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BIOPHARMING: CULTIVATING A NEW FRONTIER IN BIOTECHNOLOGY: A BRIEF OVERVIEW OF REGULATORY ASPECTS

PAVANI S, PRASANTI NL, VIDYADHARI KSL AND VENKATESWARA RAJU K*

Department of Regulatory Affairs, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram -534202, Andhra Pradesh, India

***Corresponding Author: Dr. K. Venkateswara Raju: E Mail: venkateswararaju.k@svcp.edu.in**

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ABSTRACT

The practice of utilizing plants commonly found in agricultural settings to produce beneficial proteins or metabolites for industrial or medical purposes is referred to as "Biopharming" or "Plant Molecular Farming." This approach leverages plant-based methods of heterologous protein expression to enable the large-scale synthesis of recombinant proteins with therapeutic value. Biopharming, which originated in 1997, focuses on the production of economically viable and pharmaceutically relevant proteins within plants. This article delves into the historical background of biopharming, tracing its emergence and the subsequent introduction of its products into the market. Additionally, it explores the production of edible vaccines and their significance. Furthermore, the article sheds light on the regulations governing biopharming in leading countries such as India and the USA.

Keywords: Biopharming, Edible vaccines, Protien expressioin, Metabolites, Biopharm pharmaceuticals

INTRODUCTION

Plants offer great potential in the production of complex therapeutic proteins, including monoclonal antibodies, making them a promising avenue for the treatment of human diseases. In fact, plants have been a significant source of pharmaceuticals for many years, and a considerable number of widely-used drugs (around 57 out of the top 150) contain essential active ingredients derived from plants (Grifo *et al*., 1997) **[1]**. Plant molecular farming (PMF), also known as bio-pharming, has emerged as an advanced agricultural biotechnology approach that enables the production of

medicines using plants as hosts. Bio-pharming involves using living organisms as bio-factories to produce biologic drugs externally from the human body. This method allows for the production of complex biologics on a scale that may not be achievable through existing in vitro synthesis techniques, both technically and economically. Extensive research conducted over the past two decades has demonstrated the effectiveness of plant molecular pharming in producing medically significant proteins. Notably, molecular pharming technologies have shown the potential to produce medicines that outperform their natural counterparts in terms of clinical outcomes.

Plants provide excellent molecular pharming platforms, offering various host systems such as transgenic plants, cell suspension cultures, hairy roots, and hydroponic cultures for the production of drugs, antibodies, and immunizations. Researchers have investigated numerous plant species for their ability to synthesize bio-active compounds. The Canadian Food Inspection Agency (CFIA) defines PMF as "the use of plants in agriculture to produce bio-molecules rather than food, feed, and fiber." By cultivating and selecting crops with genetically introduced traits, plants are utilized to generate bio-molecules with scientific, medicinal, or commercial importance. Throughout history, medicines have heavily relied on compounds derived from plants **[2]**.

Advantages of plant-based biopharming

- Low cost and rapid scalability
- Low manufacturing cost
- The capacity to generate innovative and intricate molecules
- Promotes safety by minimizing the risk of contamination with animal and/or human pathogens.
- Enables high-yield production of desired products.
- Offers a cost-effective alternative to expensive bioreactors.
- Utilizes the biochemical versatility of plants, which exceeds that of animals.
- Provides ecological benefits such as soil nutrient enrichment, improved soil fertility, and reduction of soil erosion.
- Helps in controlling the concentration of pests and diseases.
- Offers improved physiological compatibility for various applications.

The Emergence of plant-made pharmaceuticals

In the early stages of plant-made pharmaceutical research, the focus was primarily on utilizing food crops like corn and rice to develop cost-effective methods of delivering vaccines. Dr. J. Christopher Hall, a prominent Canadian scientist, played a crucial role in advancing plant-based technology for antibody medicine production during the early 1990s. Hall,

who held the Canadian Research Chair in Recombinant Antibody Technology at the University of Guelph from 2002 to 2014, and his team were instrumental in the development of vivoXPRESS®, a plant-based biopharmaceutical technology. This groundbreaking technology has been licensed to PlantForm Corporation, an organization co-founded by Hall in 20083. Advancements in genetic engineering have enabled the production of various types of antibodies, protein-based medicines, and vaccines using plants and plant cell systems. These products are commonly known as "plant-made pharmaceuticals" (PMPs). One notable example is Elelyso (taliglucerase alfa), which was the first PMP authorized for human use. It is an enzyme produced in carrot cells by Protalix Biotherapeutics Inc., an Israeli company (commercial rights were sold to Pfizer in 2015). In 2012, Elelyso received FDA approval for the treatment of Gaucher's disease.

