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7. Genetically Modified Crops

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Abstract:

In agriculture, recombinant DNA technology based genetically modified crops i.e. the GM crops have been a subject of intense global debate. Even though GM crops provide promising solutions in agricultural challenges, still the concerns persist due to absence of robust safety evidence and consumer acceptance in many cases. However, after meeting the regulatory requirements, the potential of GM crops to bolster food security for a rapidly growing global population cannot be ignored. This chapter provides a comprehensive exploration of GM crops, spanning from their historical origins to the contemporary landscape. Methods for creating GM crops and strategies for eliminating selectable marker genes are discussed in detail, highlighting the importance marker free transgenics. This chapter acknowledges both the advantages (e.g., increased yields, pest resistance) and potential concerns (e.g., environmental impact) surrounding GM crops. It also examines the regulatory frameworks in place to ensure the safe and responsible deployment of this technology for field cultivation of GM crops. Lastly, current scenario of GM crops, both globally and within the Indian context have been summarized.

Keywords:

Genetically modified organisms, recombinant DNA technology, transgenics, GEAC, Bt-cotton, Agrobacterium tumefaciens.

7.1 Introduction:

Genetically modified (GM) crops, also known as transgenic crops are developed through genetic modification, a process where genes with desired traits from one organism are inserted into another to introduce or enhance desirable traits or suppress undesirable ones. This technique, also termed as genetic engineering or recombinant DNA technology, may involve genes from related or unrelated species. The history of biotechnology in agriculture dates back centuries, with farmers striving to enhance crop productivity and resilience through selective breeding and the application of genetic principles. Modern biotechnology represents the latest phase in this evolution, utilizing tools such as genetic engineering to transfer genes without sexual crossing. Unlike conventional breeding, where specific genes controlling traits may not be identified, genetic engineering allows for the targeted transfer of well-characterized genes.

The journey of genetic engineering began with foundational discoveries in molecular biology, including the elucidation of DNA's role as the carrier of genetic information and the revelation of its structure. Breakthroughs in the 1960s and 1970s, such as restriction endonucleases and DNA ligase discovery, paved the way for genetic manipulation using recombinant DNA technology. Paul Berg's experiment in 1972, combining DNA from different viruses, marked the beginning of genetic engineering, led to the first GM organism (GMO) in 1973.

7.2 Historical Aspects:

The Avery-MacLeod-McCarty experiment of 1944 conclusively demonstrated that DNA serves as the biochemical carrier of genetic information. Subsequently, in 1951, the elucidation of the DNA structure was published, a pivotal moment as structure inherently governs the function. This comprehension of DNA's structure paved the way for its manipulation, although the requisite tools remained elusive. During the 1960s and 1970s, restriction endonucleases, discovered by Arber, Smith, Nathans, and Danna, revolutionized the field of DNA manipulation by enabling the specific cleavage at sequences with restriction sites. Complementing this discovery, Gellert, Lehman, Richardson, and Hurwitz (1967) identified DNA ligase, an enzyme that facilitates the joining of DNA fragments, a crucial step in recombinant DNA technology (Shuman, 2009).

The term "Genetic Engineering" was originally introduced by Nikolay Timofeev-Ressovsky (1934) in his paper titled “The Experimental Production of Mutations”. However, it wasn't until nearly 35 years later, when Paul Berg (1972), with the help of cutting and pasting enzymes, combined DNA from two virus (SV40 and lambda phase) to engineer a recombinant DNA, thus, opening the doors for recombinant DNA technology. The beginning of GMOs occurred in 1973 when Herbert Boyer and Stanley Cohen successfully inserted an antibiotic-resistant gene into the plasmid of *Escherichia coli*, thereby transforming it from an antibiotic-susceptible to a resistant strain.

In animals, first transgenic mouse was created by Rudolf Jaenisch (1974) through introduction of a foreign DNA into mouse embryo. During this period, experiments involving genetic modifications proceeded without regulatory oversight. To address concerns regarding biosafety and potential hazards, as well as to establish regulatory guidelines, Paul Berg organised the Asilomar conference (1975) in California.

This landmark meeting brought together researchers, journalists, public and various attendees from diverse groups. In 1976, the first company “Genentech” dedicated to genetic engineering was founded by Herbert Boyer and Robert Swanson. Genentech successfully employed genetically modified *E. coli* to produce human somatostatin in 1977, followed by the production of human insulin in 1978 (Rangel, 2015).

