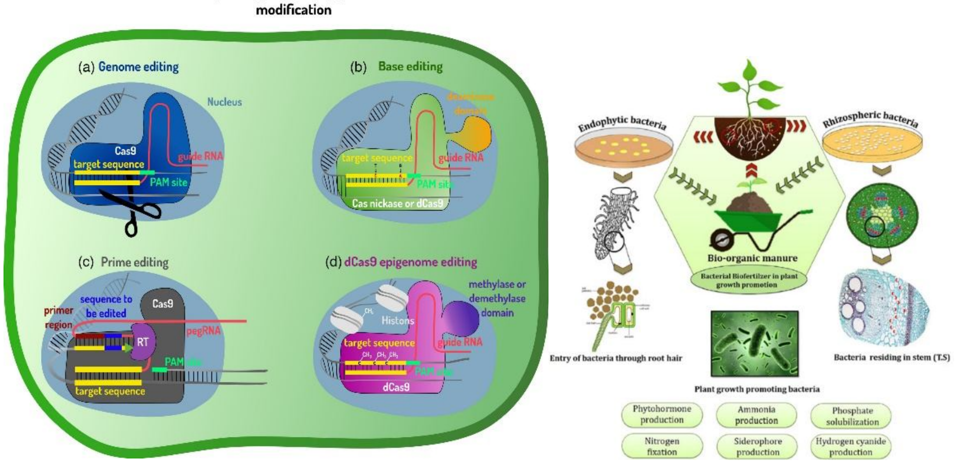


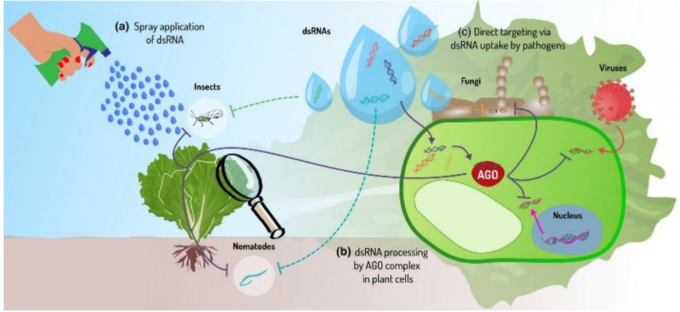
Fig .4 Approaches and possibilities in the field of plant protection against cisgenesis

Genome editing for microbial biotechnology

Genome editing is a precise technique that selectively modifies targeted DNA sequences while preserving other sections and preventing the insertion of other DNA. The process involves the use of endonucleases that identify certain DNA sequences, causing a double-strand DNA (dsDNA) break (DSB) and triggering the DNA repair pathway. One may do this by using three distinct categories of enzymes: zinc-finger nucleases (ZFNs), transcription activator-like effectors nucleases (TALENs), and Cas proteins**[30]**. The potential of genome editing approaches to enhance plant resistance to biotic and abiotic stressors is still in its early stages, however there are currently some existing instances. The workflow for developing stress-resistant crops through genome editing involves isolating and characterizing susceptibility genes, using informatics to design gRNAs that enhance specificity and minimize off-target effects, transforming plant tissue cultures or protoplasts using Agrobacterium tumefaciens-mediated transformation or ribonucleoprotein protoplast transfection, regenerating and selecting transformed plants, testing and selecting transformed lines, and finally releasing new varieties**[10]**.

