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Review Article

Biotechnological Innovations in Sustainable Food Production

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ABSTRACT

This review article explores the critical role of biotechnology in addressing the global food security crisis aggravated by the COVID-19 pandemic and Eastern European conflict. It starts by examining the profound impacts of these events on food supply chains and pricing, supported by relevant statistical evidence. The study then discusses agricultural biotechnology, comparing conventional and sustainable agriculture, with an emphasis on genome editing and modification techniques such as Zinc Finger Nucleases (ZFNs), Oligonucleotide-directed mutagenesis (ODM), and CRISPR. The application of RNA interference in agriculture and microbial biotechnology in promoting soil health and sustainable food production is discussed. Furthermore, the review shifts focus to the utilization of microbial biotechnology for soil health enhancement and sustainable food production, encompassing the development of pest-resistant and drought-tolerant crops and biotechnological methods for optimizing water use efficiency. Additionally, it examines biotechnology's applications in food processing, including enhancing nutritional content and improving shelf life and safety. The European Green Deal is analyzed, particularly its influence on agriculture through strategies like Farm to Fork, Biodiversity, and Circular Economy. Finally, the review concludes by addressing policy considerations, ethical challenges, and the necessity of international cooperation in biotechnology research, exemplified by projects like the Golden Rice and Heat-Tolerant Maize for Asia (HTMA), highlighting the multidisciplinary nature and global significance of biotechnological innovations in ensuring sustainable food production.

INTRODUCTION

Food security means the assurance of sufficient, safe, and nutritious food for all citizens so they can maintain a healthy, happy, and active lifestyle. Built on three pillars: availability, access, and right food security is the use of food. Today, it is not just an issue of a single nation or individual country, it is a global issue affecting people worldwide. The goal to achieve sustainable food production comes with multiple challenges such as climate change stands as a primary challenge that has threatened crop yields and livestock health with the shifts in growing seasons and increased prevalence of pests and diseases. Environment degradation such as water scarcity, biodiversity loss, and soil erosion further fuel the crisis. Moreover, other challenges include geopolitical instability, inequitable and inadequate food supply, and market volatility, making it difficult to achieve sustainable food production. The outbreak of the Covid-19 pandemic is the perfect example of how these challenges can be escalated and disrupt the whole food chain supply. However, in these times of food security crisis biotechnology can prove to be a potential player by deploying advanced molecular biology techniques and genetic engineering that offer promising solutions to enhance crop yield, crop resilience, and less dependency on environmentally harmful agricultural inputs. With the help of biotechnological innovations scientists have developed crop varieties that are resistant to diseases and pests, tolerant to environmental factors such as drought, salinity, and more. These innovations in agricultural biotechnology have not just increased the quantity of food crops, but also their nutritional value.

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Thus, the food crops are in adequate quantity with good quality.

The Global Food Security Crisis

In 2021, about 193 million individuals in 53 countries faced severe food shortages, marking a significant rise from past years. The FAO's (Food and Agriculture Organization) Price Index of Food noted a significant rise, averaging 157.4 points in May 2022, up from 98.1 before the pandemic. This increase indicates the broader impact on global food prices due to the pandemic. Recently covid-19 pandemic has significantly disrupted the food chain supply all over the globe. Food insecurity is exacerbated by the labor shortage due to health-related restrictions and economic shutdowns. The restrictions made it difficult for people to access food markets, and some countries even imposed export restrictions on certain food items to secure for the locals that at a large scale impacted the global food markets and contributed to food insecurity in countries that are dependent on food imports [1]. Moreover, the pandemic led to an increase in food prices, because higher demand for certain food items has severely impacted low-income groups, who are more susceptible to food insecurity and malnutrition. Ukraine and Russia are known to be the major exporters of crops, especially wheat, and corn. However, due to the recent military conflict between the two countries, the global food supply chain has been affected significantly. For instance, North African and Middle Eastern countries import over 50% of their cereals from these two nations. In 2023, it's estimated that Ukraine's agricultural output could drop by 25–50%, adding more pressure to the worldwide food supply[2].