The advent of totipotency, enabling the generation of complete plants from any living plant cell, coupled with the utilization of Ti plasmids for transformation, significantly enhanced the feasibility of genetic engineering in plants. Among agricultural crops, the first genetically engineered plants emerged in 1983, with GM tobacco and GM petunia exhibiting resistance to antibiotics.

China became a pioneer in the commercialization of GM crops with virus-resistant tobacco in 1990. The landscape shifted towards consumer products with the 1994 approval of the Flavr Savr tomato (Calgene, USA), the first FDA-approved GM food plant.

This tomato employed anti-sense RNA technology to suppress the production of the polygalacturonase enzyme, leading to delayed ripening and extended shelf life. In 2002, *Bt* cotton was approved by Genetic Engineering Approval Committee for commercial cultivation in India (Raman, 2017).

7.3 Strategies for Production of GM Crop Plants:

Approaches for creating genetically modified plants includes Agrobacterium mediated transformation, virus mediated transformation, gene gun method, in planta transformation, electroporation, microinjection, liposome mediated gene transfer etc. Agrobacterium mediated and gene gun are the most followed strategies for genetic transformation which are discussed as follows.

7.3.1 Agrobacterium Mediated Transformation:

Agrobacterium tumefaciens is a gram-negative, soil-borne bacterium known for its pathogenicity in plants, causing crown gall disease. This bacterium possesses a tumor-inducing plasmid (Ti plasmid) that contains the T-DNA with three open reading frames for auxin, cytokinin and opine synthesis spanning between the left and right border. The Ti plasmid also contains crucial other regions including the virulence region, opine catabolism region, and an origin of replication (Figure 7.1).

The virulence region and the right border are most critical for transfer of the T-DNA into host via type 4 secretion system. *Agrobacterium tumefaciens* is also known as natural genetic engineering and to harness its Ti-plasmid for creating transgenic plants, several hurdles must be addressed. Initially, the bacterium's oncogenes are removed from the wild-type T-DNA in Ti-plasmid (disarmed Ti-plasmid) to render it non-pathogenic while preserving its ability to transfer T-DNA.

Subsequently, the gene of interest and selectable markers for transgenic plants are incorporated into the disarmed Ti-plasmid, necessitating molecular biology techniques for DNA cloning in vitro. Due to the large size and typically low-copy number of the Ti plasmid, isolating and cloning it presents significant challenges.

To address this, many research groups employ a binary vector system. This system involves carrying the T-DNA region on a broad-host range replicon due to multiple *ori* sequence, while the *vir* genes for T-DNA transfer reside on the disarmed Ti-plasmid (called the helper vector).

The adoption of this binary vector system has immensely enhanced versatility and flexibility within the plant research community, leading to a notable increase in transgenic plant production (Hwang *et al.*, 2017).

