

## Crop Production And Genomic Science

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### **Abstract**

Agricultural biotechnology has emerged as a key player in crop improvement by leveraging scientific techniques to modify genes that enhance resistance to biotic and abiotic stress and improve crop quality. As the field has evolved from Mendelian genetics to molecular biotechnology, there have been significant advancements in crop improvement strategies. Recent biotechnological innovations have focused on eliminating physiological limitations in crops and increasing their yield potential.

By utilizing various agricultural biotechnological tools such as genetic engineering, tissue culture, embryo rescue, somatic hybridization, molecular marker-assisted selection, genome doubling, and omics technologies, numerous transgenic crops have been developed and approved for commercialization over the years. The application of these technologies has led to increased crop yields, reduced CO<sub>2</sub> emissions, decreased pesticide and insecticide use, and lowered crop production costs.

Despite the vast potential of biotechnology and genetically modified organisms (GMOs) to enhance global food security, there are growing concerns regarding their impact on the environment and human health. These concerns have raised questions about the safety and ecological risks of genetically modified crops. This review will explore the applications and concerns of biotechnology in crop improvement, with particular attention to potential health hazards and environmental risks.

**Keywords:** Agricultural biotechnology, genetic engineering, tissue culture, embryo rescue, somatic hybridization, molecular marker-assisted selection, genome doubling, omics technologies.

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### **INTRODUCTION**

Agricultural biotechnology is a field that applies biological organisms or a variety of biotechnological tools to improve plants, animals, microorganisms, or the food derived from them.[1] The goal is to enhance agricultural productivity, sustainability, and food security[2]. This can involve genetic modification, bioprocessing techniques, and other innovative approaches to develop crops, livestock, and microorganisms that are more resilient, nutritious, and efficient[3][10][11].

Some key objectives of agricultural biotechnology include: Improving crop yields by developing drought-resistant, pest-resistant, or disease-resistant varieties. Enhancing nutritional content to address deficiencies in staple foods, such as biofortified crops[7][8]. Reducing environmental impact through sustainable practices, such as reducing pesticide use or improving soil health. Increasing food safety by eliminating pathogens or toxins in food products. The use of biotechnology in agriculture has significantly transformed farming practices by introducing tools like genetic engineering, tissue culture, and biopesticides, which are designed to tackle some of the biggest challenges faced by farmers and food producers worldwide [4][5][6][9]. Following are some biotechnology tools used in agriculture:

Transgenesis, also known as genetic engineering or recombinant DNA (rDNA) technology, refers to the process of manipulating an organism's genetic material (specifically DNA) by introducing new genetic material from different species or by modifying existing genes.[12][13] This technique enables the creation of Genetically Modified Organisms (GMOs), which possess a combination of heritable genetic material that is not naturally found in their species.[17][18] Approximately 530 distinct transgenic events in 32 different crops have been authorized for cultivation in various regions around the world [19][15]. Of these, maize has the highest number of events, totaling 240, followed by cotton with 67, potato with 50, soybean with 42, and carnation with 19, among others. Transgenesis has been used to create crops with specific traits, including herbicide-tolerant (HT) crops, insect-resistant (IR) crops, abiotic stress-tolerant (AST) crops, disease-resistant crops, and nutritionally enhanced crops. The first herbicide-tolerant transgenic crop to be commercially introduced was the glyphosate-tolerant soybean (Roundup Ready

soybean), which contained the EPSPS gene from the CP4 strain of *Agrobacterium tumefaciens*. [14] Most of the commercially available glyphosate-resistant crops carry this gene [16]. Additionally, two genes from *Streptomyces* species, namely *pat* and *bar*, have been used to develop glufosinate-resistant crops. Other herbicide-tolerant transgenic crops, resistant to herbicides such as 2,4-D, Isoxafutole, Oxylin, and Sulfonylurea, have also been commercialized in recent years [21]. In total, 351 herbicide tolerance events have been approved for cultivation [19]. Among these, the largest number of herbicide-tolerant events (212) have been commercialized in maize, followed by cotton (45) and other crops.