Biotechnology in Agriculture and Sustainability

Sustainable food production needs sustainable agriculture methods. The word sustainability is derived from the word "sustain" which means endure, maintain, or uphold. Sustainable food production and agricultural development aim to produce food that should be enough to meet the nutrient and fiber needs of the growing population and long-term food security for current and future generations. It also focuses on the aim that the technology or method used for food production should not harm the living organisms, environment, and overall ecosystem. The methods should be cost-effective and eco-friendly. Hence, biotechnology has emerged as a promising tool to address all these concerns. It is an emerging field that helps in sustainable food production besides its application in health, plant and pharmaceutical sectors. It can increase crop yield, more nutrient benefits, biotic and abiotic resistance to plants, and enable modern techniques for crop improvement [3]. The domain "agricultural biotechnology" of this field is the solution to all food and crop-related problems. Agricultural biotechnology is defined as; The use of biotechnological techniques to improve plants, crops and animals, or modify microorganisms for specific agricultural uses.

Conventional Versus Sustainable Agriculture and Food Production

To understand the importance of sustainable agriculture, it is important to discuss the conventional agricultural system for food production and compare it with advanced sustainable methods. Conventional systems focus on maximized production and yield with little regard to environmental impacts. Whereas, sustainable systems offer economically beneficial and environment-friendly approaches, promoting stewardship and conservation of resources. The two perspectives on the use of sustainable methods are – modifying current methods with modern technologies and advocating changes in agricultural systems by incorporating societal values. While conventional methods prioritize technologies for only better yield and financial gains without considering environmental and soil impacts [4]. It often causes environmental repercussions such as soil contamination and degradation. It affects the future of growing plants and causes nutrient deficiency. Sustainable methods, on the other hand, offer a balanced ecological and biodiversity-beneficial approach and preserve water quality, and soil health with optimized yields. The difference between these two agricultural methods also lies in the economic implications. Conventional farming uses mechanization and requires immediate financial returns leading to increased farmer indebtedness. Whereas, sustainable agriculture has the potential to offer cost-friendly systems by efficient use of resources[5].

BIOTECHNOLOGICAL TOOLS FOR CROP IMPROVEMENT

Conventional breeding for improving genetic resources is a difficult and slow process and has many drawbacks such as a long juvenile period, heterozygosity, auto-incompatibility and a time-consuming process. New biotechnological tools (NBTs) such as genetic engineering facilitate the integration of genes into the plant genome, eradicating all conventional drawbacks and giving stability to most of the cloned traits. (5) Sustainable food production by using biotechnology follows almost all the sustainable development goals (SDGs) such as zero hunger, no poverty, promoting healthy lives and well-being, water availability, sustainable economic growth, sustainable consumption and production patterns etc. Different techniques of biotechnology are being used to improve yield and produce sustainable food all over the world. Some of the techniques are listed below.

Genome Editing and Modification Techniques

In genetic engineering, scientists manipulate the DNA of organisms by adding foreign genes of interest to make

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recombinant DNA. Integration of foreign genes allows for the production of desired products and the expression of specific qualities in the plant. For this purpose, the genes of bacteria are most commonly used. First, the DNA of the host and gene of interest is cut by the endonuclease restriction enzyme that produces sticky ends in these sequences. Then, these two fragments are joined by DNA ligase. This ligation process proceeds by making the cell compatible to take foreign genes. For this purpose, calcium chloride solution is used to bring a positive charge on the cell. As DNA is negatively charged, the cell easily attracts the DNA and heat is given for proper recombination. Now, recombinant DNA is produced and the cell is grown in culture for cloning (make several copies). An example of genetic engineering in plants is the production of BT cotton. These cottons are specialized as insect and pest-resistant [6]. The cry gene is taken from the bacteria Bacillus thuringiensis and incorporated into the plant, serving as an insecticide. Similarly, maize, potato, alfalfa, canola, papaya, tomato, and sugar beet are all genetically engineered plants. The advantage of this technique is to make biotic (insects, pests, herbs) and abiotic (temperature, light, pH, gasses) stress-tolerant crops and to increase the shelf life of plants.