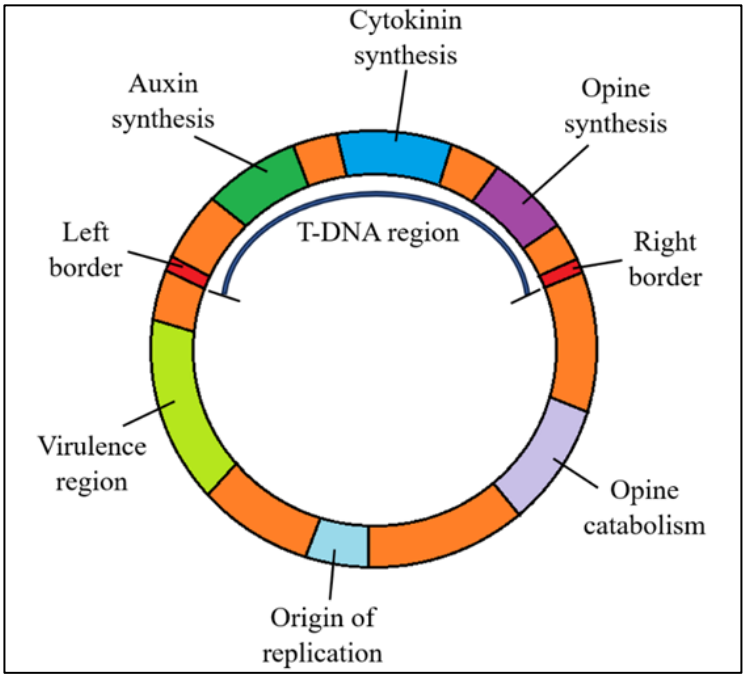

Figure 7.1: A schematic diagram of Agrobacterium tumefaciens Ti- plasmid

7.3.2 Gene Gun Method:

Beyond Agrobacterium-mediated transformation, which is predominantly suited for dicotyledonous plants due to host specificity, physical methods resolve this issue among which the gene gun method is the most followed. The method possesses several synonyms, such as particle bombardment, biolistic, and microprojectile bombardment. In this technique, the gene of interest (GOI) is coated onto microcarriers ranging from 0.6 to 2 μm in size, typically composed of gold, tungsten, or platinum. Following coating, the microprojectiles are propelled at high velocities into the explant using either gunpowder or a helium gas-driven pressure chamber. Microprojectiles that successfully penetrate the nucleus release the GOI, facilitating its uptake and potential integration into the host genome. Gene gun method proves particularly advantageous for studies involving organelle genome transformation, transient gene expression analysis, and co-transformation experiments (Gantait *et al.*, 2022).

7.4 Selectable Marker Gene (SMG) and SMG Free Transgenics:

In molecular biology and genetic engineering, a selectable marker is a gene that is inserted into a cell or organism alongside the intended gene for identification and selection of cells that have effectively incorporated the desired gene.

Selectable marker gene (SMG) are commonly employed in methods involving transfection, transduction, or transformation to make it easier to identify and separate the cells with integrated desired gene into their genome.

A. Types of SMGs:

SMGs are grouped into three categories i.e. antibiotic resistance genes, antimetabolite marker genes and herbicide resistance genes (Miki *et al.*, 2009)

a. Antibiotic Resistance Genes: Genes resistant to antibiotics, especially those found in *E. coli*, are utilized as SMG such as the *npt II* gene, which codes neomycin phospho-transferase II enzyme. Kanamycin resistance is conferred by this marker gene.

b. Antimetabolite Marker Genes: Dihydrofolate reductase enzyme is encoded by a mutant mouse *dhfr* gene. It has a poor affinity for methotrexate, when fused with cauliflower mosaic virus 35S promoter, thus a methotrexate-resistant marker is produced that is used to identify transformed plants.

c. Herbicide Resistance Markers: Eg: Enolpyruvylshikimate phosphate synthase (*epsps*/*aroA* genes). The genes *epsps*/*aroA* confer resistance to the herbicide "glyphosate."

B. Why to Eliminate Selectable Markers in GM Crops:

In many jurisdictions, regulatory bodies have strict guidelines regarding the presence of SMGs in GMOs intended for commercial release. The presence of SMGs, especially those conferring antibiotic resistance, can raise concerns among consumers about potential health risks upon consumption of genetically modified foods. There are concerns about the environmental impact of GMOs containing selectable markers, particularly the potential for horizontal gene transfer to wild or non-target organisms. While SMGs are useful for the initial selection of transformed cells, their continued presence in GMOs may pose biosafety risks (Miki and McHugh, 2004)

C. Strategies to Obtain Marker Free Transgenics:

Several approaches have been developed for generating marker-free transgenic organisms, including plants (Puchta, 2003).

a. Recombinase-Mediated Excision: This approach involves the use of site-specific recombinases, such as Cre-lox or FLP-FRT systems, to catalyze the excision of SMGs from the transgenic organism's genome after transformation. Recombinase recognition sites flanking the SMG allow for precise removal. This method leaves behind only the gene of interest (GOI) integrated in genome. This approach has been followed to remove kanamycin resistance gene from GM tobacco creation using Cre-lox site and Cre recombinase enzyme (Dale and Ow, 1991).

b. Selectable Marker Recycling: In this approach, the selectable marker gene is flanked by direct repeats of a recombinase recognition site.
After successful transformation and selection, a recombinase mediates recombination between the repeats, excising the selectable marker gene. This leaves behind a single recombinase recognition site, which can be reused for subsequent rounds of transformation.

c. Co-transformation with Unlinked Selectable Marker: Instead of integrating the SMG at the same locus as the GOI, this method involves co-transforming the organism

with two separate DNA constructs: one containing the GOI and another with SMG. After selection for transformed cells, subsequent breeding or segregation will be able to generate of marker-free progeny (Daley *et al*., 1998).