#### Insect Resistant Transgenic Crops

The majority of insect-resistant transgenic crops are developed using *cry* genes from *Bacillus thuringiensis* (Bt), which provide resistance to a wide range of insect pests, including Lepidopterans, Coleopterans, and Dipterans. These *cry* genes not only confer resistance to insect pests but are also non-toxic to mammals [22]. The first commercially successful crop to incorporate the *cry* gene was cotton, which gained resistance against lepidopteran insect pests. Following the success of transgenic cotton, *cry* genes have been introduced into a variety of other crops, including potato, rice, canola, soybean, maize, chickpea, alfalfa, and tomato. Additionally, *vip* genes, isolated from *Bacillus* species like *B. thuringiensis* and *B. cereus*, have been used in cotton and maize to confer insect resistance [23].

Genes encoding protease inhibitors (PI) from various sources, including plants, bacteria, and fungi, have also been utilized to develop insect-resistant plants. For example, the *cptII* and potato protease inhibitor II (PI2) genes have been introduced into tobacco, rice, and cotton to provide resistance against insect pests. To date, a total of 305 insect resistance events have been approved for cultivation [19]. Among these, the highest number of insect-resistant events (208) have been commercialized in maize, followed by cotton (50), potato (30), and other crops.

#### Abiotic Stress-Tolerant Transgenic Crops

The impact of abiotic stresses, such as drought, heat, and salinity, on crops is increasing due to changing climatic conditions. Some plants are able to adapt to these stresses at the molecular level by altering the expression of various genes, which helps create near-optimal conditions for plant growth and development. However, because the adaptation to abiotic stresses involves the interaction of many genes, there are fewer abiotic stress tolerance events commercialized compared to traits like disease, insect, and herbicide resistance.

To date, 12 abiotic stress tolerance events have been approved for cultivation, with maize (7), sugarcane (3), and soybean (2) being the primary crops involved. One example of abiotic stress tolerance in plants includes the use of bacterial cold shock proteins (*csp*) to mitigate the effects of environmental stresses like cold, heat, and water deficit. For instance, Castiglioni et al. (2008) [24] demonstrated that introducing *cspA* from *E. coli* and *cspB* from *Bacillus subtilis* into maize helped the crop better adapt to water scarcity without causing pleiotropic effects.

More recently, the *Hahb-4* gene from *Helianthus annuus* (sunflower) was introduced into Verdeca's drought-tolerant transgenic soybean, marketed as Verdeca HB4 Soybean. This gene produces a transcription factor that binds to a dehydration-responsive regulatory region in plants, enhancing drought resistance. Additionally, drought-tolerant sugarcane has been developed by incorporating the *beta* gene from *E. coli* and *Rhizobium meliloti*. These genetically modified sugarcane plants can withstand drought for up to 36 days and produce 10-30% more sugar than non-transgenic plants under drought conditions in field trials [25,26].

#### Disease-Resistant Transgenic Crops

Plant diseases, caused by pathogens like fungi, bacteria, and viruses, can result in significant crop losses, threatening food security worldwide. Traditionally, these diseases have been controlled using agrochemicals. However, the widespread use of these chemicals not only harms the environment but also leads to the development of pests that are resistant to them. To overcome these challenges, scientists have turned to transgenesis, a technique that allows plants to be bred with built-in resistance to various diseases. So far, 29 disease resistance events have been approved for commercial cultivation, with the majority of these events found in potatoes (19), followed by papaya (4), squash (2), and a few other crops.

The majority of these transgenic crops are resistant to viruses, which have been particularly problematic for crops.

One of the first breakthroughs in disease-resistant plants was the development of a plant resistant to Tobacco Mosaic Virus (TMV) by incorporating a gene that encodes the viral coat protein of TMV. This gene made the plant immune to TMV infection [27]. Building on this success, transgenic papaya was developed to resist the Papaya Ringspot Virus (PRSV) using a method known as pathogen-derived resistance. In this approach, the prsv cp gene was introduced into papaya via a process called microparticle bombardment [28].