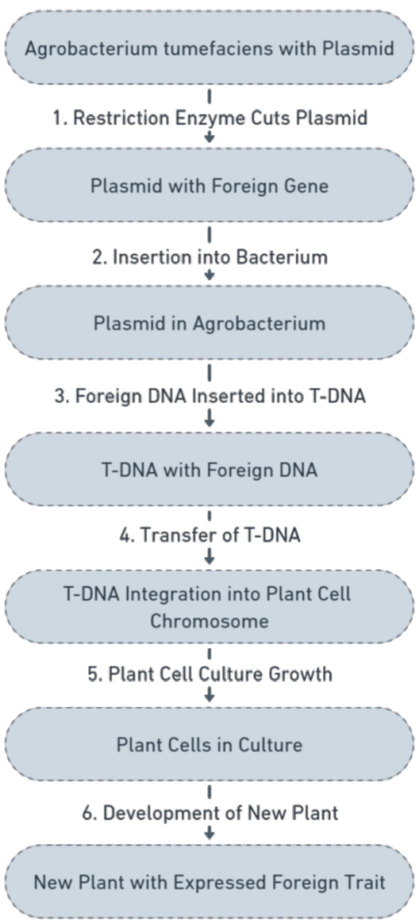

Figure 1: Flowchart of steps of production of genetically engineered plants by genetic engineering technique [6]

Mechanism of Plant Genome Editing

Genome editing tools, also known as GEEN (genome editing with engineered nucleases), allow the cleavage and rejoining of DNA material at target sites to change the expression of genes. For this purpose, special enzymes are being used such as restriction endonucleases for cleavage and DNA ligase for joining of DNA segments after insertion and deletion of nucleotide sequences. These enzymes normally target short DNA segments, i.e., segments of bacteria and viruses etc., so it is challenging to manipulate the DNA of higher organisms by using these enzymes. The first effort to resolve this issue was associated with the designing of artificial enzymes called oligonucleotides. This is relatively a short nucleotide sequence capable of binding with DNA sequence and can cleave it from specific sites. Secondly, the target approach for this problem is the chimeric nucleases which are protein structures comprising one or two structural units. One unit is catalyzed for the cleavage of specific DNA sequences and the other is responsible for selectively binding to specific sequences of nucleotides. These nucleases can either be produced in the cell or can be inserted by different techniques [7].

Zinc Finger Nucleases (ZFNs)

Zinc finger nucleases are the first generation of genome editing tools. It is mainly an engineered nuclease based on the functional domain of the Cys2-His2 zinc finger (ZF). Each domain of ZF consists of three tandem nucleotides and 30 amino acid residues which are arranged in ββα configuration. It binds to DNA by incorporating the α-helix in the major groove of the helix. This is revealed by crystallographic structure analysis. Its structure consists of two functional domains i.e., the ZF Cys2-His2 domain and the FokI DNA cleavage domain. The first domain is at the N-terminal while the former is at C-terminal. These domains are necessary for proper enzymatic activities. It recognizes the target sequences of base pairs 18 to 24 [8]. It is a highly revolutionized genome editing technique, having multiple advantages such as high efficiency, specificity and minimum non-target effects. This technique has been successfully employed since 1996 for higher organisms including plants for gene modification, inactivation and targeted edition [9]. Examples include using this technique in tobacco, Arabidopsis and maize for stacking traits.

Oligonucleotide-directed mutagenesis (ODM)

Oligonucleotide -Directed Mutagenesis (ODM) is another novel gene editing technology for plants after

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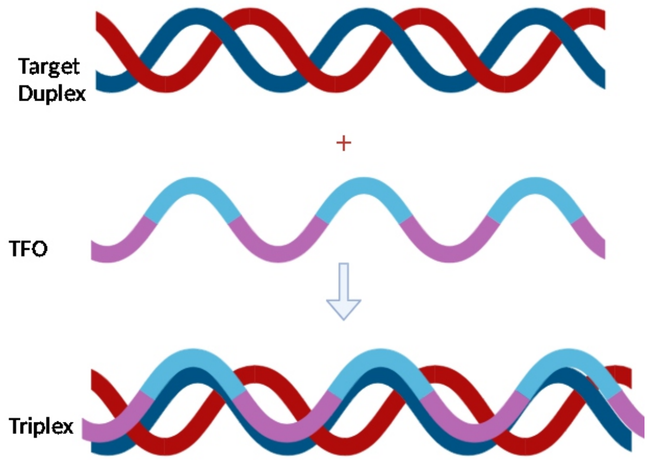