CRISPR-Cas systems, initially identified as conserved defense mechanisms in bacteria against viral attacks, consist of three essential components: a nuclease protein, a single guide RNA (sgRNA) that directs the Cas protein to specific target sites, and a protospacer adjacent motif (PAM) along with a short sequence preceding the complementary DNA strand, which serves as a marker for the target site. The sgRNA-Cas complex surveys the genomic DNA in search of the corresponding sequence, and upon recognition, the Cas protein triggers a double-stranded DNA cleavage at a specified location indicated by the Cas type**[31]**. The CRISPR-Cas system has remarkable versatility in generating knockout mutants, introducing a DNA fragment via a donor vector utilizing the homologous recombination (HR) system, performing base editing on a target region, inducing mutations in regulatory sequences, and modifying the epigenome. When targeting multiple closely related genes, there are two strategies to choose from: using multiple guide RNAs controlled by a single promoter, or using multiple guides controlled by their own specific promoters. Another option is to use one or a few sgRNAs that can activate the Cas protein on different genes**[59]**. In 2013, the first instance of employing CRISPR/Cas systems for genome editing in plants was reported. This achievement used two model species, Arabidopsis thaliana and Nicotiana benthamiana, and utilized reporter genes that were clearly visible. Over time, significant advancements have been achieved, with several studies conducted on various herbaceous plant species such as tomato, rice, soybean, and wheat. These advancements have also extended to woody species including citrus, apple, and grape. Various research facilities are dedicated to the development of novel transportation techniques for plant systems**[32]**.

Fig .5 Genome editing for microbial biotechnology



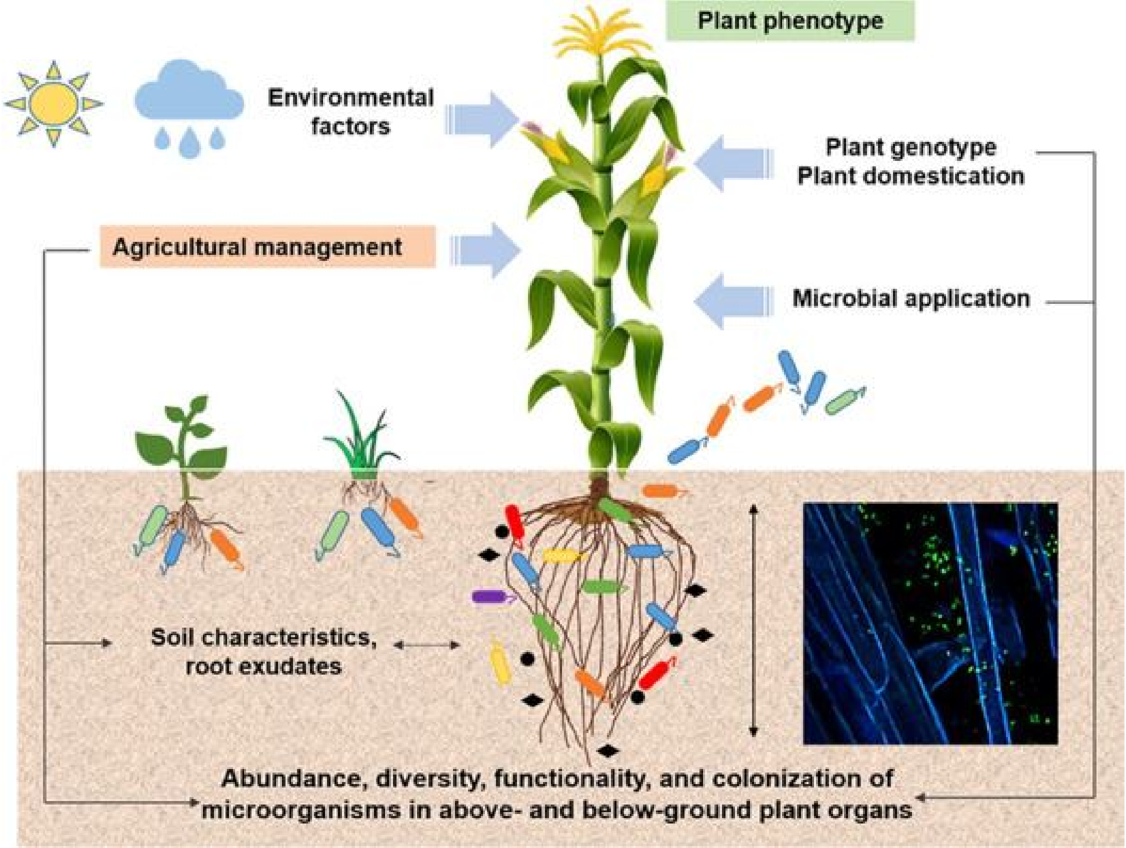
CRISPR technology has been extensively used to augment plant resistance against fungal and bacterial diseases, including Theobroma cacao and several other species. Genes that make plants vulnerable to infections are crucial for the interaction between plants and diseases, particularly in the case of biotrophic pathogens**[33]**. Genetic mutations or the absence of a S gene may restrict the pathogen's capacity to induce illness. As an example, in their study, Paula de Toledo Thomazella et al. (2016) deliberately induced a genetic alteration in the DMR6 gene of Solanum lycopersicum, resulting in a reduced vulnerability of tomatoes to downy mildew, Pseudomonas syringae, Phytophthora capsici, and Xanthomonas spp**[11]**.The use of CRISPR technology in rice has been utilized to develop types that are resistant to bacterial blight. This is achieved by using Cas9-mediated genome editing to induce mutations in one or more vulnerable genes. An further use of CRISPR to mitigate biotic stress was shown in tomato by directing a microRNA (miRNA), augmenting plant resistance against Fusarium oxysporum f. sp. Lycopersici, the pathogen responsible for tomato wilt disease**[60]**. CRISPR technology offers a viable approach to developing virus-resistant plants by targeting fungal and bacterial diseases. One approach to combat viral replication is to create genetically modified plants that produce a constant supply of Cas protein and gRNA(s) that specifically target viral sequences. Another method is to modify plant genes to develop cultivars that are resistant to viruses. Plant genes related to stress response may be modified to address abiotic challenges such water deficiency, high temperature, and soil salinity**[61]**. The OST2/AHA1 locus, which controls the reaction of stomata to abscisic acid, was modified to produce Arabidopsis plants with enhanced stomatal responses under drought conditions, resulting in a reduced rate of water loss**[34]**.