In beans, RNA interference (RNAi) technology was used to develop resistance to the Bean Golden Mosaic Virus (BGMV). By silencing the viral gene AC1, which is essential for virus replication, the plant was able to prevent the virus from spreading. In potatoes, resistance to late blight was achieved by introducing the Rpi-vnt1.1 gene from *Solanum venturii*. [30] This gene produces a protein that protects the potato from the destructive disease.

Another example is the use of chitinase enzymes, which break down chitin and  $\alpha$ -1, 3 glucan, key components of fungal cell walls. By inserting the chitinase gene into crops like tobacco and rice, researchers were able to enhance their ability to resist fungal infections.[31]

These advancements in disease-resistant crops help reduce dependency on harmful agrochemicals, improve yields, and ensure more sustainable farming practices.

#### Nutritionally Improved Transgenic Crops

There have been numerous successful efforts to improve the nutritional value of crops through transgenesis, with several innovative examples leading to more nutritious foods for global consumption. One of the most well-known examples is Golden Rice (GR2E), a biofortified variety developed by introducing two key genes: *crt1* from *Pantoea ananatis* and *psy1* from *Zea mays*. These genes enable the rice to produce carotenoids, which are converted into vitamin A in the rice endosperm. Golden Rice has been approved for human consumption in several countries, including the Philippines, Australia, New Zealand, Canada, and the United States, providing a potential solution to vitamin A deficiency in developing regions [19].

Another example of transgenic crops aimed at improving nutrition is the development of nutritionally enhanced potatoes. By introducing the *AmA1* gene from *Amaranthus* seeds, researchers have created potato tubers that are rich in essential amino acids, meeting the dietary requirements set by the World Health Organization [32]. This could significantly improve protein quality in diets that rely heavily on potatoes as a staple food.

In tomatoes, efforts to enhance the pro-vitamin A content have led to the creation of transgenic plants that convert phytoene into lycopene, a form of vitamin A [33] By transferring a bacterial gene for the enzyme phytoene-desaturase, these tomatoes produce three times more  $\beta$ -carotene than their non-transgenic counterparts, offering a richer source of this important nutrient.

Additionally, in Brassica species like canola (*Brassica napus*) and mustard (*Brassica juncea*), the introduction of an antisense *fae1* gene has resulted in lower levels of erucic acid, a compound that is undesirable for human consumption [34]. This improvement makes the oil from these plants safer and healthier for consumption.

Lastly, in maize, the incorporation of the *cordapA* gene from *Corynebacterium glutamicum* has led to an increase in the production of lysine, an essential amino acid that is often limited in maize. This genetic modification holds great potential for improving the nutritional value of maize, particularly in regions where it is a primary food source.

These advances in nutritionally improved crops offer promising solutions to addressing malnutrition and food security, particularly in areas where certain nutrients are scarce or deficient in local diets.

#### Tissue Culture

Tissue culture is a technique used to grow cells, tissues, or organs in a controlled nutrient medium under sterile conditions.[35] It typically involves taking small pieces of plant tissue, known as explants, and culturing them in an aseptic environment to develop into whole, living organisms.[36] Through this

method, genetically engineered cells can be transformed into genetically modified plants, offering new possibilities for improving crops.

Tissue culture plays a key role in creating genetic variability, which is vital for advancing plant breeding. By using techniques like protoplast culture, anther culture, and embryo culture, scientists can improve crops and increase the availability of desirable genetic traits for breeding programs. It's one of the essential tools in agricultural biotechnology.[37][38]

One of the key applications of tissue culture is in the germination of seeds that are difficult to sprout naturally, such as those of the banana. For instance, the Grand Naine (G9) variety of banana is propagated through tissue culture, leading to the mass production of disease-free, high-yielding clones that are genetically identical to the parent plant [39]. Similarly, meristem tip culture is used to produce banana plants that are free from diseases like banana bunchy top virus (BBTV) and brome mosaic virus (BMV). Tissue culture is also a powerful tool for the conservation of endangered plant species and germplasms. For plants that either do not produce seeds or produce seeds that cannot be stored long-term (like recalcitrant seeds), tissue culture techniques provide a way to preserve these species in gene banks, ensuring that valuable genetic material is maintained for future use.