successful exploitation in animal cells (mammalian system). It is the tool for precise targeted editing in the DNA of plant systems. It uses an oligonucleotide of 1-100 base pairs, identical to the target sequence except there is a change in one base pair (mutation is inserted). It detects and targets mutagenesis. They bind to the target and activate the cell machinery of the natural repairing process. The machinery recognizes the mismatch and makes copies of detected mismatches through the repair process. This results in the production of the target sequence in the plant required for novel traits or functioning. The oligonucleotide sequence is then degraded by the plant after the process. This technique is now revolutionized by utilizing a fluorescent conversion system. By editing a single nucleotide, BFP (Blue fluorescent protein) is converted to GFG (Green fluorescent protein) [10].

Figure 2: Combination of target duplex and TFO to detect and remove mutagenesis

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

Another novel technique of genome editing in crops is clustered regularly interspaced short palindromic repeats. It emerged recently and became popular in recent years. CRISPR-Cas 9 (CRISPR-associated protein) is significant among all types. This method uses the immune system of bacteria and archaea which relies on the presence of CRISPR loci, a special site in the bacterial genome [7]. These sites consist of repeated spacer sequences and operons containing Cas9 protein. The spacers are short base pair sequences that are integrated into bacterial DNA from foreign DNA following recombination. It differs from TALENS as it targets the recognition site by complementary-based interaction between the DNA of the target site and guide RNA. This forms a complex with nuclease activity, leading to precise cleavage of double-stranded DNA via Cas endonuclease enzymes [11]. This system is present in various types of bacteria but the most popular type is the CRISPR/Cas type II-A system which is present in the bacterium Streptococcus pyogenes. It consists of three genes i.e., trans-activating crRNA (tracrRNA), CRISPR RNA (crRNA) and Cas9 protein [12]. This method is used to produce genetically modified cells grown in the culture medium.

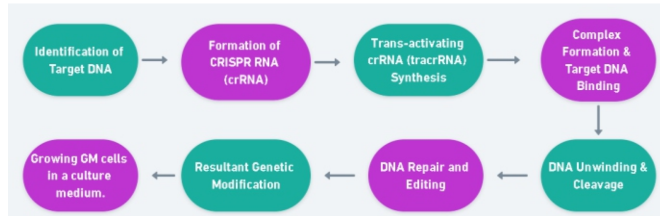

Figure 3: Modification of plants by using CRISPR technique

RNA Interference in Agriculture

RNA interference (RNAi) was discovered before gene editing techniques and it is considered a natural process for controlling gene expression in plants and animals (higher organisms) at the post-transcriptional level. It was observed after its discovery that the RNAi technique has the potential to enhance crop improvement choices. It can contribute greatly to the production of sustainable food with little environmental impact on the crops [13]. The manipulation of gene expression in plants can be exploited by either spray-induced gene silencing or host-induced gene silencing. It is one of the best methods to study gene expression. It is used widely to promote quality traits, modify the physical appearance of plants to attract more insects for pollination or ornamental purposes, male sterility control, make plants resistant to biotic and abiotic stresses, delay ripening, nutritional improvements related to bio-fortification and other changes depend upon the requirements [14].

Usage of Microbial Biotechnology for Sustainable Food Production

'Soil is essential for the maintenance of biodiversity above and below ground. The wealth of biodiversity below ground is vast and unappreciated: millions of microorganisms live and reproduce in a few grams of topsoil, an ecosystem essential for life on earth [15]. The microorganisms which help to maintain soil health for better food production encompass bacteria, actinomycetes, fungi, algae, protozoa, and viruses, each group possessing distinct traits that characterize them and specific roles within their soil environment. Crucially, these organisms don't operate independently; their interactions with one another significantly influence soil fertility, often to a greater extent than the individual activities of each organism. Biological fertility pertains to the organisms inhabiting the soil, engaging in interactions with other components. These organisms, residing on soil, organic matter, or fellow soil