d. Positive Selection Systems: Positive selection systems utilize a positive selection marker that allows the identification of transgenic cells or organisms without relying on selectable marker genes. Examples include the use of reporter genes such as GFP (Green Fluorescent Protein) or GUS (β-glucuronidase), which can be visualized or detected through enzymatic assays, respectively. Positive selection systems enable the identification of transgenic cells based on the expression of the reporter gene rather than the presence of a selectable marker (Zuo *et al.*, 2002).

e. Homologous Recombination-Mediated Gene Targeting: Homologous recombination is used for precise integration of the transgene into a specific genomic locus without the need for selectable markers. This approach relies on the introduction of DNA sequences homologous to the target locus, facilitating the precise insertion of the transgene through homologous recombination. Selectable markers are not required because only cells with the desired genomic modification will survive the selection process (Tuteja *et al*., 2012).

f. Transposition: This approach utilizes mobile DNA segments (transposable elements) to detach the desired gene or the SMG from each other after the initial transformation and selection process. Both methods have proven effective. In the first approach, the selection marker hitches on mobile element and jumps out after it function for selection is done. Alternatively, the desired gene itself can be used to relocate into a new location, separating it from the original locus where it was inserted along with the selection marker (Gorbunova and Levy, 2000).

These approaches offer strategies for creating transgenic organisms leaving behind selectable marker genes, addressing concerns related to biosafety, regulatory approval, and public acceptance of GM crops.

7.5 Rules and regulations for GM crops in India:

India employs a comprehensive regulatory framework for Genetically Modified Organisms (GMOs) and their products. The cornerstone of this system is the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989" (often abbreviated as Rules 1989).

Established under the Environment (Protection) Act, 1986, these regulations govern a broad spectrum of activities related to GMOs including research, development, production, processing, storage, packaging, transportation, sale, import, and export.

The Ministry of Environment, Forest and Climate Change (MoEFCC), the Department of Biotechnology (DBT) under the Ministry of Science & Technology, Government of India, and respective State Governments are entrusted with overseeing the implementation of these rules.

The Rules 1989 designate six competent authorities, each assigned specific responsibilities and compositions for effective administration (Table 7.1).

Table 7.1: Competent authorities and their roles as per Rule 1989

Committee	Functions
RDAC (Recombinant DNA Advisory Committee)	Reviews advancements in biotechnology and propose suitable safety protocols for the research, utilization, and applications of recombinant DNA technology.
IBSC (Institutional Biosafety Committee)	Tasked with ensuring strict adherence to safety protocols during experiments conducted at specified institutional sites.
RCGM (Review Committee on Genetic Manipulation)	Oversee projects related with genetic modification, approval of experiments categorized as risk level III and higher, and develop manuals outlining protocols for GMO research and utilization.
GEAC (Genetic Engineering Appraisal Committee)	Review, monitor, and authorize all actions related to the import, export, transportation, manufacturing, utilization, or sale of GMOs and their derivative products, with a focus on environmental considerations.
SBCC (State Biotechnology Coordination committee)	State level monitoring and supervision
DLC (District Level Committee)	District-level supervision and adherence

RCGM and GEAC establish specialized sub-committees and expert panels as per the requirement which consist of experts from diverse fields within public sector institutions to develop and assess guidelines and biosafety. Furthermore, case-specific Central Compliance Committees (CCC) can be formed to provide additional oversight during confined field trials of regulated genetically engineered plants. The specific approval process for these trials and the release of GM crop plants is detailed in Figure 7.2.