CRISPR technology may be used to investigate the role of gene(s) in intricate regulatory processes, such as the non-expressor of pathogenesis-related gene 1 (NPR1), which acts as a specific receptor for salicylic acid (SA). The application of Cas9 was used to acquire NPR1 tomato mutants, which exhibited reduced drought tolerance, so indicating the involvement of NPR1 in abiotic stress resilience as well. In a more recent work, researchers used the CRISPR activation (CRISPRa) system to investigate stress-related reactions and improve the ability of Arabidopsis plants to withstand drought**[62]**. The use of genome editing in modifying particular plant genes has been extensive. However, a significant limitation persists: Cas proteins have the ability to identify PAM sites in non-target regions and cause double-strand breaks (DSBs), resulting in unwanted characteristics or traits. In order to reduce unintended actions, several bioinformatic methods have been created to computationally forecast the activity of Cas enzymes on certain genomes. Plant genome editing techniques have previously used Cas variants with enhanced specificity, including Cas12a and b, eSpCas9, HiFi-Cas9, and HypaCas9**[12]**.

Authors have also been prioritizing the use of dead Cas9 (dCas9), in addition to enhanced Cas variants. dCas9 has the ability to disrupt transcription without carrying out endonuclease activity. This system may be designed by connecting it to a transcription activator or repressor, enabling the manipulation of gene expression in animals that do not have a programmable expression system. It is useful for examining the overexpression or down-regulation of target genes without altering the genomic context or introducing a transgenic**[35]**.

The CRISPR-Cas system has been modified to enable base editing, a process that facilitates the conversion of one base pair to another without the need for a double-strand break (DSB) to occur. An article conducted a recent experiment to evaluate the efficacy of plant prime editing (PPE) in rice and wheat, providing the first validation of this technique in plants. A novel category of CRISPR-Cas systems has been developed that selectively target RNA rather than DNA. These systems have been effectively used in plants to trigger interference against RNA viruses**[63]**. An engineered form of the dCas13 protein, which is linked to a deaminase enzyme, was shown to be effective in changing the nucleotide A to G during RNA editing. This approach enables the editing of whole transcripts that include disease-causing mutations. The rapid advancement of these potent and inventive methods serves as the foundation for enhanced agricultural productivity, robust crops that can withstand both living organism-related and non-living organism-related pressures, and resolving consumer apprehensions about genetically modified organisms and dietary requirements**[36]**.