The Evolution Biopharm Medicines

1982 - The first recombinant protein, insulin, is produced using E. coli bacteria.

1986 - Chimeric human growth hormone is generated using transgenic tobacco and sunflower.

1989 - The first plant-made monoclonal antibody, Orthoclone OKT3® (muromonab-CD3), is developed.

1990 - Human serum albumin becomes the first plant-derived pharmaceutical protein, produced using transgenic tobacco and potato plants.

1997 - Avidin is reported as the first protein used for extraction and purification.

1998 - Human Tissue Plasminogen Activator, an animal-derived transgenic protein, is produced in the milk of mice.

1999 - Aprotinin becomes the first molecularly farmed pharmaceutical protein.

2003 - Trypzean becomes the first large-scale transgenic plant product.

2006 - Dow AgroSciences develops the world's first licensed injectable plant-made vaccine for Newcastle disease, using tobacco plants.

2008 - Plant Form Corporation is established, introducing an innovative manufacturing platform for producing therapeutic biologics and biosimilar versions of approved antibody medicines.

2009 - The first licensing of an animal-made pharmaceutical anticoagulant, antithrombin, is achieved using genetically engineered goats.

2012 - Elelyso, manufactured in carrot cells, becomes the first plant cell-based enzyme replacement therapy approved by the FDA for treating Gaucher disease. It is developed by Protalix Bio-Therapeutics and Pfizer.

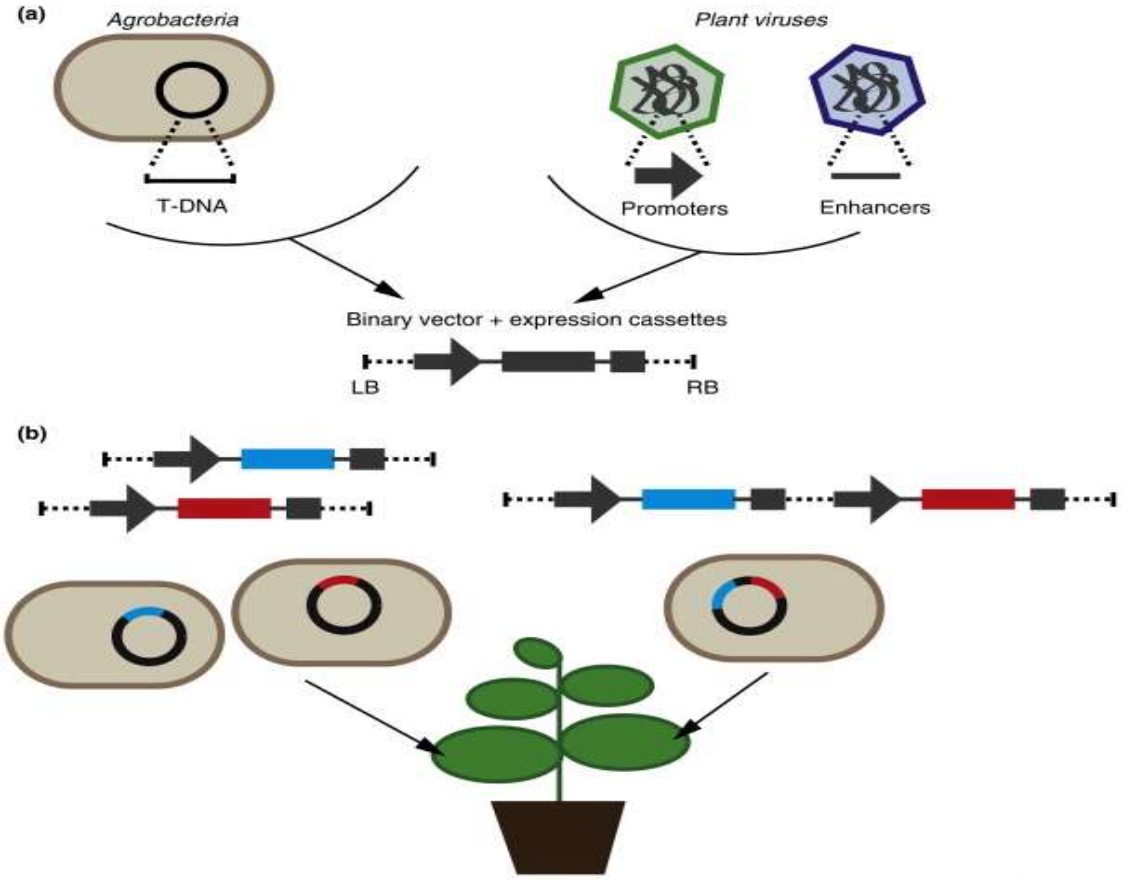
2014 - Zmapp, a highly successful vaccine for the Ebola virus, is produced using tobacco plants.