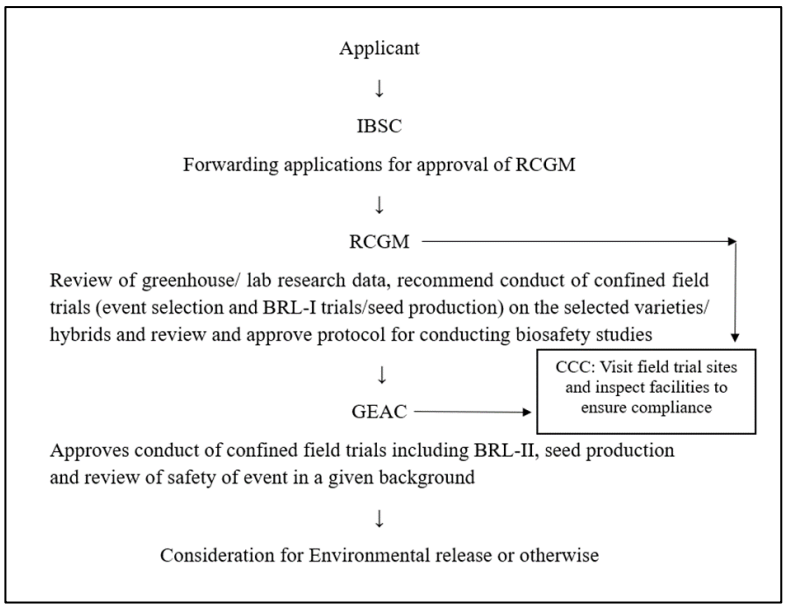

Figure 7.2: Protocol of approval process for confined field trials and GM crop release

The initial framework for regulating food products derived from biotechnology was established under the Rules 1989, specifically Rule 11. This rule mandated approval from the GEAC before producing, selling, importing, or using any food item, ingredient, additive, or processing aid containing GMOs or cells. However, a significant shift occurred in 2006 with the implementation of the Food Safety and Standards Act (FSSA).

This act encompassed GM foods within its broader definition of "food." The Food Safety and Standards Act (FSSA) establishes a comprehensive framework to ensure the safety and quality of food products in India.

This act restricts the manufacturing, distribution, sale, or import of certain food categories unless specifically permitted by the FSSA or its regulations. The definition of "genetically engineered or modified food" under the FSSA is broad.

With the inclusion of "genetically modified or engineered food" within the broader definition of "food" under the Food Safety and Standards Act (FSSA) of 2006, the Food Safety and Standards Authority of India (FSSAI) taken the responsibility of overseeing these products.

In line with this legislative change, the FSSAI has established a dedicated scientific panel specifically for evaluating and regulating Genetically Modified Organisms (GMOs) in food items.

7.5.1 Advantages of GM Crops:

Genetically modified organisms (GMOs) are organisms that have been altered to contain new DNA sequences and in case of crop plants, novel genetic traits in GM crops have several potential advantages. They provide various benefits, including increased yield, enhanced quality, resistance to diseases and pests, and other valuable characteristics. GM crops offer both direct and indirect advantages, including:

- **Improving yield:** Boosting plant yields face challenges from various biotic and abiotic factors. Therefore, GM technology emerges as a promising strategy for addressing these limitations and enhancing crop productivity.
- **Biotic stress resistance:** Recombinant DNA technology plays a pivotal role in enhancing yields and minimizing chemical usage through the creation of resistant and tolerant crop varieties. This results in substantial reductions in environmental pollution. An illustrative example of this is *Bacillus thuringiensis* (Bt) plants, which contain the *Cry* gene, enabling them to produce toxins that effectively combat insects without the need for pesticides (Meftaul *et al.*, 2020).
- **Abiotic stress tolerance:** In addition to addressing biotic stresses, GM crops have also been engineered to withstand various abiotic stresses such as salt, cold, drought, and heavy metals. For instance, soybeans have been genetically modified to tolerate high salt levels, while tomatoes have been enhanced for cold stress tolerance (Uslu, 2021).
- GM crops have been tailored to elevate their nutritional value, exemplified by the creation of golden rice enriched with substantial amounts of vitamin A, and have played a crucial role in enhancing the phytochemical composition and biological functionalities of plants.

This includes augmenting the levels of phenolic compounds, triterpenes, saponins, flavonoids, and other antioxidants, resulting in heightened antimicrobial properties and resistance to insect pests (Ghimire *et al*., 2023).

- GM crops have also supplemented in improving crop quality such as increased shelf life, improved taste, and fruit texture.
- Additional benefits encompass the elimination of allergens, phytoremediation through the expression of hyperaccumulation traits, and the industrial-scale production of vaccines and biofuels (Ghimire *et al.*, 2023).