Another important application of tissue culture is embryo rescue in wide hybridization. Sometimes, hybrid embryos resulting from crossbreeding between different species or genera may not develop due to fertilization incompatibility. In such cases, scientists can rescue the embryos by culturing them in vitro, allowing the production of a viable plant. This method facilitates the transfer of beneficial traits from wild relatives into cultivated crops, as seen in the *Capsicum* (pepper) plant, where embryo rescue was used to transfer fruit rot resistance traits, as demonstrated by Debbarama et al. in 2013.[18][38][42]

Overall, tissue culture is a versatile and essential technique that has revolutionized plant breeding, conservation, and the production of genetically improved crops, benefiting both agriculture and biodiversity.

#### Somatic Hybridization

Somatic hybridization is a technique that combines somatic cells (the non-reproductive cells) from two different plant cultivars, species, or genera to manipulate their genetic material. [43] This process, often achieved through protoplast fusion, allows scientists to regenerate new and unique plant varieties by merging cells that wouldn't normally be able to crossbreed due to natural barriers. The new hybrid cells can then be cultured into whole, viable plants through tissue culture.[44][45]

One of the key advantages of somatic hybridization is its ability to overcome incompatibility barriers between different plant species or genera. For example, a fusion between the protoplasts of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) has led to the creation of a Pomato plant—a hybrid that combines the characteristics of both species. This process not only bypasses the normal sexual incompatibility but also results in novel genotypes with beneficial traits.[46]

In another example, somatic hybridization has been used to create a salt-tolerant hybrid by fusing the protoplasts of rice (*Oryza sativa*) and mangrove grass (*Myriostachya wightiana*). [47]. This hybrid is a significant step toward developing rice varieties that can thrive in salty soil conditions, offering new solutions for farming in coastal or drought-prone areas.[48]

Somatic hybridization is also valuable in transferring specific genetic traits, especially those that are controlled by the cytoplasm, such as male sterility or resistance to antibiotics and herbicides. For instance, in rice, cybridization has been used to transfer Cytoplasmic Male Sterility (CMS), a trait that is important for hybrid seed production.[49]

Additionally, this technique has been applied to disease resistance, where asymmetric somatic hybridization was used to transfer bacterial blight resistance from a wild rice species, *Oryza meyeriana*, to a cultivated rice species, *Oryza sativa*. This approach opens up opportunities to develop crops with improved resistance to pests and diseases without relying on chemical treatments.

Overall, somatic hybridization is a powerful tool in plant breeding, allowing scientists to create plants with improved characteristics, such as better disease resistance, stress tolerance, or desirable agronomic traits, by merging the genetic potential of different species in ways that would not occur naturally.

#### Molecular Marker-Aided Genetic Analysis and Selection

Molecular marker-aided genetic analysis plays a crucial role in identifying genes and understanding the genetic makeup of organisms. It involves studying DNA sequences to pinpoint genes, Quantitative Trait Loci (QTLs), and molecular markers, and linking them to specific traits or characteristics in plants or animals. This analysis helps researchers uncover how certain traits are inherited and how genetic variation influences traits like disease resistance or yield.

Molecular marker-assisted selection (MAS) helps track and select specific DNA markers across generations, making it easier to identify desirable traits in plants or animals. By using molecular markers, breeders can select individuals with the best genetic traits without waiting for the traits to appear physically, which speeds up the breeding process.[37]

Marker-assisted breeding combines molecular markers with linkage maps and genomic information to enhance plants and animals with specific traits [50]. For example, scientists have used MAS to identify rice genotypes resistant to Bacterial Blight (BB) and those with Basmati quality traits. These markers help select the best candidates for developing new commercial varieties, as well as identifying potential parents for Basmati breeding programs to introduce disease resistance[51].

In coffee, MAS has been used to identify genetic sources of resistance to Coffee Berry Disease and Coffee Rust, two major diseases that impact coffee production. By using genes from different *Coffea* species, researchers have been able to pyramid (combine) these resistance genes into breeding programs, helping create coffee plants with durable resistance to these diseases.