Fig .6 agricultural productivity



Exploring alternative methods for crop protection without the use of genetically modified organisms (GMOs)

Small RNAs (sRNAs) and RNA interference (RNAi) are now recognized as regulators of gene expression in plant immune responses, pathogen pathogenicity, and plant-microbe interactions. Since the identification of the RNA interference (RNAi) mechanism, several endeavors have been undertaken to enhance its suitability in the field of plant protection**[64]**. In the realm of plants, RNAi is well recognized as a conserved regulatory mechanism that plays crucial functions in controlling endogenous gene expression and defending against viral infections. This mechanism leads to the post-transcriptional suppression of certain RNA sequences. The RNA interference (RNAi) mechanism is activated by double-stranded RNA (dsRNA) molecules. These dsRNA molecules are created inside the cell and then undergo processing by RNase III DICER-LIKE endonucleases. As a result of this processing, the dsRNA molecules are cleaved into small interfering RNAs (siRNAs) that are 21-24 nucleotides in length**[65]**. Following cleavage, one of the two siRNA strands binds to ARGONAUTE (AGOs) proteins to create RNA-induced silencing complexes (RISCs). These complexes selectively interact with transcripts that have complementary sequences, leading to either cleavage of the mRNA or repression of translation**[37]**. This process is referred to as post-transcriptional gene silencing (PTGS). In addition, siRNAs may induce the placement of inhibitory chromatin markers on specific DNA sequences, leading to the suppression of gene expression (TGS). In plants and invertebrates, siRNAs play a crucial role in plant host-pathogen interactions. Specifically, during viral infections, siRNAs are generated inside infected cells by the processing of double-stranded RNA molecules that originate from the viral genome**[66]**. There is evidence to suggest that siRNAs, once generated in a particular cell, may travel via plasmodesmata to neighboring cells and, through the vascular system, reach distant areas of the plant, causing systemic silence. Both the siRNA short-distance and longdistance transport systems across the whole plant have been recorded and are now being examined. RNAi activities play a crucial role in activating plant defense against pests and pathogens, as well as regulating their growth and ability to cause disease. There is evidence indicating that small RNAs (sRNAs) may be transferred in both directions between interacting partners, such as plants and fungi. This exchange of sRNAs induces gene silencing in each other, resulting in a process known as cross-kingdom RNAi**[38]**. The latter process is facilitated by exosome-like extracellular vesicles that have the ability to transport small RNAs into the organisms that are interacting with each other. This has been recently shown in the Arabidopsis-B. cinerea pathosystem. This research suggests that cross-kingdom RNAi may be used to manage plant illnesses caused by many pathogens, including fungus, viruses, nematodes, and insects. Furthermore, it promotes the use of RNAi as a technique to combat agricultural pathogens**[83]**. RNAi is a potentially effective and eco-friendly method that may be used to combat agricultural pests and diseases. It offers a viable alternative to the usage of insecticides. Currently, RNAi has mostly been used in plants for functional genomic investigations and to provide resistance against insects in genetically modified plants. The technique of Agrobacterium-mediated transformation has been used to introduce pathogen/pest gene-targeting small RNAs (sRNAs) or double-stranded RNAs (dsRNAs) against a specific target**[84]**. Alternatively, the use of a virus-induced gene silencing (VIGS) technique may be used to produce specifically designed RNAs that target pathogens in plant tissue, thereby avoiding the need to create genetically modified organisms (GMOs)**[13]**.