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inhabitants, play essential roles in various soil processes, contributing significantly to nutrient and carbon cycles. Importantly, very few of these soil organisms pose any threat as pests. Soil microbial communities play a fundamental role in supporting the productivity of agricultural endeavors and drive ecological processes such as nutrient and carbon cycling, contaminant degradation, and the suppression of soil-borne diseases. Moreover, they engage in essential and often mutually beneficial relationships with plants. Once the microorganisms have enhanced the fertility of the soil, better food crops can be grown sustainably to ensure vast food production [16]. Modern agriculture's heavy reliance on synthetic chemical fertilizers has led to environmental problems, necessitating sustainable practices for cost-efficient and eco-friendly food production. Biofertilizers, containing microorganisms like bacteria, fungi, and algae, offer a natural and economical solution. These alternatives not only enrich soil nutrients but also promote plant growth, suppress diseases, and maintain soil structure and biodiversity. Nitrogen-fixing bacteria benefit various crops, while blue-green algae and Azolla contribute to nitrogen availability. Arbuscular mycorrhizal fungi aid in phosphorus uptake, and phosphorus-solubilizing bacteria enhance phosphorus availability, resulting in increased crop yields. Azospirillum, a nitrogen-fixing bacterium, provides additional benefits such as growth-promoting substances and resistance to diseases and drought [17]. The application of microbial biofertilizers proves effective in maintaining soil nutrient balance, reducing the need for chemical fertilizers and fostering sustainable agriculture.

Biotechnology in Crop Protection

Biotechnology plays a crucial role in crop protection by leveraging scientific advancements to develop innovative and effective strategies for managing pests, diseases, and environmental stressors in agriculture. Through genetic engineering, molecular biology, and other biotechnological tools, researchers and scientists have been able to enhance crop resistance, develop biopesticides, and create genetically modified organisms (GMOs) with improved traits. Pest infestations pose a widespread challenge for various crops globally, encompassing fodder crops and those cultivated for food. One illustrative case is BT-Cotton, where genes from Bacillus thuringiensis (BT), a commonly found bacterium, are integrated into the cotton crop to induce the production of a specific protein. This protein exhibits toxicity towards numerous insects. The resulting BT-Cotton experiences reduced susceptibility to pests, resulting in a notable increase in overall crop yield [18]. Additionally, the application of naturally occurring pathogens, such as bacteria, fungi, or viruses, serves as a biopesticide to target specific pests. Botanicals, derived from plant extracts with pesticidal properties, contribute to pest deterrence. Microbial inoculants enhance soil health, suppress diseases, and indirectly aid in pest resistance. Genetic control involves the use of genetically modified organisms (GMOs) with traits that enhance pest resistance. Biotechnology plays a crucial role in fortifying crops against the adversities of drought and salinity. Through genetic engineering, crops can be tailored to express genes that amplify their resilience to water scarcity and soil salinity. Biotechnological advancements also facilitate the creation of crops with superior water use efficiency, the synthesis of Osmo protectants for cellular safeguarding, and the activation of stress-responsive gene pathways [17]. Furthermore, biotechnology contributes to the development of crops capable of thriving in nutrient-poor soils by enhancing biological nitrogen fixation and utilizing marker-assisted breeding programs. Biotechnological approaches to enhance water use efficiency include introducing genes that regulate water uptake, reduce transpiration, and improve overall water utilization for essential physiological processes [19]. By leveraging biotechnology, researchers seek to develop crops that can thrive with reduced water inputs, addressing challenges associated with water scarcity and promoting sustainable agriculture.

Biotechnology in Food Processing

Applications of biotechnology in enhancing nutritional content involve genetic modifications to improve the nutritional profile of crops. This includes biofortification, where essential nutrients such as vitamins and minerals are increased in staple crops through genetic engineering. Biofortified crops are plants that have been genetically modified to contain higher levels of essential nutrients, such as vitamins and minerals. The goal of biofortification is to enhance the nutritional content of staple crops, addressing micronutrient deficiencies in human diets. Through biotechnological approaches, specific genes related to nutrient accumulation are introduced or modified in the crops. For example, biofortified rice may contain increased levels of vitamin A, while biofortified wheat may have elevated zinc content. These crops aim to provide a sustainable solution to global malnutrition by delivering crucial nutrients through commonly consumed foods. Genetic modifications can reduce anti-nutritional factors in crops, making them more nutritious and healthier for consumption. Biotechnology is being employed by scientists to produce fruits with improved taste. Genetically modified (GM) foods with enhanced taste include eggplant, cherries, peppers, seedless watermelon, and tomatoes. In these fruits, the seeds are eliminated, resulting in favorable outcomes such as higher sugar content in a soluble form, thereby enhancing sweetness.