7.5.2 Concerns with GM Crops:

The production and use of GM crops in commercial markets raised concerns about human health, the environment, and the threat to genetic diversity as well. Concerns surrounding human health risks related to GM crops involve potential adverse effects such as the unintended consequences of inserted foreign DNA, toxicity associated with inserted genes, and worries regarding the allergenic traits. Effects attributed to inserted genes encompass gene silencing, unintended genomic alterations, and gene overexpression (Conner and Jacobs, 1999). The worry over the accumulation of toxic chemicals resulting from herbicide-tolerant GM crop was initially more of a perception than an established issue.

Concerns regarding the allergenic potential of marker genes, such as the antibiotic resistance gene and green fluorescent protein gene, were prevalent. Allergies to GM foods were a significant public concern.

To assess allergenic reactions, both GM and non-GM foods were tested, revealing the possibility of GM crops acquiring allergenic traits through novel gene transfers.

The environmental impacts of GM crops encompass concerns such as the potential threat to genetic diversity, as newly developed GM varieties might become invasive over time. Additionally, there's the risk of crossbreeding between GM crops and their closely related species, which could result in the transfer of transgenes from GM crops to other plants however it could take years to happen. Additional potential risks entail the emergence of superbugs, such as *Bt*-resistant bollworms, and superweeds, characterized by herbicide tolerant weeds. Concerns also extend to the impact on non-target organisms, which may experience lethality. Indirect consequences of herbicide-tolerant plants may include the widespread use of herbicides and subsequent accumulation in the environment.

Economic apprehensions arise regarding the widespread cultivation and commercialization of GM crops, potentially impacting export markets and the organic status of crops. Furthermore, concerns encompass religious beliefs, sociocultural acceptance of GM crops, and various political considerations (Uslu, 2021).

7.5.3 Current Scenario of GMO Crops (ISAAA, 2018):

- As of 2018, over 17 million farmers in 29 countries worldwide engaged in the cultivation of various GM crops, contributing to a global market valued at US$18.2 billion.

- Predominantly, the USA, Brazil, Argentina, Canada, and India collectively account for 91% of the global GM crop cultivation.
- Currently, the total area dedicated to GMOs stands at 190.4 million hectares.
- Major GM crops cultivated globally include soybean (95.9 million hectares, approximately 50% of the transgenic area), maize (58.9 million hectares, around 31% of the transgenic area), cotton (24.9 million hectares, constituting about 13% of the area), and canola (10.1 million hectares, making up approximately 5.3% of the area).
- There have been 32 plant varieties of GMO crops released for commercial cultivation, with key traits including herbicide tolerance (47% of the total area), insect resistance, disease resistance, abiotic stress tolerance, and nutritional enhancement.
- In India, the states of Gujarat, Maharashtra, and Telangana have received approval from GEAC for testing transgenic cotton containing the *Cry* gene.
- While transgenic brinjal, tomato, maize, and chickpea are undergoing different trials, cotton remains the only crop under large-scale commercial cultivation.
- In 2010, GM brinjal received approval from GEAC for testing and release but was subsequently placed under an "indefinite moratorium" by Government of India.
- More recently, GM mustard DMH-11 and its parental lines obtained approval for environmental release, seed production, and testing on October 18, 2022.

7.6 Conclusion:

GM crops represent a significant advancement in agricultural technology, offering the potential to address challenges related to food security, environmental sustainability, and crop resilience. By harnessing the power of biotechnology, scientists have been able to introduce desirable traits into crops through the creation of GM crops. These crops are engineered to exhibit characteristics such as resistance to pests and diseases, tolerance to herbicides, and enhanced nutritional profiles, thereby addressing the growing demands of an expanding population and changing environmental conditions. While GM crops hold immense potential for addressing key agricultural and societal needs, they also evoke significant concerns. One major concern revolves around the presence of selectable marker genes (SMGs) in GM crops, particularly those conferring antibiotic resistance. The use of SMGs has raised apprehensions among regulatory bodies and consumers regarding potential health risks associated with consuming GM foods, as well as environmental implications such as horizontal gene transfer to non-target organisms.

Despite these concerns, the adoption of GM crops has proliferated globally, driven by their perceived benefits and contributions to agricultural productivity. However, as the cultivation and consumption of GM crops continue to increase, there is a growing emphasis on addressing biosafety considerations and regulatory requirements to ensure the safety and sustainability of GM foods. This necessitates understanding of the advantages and concerns associated with GM crops, along with concerted efforts to develop strategies that mitigate risks while harnessing the potential of genetic engineering in agriculture.

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