Novel techniques, such as GMO-free RNAi methods, use exogenous dsRNA/siRNA to directly treat plants, aiming to address the challenges associated with plant transformation and its constraints. There are documented instances in the literature where the application of exogenous dsRNAs has been shown to modulate the internal processes of plants. Multiple papers have shown that the external administration of artificially produced long double-stranded RNAs, hairpin RNAs (hpRNAs), or small interfering RNAs (siRNAs) may reduce the expression of crucial genes in pests, hence managing detrimental insects, fungal infections, and viral diseases in agricultural plants**[67]**. Exogenously administered dsRNAs have been effective in managing fungal-plant pathosystems, with intact dsRNAs demonstrating greater efficacy in inhibiting pathogen growth. Koch et al. (2016) found that intact dsRNAs are more effective in suppressing pathogen growth, as shown by the findings in insects and fungus. Furthermore, SIGS has shown efficacy against other fungal diseases, including Sclerotinia sclerotiorum in Brassica napus, Fusarium asiaticum in wheat coleoptiles, and Botrytis cinerea in multiple plant species, including grapevine, in both natural and post-harvest circumstances**[39]**. Nevertheless, more research and development are necessary to fully explore and enhance the use of dsRNAs as bio-based insecticides, particularly in terms of uncovering the cell regulatory features that have not yet been extensively studied**[85]**. Several data indicate that most plant endo-genes have a low sensitivity to RNA interference (RNAi), which is influenced by the presence of introns. Introns are well-known for their ability to block RNA silencing mechanisms**[68]**. To achieve widespread use of dsRNAs as protective agents in crops, it is necessary to develop new technologies. These technologies include the use of nanoparticles or other synthetic carriers to slow down the degradation of dsRNA, innovative delivery methods such as high-pressure spraying or brush-mediated leaf applications, and the establishment of a science-based risk assessment procedure for the external application of dsRNA. Obstacles in using exogenous dsRNAs for crop protection include problems with formulation, understanding the target species, and variations in sensitivity to dsRNAs across different taxa and genera within the same family**[86]**. The quantity of dsRNA applied or administered varies significantly across various studies, with effective amounts ranging from picomoles to milligrams per treated organism. Encapsulation technologies have the ability to mitigate this issue by safeguarding against degradation and/or enabling the entry of dsRNAs into the desired tissues. The cellular absorption processes of double-stranded RNAs (dsRNAs) and the detection of certain patterns or sequences by the target RNA interference (RNAi) machinery are crucial factors to consider. C. elegans has been shown to possess Systemic RNAi deficient (SID) proteins. These proteins play a role in obtaining and carrying double-stranded RNA (dsRNA) and the resulting small interfering RNA (siRNA) throughout the nematode's body. However, further research is required to elucidate the process in greater depth[87]. A comprehensive understanding of adsorption and transportation is crucial for comprehending the emergence of resistance mechanisms in pests and diseases. For instance, in the case of D. virgifera, it has been shown that resistance to treatment grew during only 11 generations, accompanied by a decrease in the absorption of dsRNAs[88]. The mechanism by which the RNAi pathway of the target organism recognizes the dsRNAs is a crucial element that is not well understood. Unlike fungus, insects exhibit a perplexing array of reactions that are not consistently associated with evolutionary characteristics and vary within species within the same family. In order to effectively use dsRNAs as long-lasting plant protection methods, it is necessary to gather information on the size and concentration of dsRNAs in formulations, as well as the processes by which they are taken up by pests/pathogens and the characteristics of the RNAi machinery in these target organisms[14].

Conclusion

In conclusion, microbial biotechnology is a revolutionary technique to crop protection and enhancement that provides environmentally friendly alternatives to the traditional agricultural approaches that are now in use. Not only does the creative use of microorganisms as biofertilizers, biopesticides, and soil health enhancers have the effect of reducing dependency on chemical inputs, but it also encourages environmental stewardship. Microbial solutions have the ability to make crops more resistant to both biotic and abiotic challenges, and the improvements that have been made in genetic engineering and microbial genomics have further strengthened this potential. The incorporation of microbial biotechnology into conventional agriculture offers the potential to improve production, guarantee food safety, and provide support to ecological equilibrium. This is particularly relevant in light of the growing number of threats to global food security. The adoption of these advances is very necessary in order to construct a sustainable future in agriculture, one that is able to successfully satisfy the requirements of a rising population while also protecting natural resources.

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