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Biotechnology is also utilized to modify fermentation pathways, contributing to the infusion of flavor and aroma in wines. To extend the shelf life and improve the transportation of tomatoes, the California-based company Calgene developed the Flavr Savr variety through genetic engineering. Traditional harvesting and ripening processes were addressed by modifying the enzyme polygalacturonase, responsible for pectin breakdown during ripening. Using antisense RNA, scientists reduced the quantity of this enzyme, resulting in tomatoes with a firmer texture, enhanced shelf life, and improved transport qualities[20].

CHALLENGES AND LIMITATIONS

One of the foremost challenges revolves around ethical considerations tied to genetic modification and other biotechnological interventions [21]. Striking a balance between technological advancement and ethical boundaries, particularly in areas like genetic engineering, poses a substantial challenge for policymakers. Despite the potential benefits, the environmental impact of biotechnological applications, such as the release of genetically modified organisms (GMOs), raises concerns. Unintended consequences, such as gene flow and potential disruptions to ecosystems, underscore the need for thorough risk assessments. Biotechnological approaches often focus on a limited number of traits, such as resistance to specific pests or tolerance to certain environmental conditions. This narrow scope may result in an overreliance on a few genetic characteristics, increasing the vulnerability of crops to unforeseen challenges [22]. The intricate nature of biotechnological processes and their reliance on sophisticated techniques can present limitations, particularly for smaller-scale farmers or regions with limited technical infrastructure. Implementation may require substantial investment in training and infrastructure development.

GLOBAL COLLABORATION IN BIOTECHNOLOGY FOR SUSTAINABLE FOOD PRODUCTION

Global collaboration in biotechnology is indispensable for advancing sustainable food production on a global scale. The complexities of modern agriculture and the challenges posed by climate change, population growth, and resource limitations require a collective effort from countries around the world. By pooling resources, expertise, and research findings, nations can leverage the power of biotechnology to develop innovative solutions that enhance crop yields, improve nutritional content, and mitigate environmental impacts [23]. Let's discuss a few projects; The Golden Rice project, pioneered by the International Rice Research Institute (IRRI), illustrates a remarkable instance of international collaboration in harnessing biotechnology for sustainable food production. The primary objective of this initiative was to tackle the prevalent issue of vitamin A deficiency in numerous developing countries, where it contributes to intense health problems [24]. The Heat-Tolerant Maize for Asia (HTMA) project, led by the International Rice Research Institute (IRRI), illustrates the power of global collaboration in harnessing biotechnology for sustainable food production. The initiative focuses on developing heat-tolerant maize varieties to counter rising temperatures that threaten crop cultivation. The project employs a collaborative approach, merging traditional breeding methods with advanced biotechnological tools. Field trials across diverse agroecological zones in Asia evaluate maize varieties under elevated temperatures, using genomic insights to identify key genes associated with heat tolerance. Beyond developing heat-tolerant maize, the project aims for sustainable agricultural practices for food sovereignty and improved livelihoods [25]. Vertical farming, coupled with advanced biotechnology, is revolutionizing agriculture by offering a more economical and sustainable approach to food production. This approach, stacking crops indoors, makes the most of space in controlled environments, which is crucial in crowded cities. Genetic modifications optimize crop growth in confined spaces, minimizing environmental impact [26]. Innovative methods like hydroponics and aeroponics further enhance sustainability, allowing precise nutrient control and efficient space utilization. Vertical farming, integrating these technologies, ensures year-round production in controlled environments, providing a stable supply of fresh produce.

CONCLUSIONS

Biotechnology can enhance the production of sustainable food by improving crop features, tackling issues with global food security, and reducing reliance on destructive agricultural methods. But it's important to take a balanced approach that incorporates ethical and environmental concerns with technological advancement. Transparency, social and economic repercussions, and responsible behaviors are crucial. Ensuring congruence with sustainable agricultural principles is crucial for optimizing the potential of biotechnological breakthroughs.

Authors Contribution

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Writing-review and editing: MA, WR, MI, MF, FT
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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