In wheat breeding, genetic analysis has been applied to study Fusarium Head Blight (FHB) resistance. A study on CIMMYT's bread wheat line C615 used both traditional breeding methods and advanced QTL mapping to understand the genetic basis of FHB resistance.[52] The findings provided valuable insights into the relationship between FHB resistance and related traits, guiding marker-assisted selection in wheat breeding programs to improve disease resistance over time.

Through molecular marker-aided selection, scientists can more efficiently enhance desirable traits in crops and livestock, leading to better yields, improved disease resistance, and higher quality products.[53] This technology is transforming breeding practices and helping to create more resilient and productive agricultural systems.

#### CRISPR/Cas-mediated gene enhancement

Modern crop production depends heavily on the precise and effective change of plant DNA made possible by the potent gene-editing technology CRISPR-Cas. It makes it possible to create crops with better qualities including increased yields, disease and pest resistance, and drought or extreme weather tolerance. Additionally, this approach lowers the demand for chemical inputs like fertilizers and pesticides while improving the nutritional value of food. CRISPR-Cas promotes sustainable agriculture and worldwide food security by accelerating the breeding process and producing more hardy crops.

#### Doubled Haploid / Genome Doubling

A doubled haploid (DH) is a special type of plant that results from the doubling of the chromosome number in a haploid cell. Haploid cells, like pollen or egg cells, typically contain only one set of chromosomes. When these cells undergo chromosome doubling, they become doubled haploid cells [54]. These cells are then grown into plants that are genetically uniform and pure, which can accelerate the development of inbred lines or pure-line varieties compared to traditional breeding methods [55].

One of the key advantages of DH technology is its ability to speed up breeding programs. For example, in wheat breeding, DH technology has helped accelerate genetic gains in both yield and disease resistance, allowing for faster development of new wheat varieties.[56][57] This technology reduces the time it takes to bring new crop varieties to market, which is crucial for meeting growing food demands in the face of climate change.

In crops like rice, DH plants created through techniques such as anther-culture offer a fast and efficient way to produce homozygous lines, which are more stable and have better performance than other types of lines.[58] Similarly, in a study conducted by Bakhshi et al. in 2017, researchers used chromosome elimination methods to create DH wheat lines by crossing wheat with maize as the male parent [59]. These wheat lines were further developed to perform well under heat stress conditions, showing the potential for DH technology to help crops adapt to changing environmental factors.[60]

### 'Omics' Technologies

'Omics' technologies are a set of advanced tools in bioinformatics that include genomics, proteomics, transcriptomics, metabolomics, and phenomics. These technologies help scientists study various aspects of an organism at a molecular level, providing deep insights into the structure, function, and evolution of genes, proteins, and other biological molecules.

Genomics focuses on the DNA of organisms and helps identify the genes responsible for specific traits.

Proteomics analyses the proteins in tissues to understand gene expression and the specific functions of proteins.

Transcriptomics looks at the RNA molecules that are transcribed from genes, providing clues about gene activity in different tissues.

Metabolomics studies the metabolites produced by plants and helps identify the chemical pathways that drive plant growth and development.

By combining these approaches, scientists can gain a comprehensive view of plant molecular responses and develop more targeted strategies for improving crop traits. For example, herbicide-tolerant maize has been developed by precisely inserting a target gene through site-directed mutagenesis, a technique made possible by these 'omics' technologies.[60] This precision helps breed crops with specific traits more efficiently, contributing to better yields, disease resistance, and adaptability to environmental changes.[61] In summary, DH technology and omics approaches are reshaping crop breeding, making it possible to develop more resilient and high-yielding plants in less time, which is essential for global food security.[62][63]

### Concerns of Agricultural Biotechnology

In 2019, biotech crops were grown in 29 countries, playing a significant role in improving food security, supporting sustainable farming practices, addressing climate change, and enhancing the livelihoods of farmers and their communities worldwide. However, there are some concerns related to the genetic modification of crops, particularly regarding their potential ecological impact and safety for human consumption.[64] These concerns have sparked ongoing debates. Below, we highlight some of the key issues surrounding agricultural biotechnology:

#### Adverse Effects on Non-Target Organisms

While transgenic crops are designed for specific purposes like disease or pest resistance, they can sometimes have unintended consequences for non-target organisms. For example, the introduction of glyphosate-resistant transgenic crops in the U.S. and Mexico has been linked to a decline in monarch butterfly populations.[65] Studies showed that monarch larvae had a higher mortality rate when feeding on milkweed leaves that were dusted with genetically modified Bt maize, compared to laboratory conditions. Similarly, in China, the widespread use of Bt cotton initially reduced pest populations, but eventually, it led to an increase in the population of a minor pest, the Mirid bug, which later became a major pest itself.[66][67]

#### Biosafety Issues

Concerns have been raised about the safety of transgenic foods, both for human health and the environment. Health risks include potential allergenicity, toxicity, and the possibility of horizontal gene transfer. For instance, when genes were introduced to bean crops to increase levels of cysteine and methionine, the modified beans were discarded after it was found that the expressed protein triggered allergic reactions. Similarly, the World Health Organization (WHO) has highlighted the possibility that genetic material from transgenic foods could be transferred to human cells, intestinal bacteria, or soil microbes, since not all ingested DNA is completely broken down during digestion. There is also a potential concern about the transfer of antibiotic-resistant marker genes from transgenic foods to gut microbes, though the likelihood of this occurring is very low.[68][69] Moreover, the widespread cultivation of genetically modified crops could lead to "genetic erosion," where farmers focus on a few popular varieties, reducing genetic diversity. This, in turn, could lead to unpredictable ecological changes, including the resurgence of pests and the emergence of "superweeds."

#### Resistance Breakdown

The large-scale cultivation of insect-resistant and herbicide-tolerant crops increases the likelihood that targeted pests will develop resistance over time. This high selection pressure may result in the evolution of new pest biotypes that are resistant to the transgenic technology. For instance, in Brazil, there have been reports of resistance in Fall armyworms (*Spodoptera frugiperda*) to Bt maize and Bt soybeans. In China, cotton bollworm resistance to Bt cotton has also been observed. The development of herbicide-resistant "superweeds" is another concern, as these weeds can adapt to the herbicides used with genetically modified crops, further complicating weed management.[70]

#### Economic, Social, and Political Concerns

Economically, the high cost of genetically modified seeds could make them unaffordable for small farmers, especially those in developing countries. There is also concern that rapid technological advancements in farming could negatively impact rural communities and farm structures. Politically, one of the major debates surrounding GM crops is whether or not to label genetically modified foods.[71] For example, in the U.S., GM foods are not labeled, while many other countries, particularly in the European Union, have stricter regulations requiring labeling.[68] This discrepancy reflects differences in consumer preferences and concerns, making the issue of labeling a significant point of contention in the global discussion on agricultural biotechnology.

#### Conclusion

Agriculture has evolved significantly, transitioning from the Green Revolution to the Gene Revolution, and continues to advance rapidly. With the power of biotechnology, we can now better understand and modify the genetic makeup of plants and animals. This ability helps us meet the growing demand for food by developing new crop varieties that are higher-yielding, more resilient to environmental stresses, and better able to withstand pests and diseases, all while promoting sustainability. Biotechnology has not only boosted crop productivity but also reduced production costs by minimizing the need for pesticides, ultimately improving farmers' livelihoods.

In addition, biotechnology has enabled the creation of plant varieties that are adaptable to different environments, require fewer resources, and contribute to better crop rotation practices, conserving natural resources. However, as these advancements continue, there are ongoing concerns about the safety of genetically modified (GM) crops, including potential risks to human health, food security, and the environment. Social, economic, and political issues also remain points of discussion. It's essential that the use of GM crops is fully and transparently assessed, with robust regulations in place to ensure their safe application. Furthermore, emerging techniques like cisgenesis, intragenesis, and genome editing offer alternative methods for developing improved crops in the future.

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