2015 - Transgenic plants are utilized to produce a recombinant monoclonal

antibody for rabies post-exposure prophylaxis.

PMF production platform

The production of desired recombinant proteins in plants that have undergone stable transformation can be challenging due to random insertion and the need to identify suitable promoters for achieving high-level expression of foreign genes. This often leads to low yields of the target protein. However, in situations such as outbreaks of viral diseases like Ebola, severe acute respiratory syndrome (SARS), or MERS-CoV, conventional plant molecular farming strategies may not be feasible due to time constraints. To overcome this challenge, transient expression systems have emerged as an alternative approach, allowing for the rapid production of recombinant proteins within a shorter timeframe of three to five days. These systems offer a faster production method compared to stable transformation. In transient expression, various viral vectors have been developed for small- or medium-scale production of plant-made pharmaceuticals (PMPs) **[3]**. The process involves the insertion of genes into genetically engineered plants, which are then cultured to facilitate protein production, as shown in **Picture 1**.



Picture 1

Here are some examples:

1. The limited availability of the Ebola vaccine is largely attributed to the labor-intensive agrobacterium infiltration method used for temporary expression by infiltrating leaves with the bacterium.

2. A single-vector DNA replicon system based on the Bean yellow dwarf virus (BeYDV) was developed by Mason *et al*. (2010). It utilizes multiple DNA replicon cassettes.

3. ZMab and MB-003 were later combined to create ZMapp, which demonstrated complete cure of Ebola virus-infected rhesus macaques when used as a pharmaceutical treatment.

Bioreactor-based platforms:

Currently, plant-cell-culture-based bioreactors are showing more promise compared to conventional plant molecular farming (PMF) approaches that rely on utilizing whole plants for medicine production. These bioreactors offer several advantages, including addressing concerns related to biosafety. With plant-cell cultures, the risk of cross-fertilization and unintended spread of pollen, which can be a concern with whole plants, is alleviated. Moreover, operating cultured plant cells in bioreactors is more cost-effective than using mammalian or microbial bioreactors. Plant cells in culture require basic nutrients to grow and develop, making their cultivation and maintenance significantly more affordable.

Enhanced outcomes of Biopharming

A crucial aspect of biopharming is the careful selection of an appropriate plant species for the specific pharmaceutical (vaccine and/or drug) being developed, along with the implementation of an efficient method to achieve high-yield production. Common techniques employed for introducing the desired gene into plant cells during the transformation process include Agrobacterium tumefaciens and the gene gun method.

The use of genetically modified (GM) plants in plant molecular farming (PMF) opens up possibilities for producing a wide range of substances. This includes both primary products and derived products or by-products, significantly expanding the potential scope of plant-made pharmaceuticals (PMPs) and plant-made industrial products (PMIs).

One particularly promising application of biopharming is the development of edible vaccines. Edible vaccines offer the potential for immunization without additional adverse effects, making them a highly regarded biopharmaceutical technique.

Primary products are proteins that includes -

- Antibodies
- Antibody fragments

- Enzymes (industrial, therapeutic, diagnostic, cosmetic)
- Structural proteins (peptides, hormones)
- Antigens (vaccines)
- Therapeutic agents
- Drugs and
- Enzyme inhibitors

Derived products or By-products are non-protein molecules that include -

- Bioplastics,
- Vitamins,
- Cofactors
- Nutraceuticals
- Secondary plant metabolites (phenolic compounds, glucosinolates, tannins, starches, sugars, perfumes, scents and aromas, alkaloids) and
- Fibers

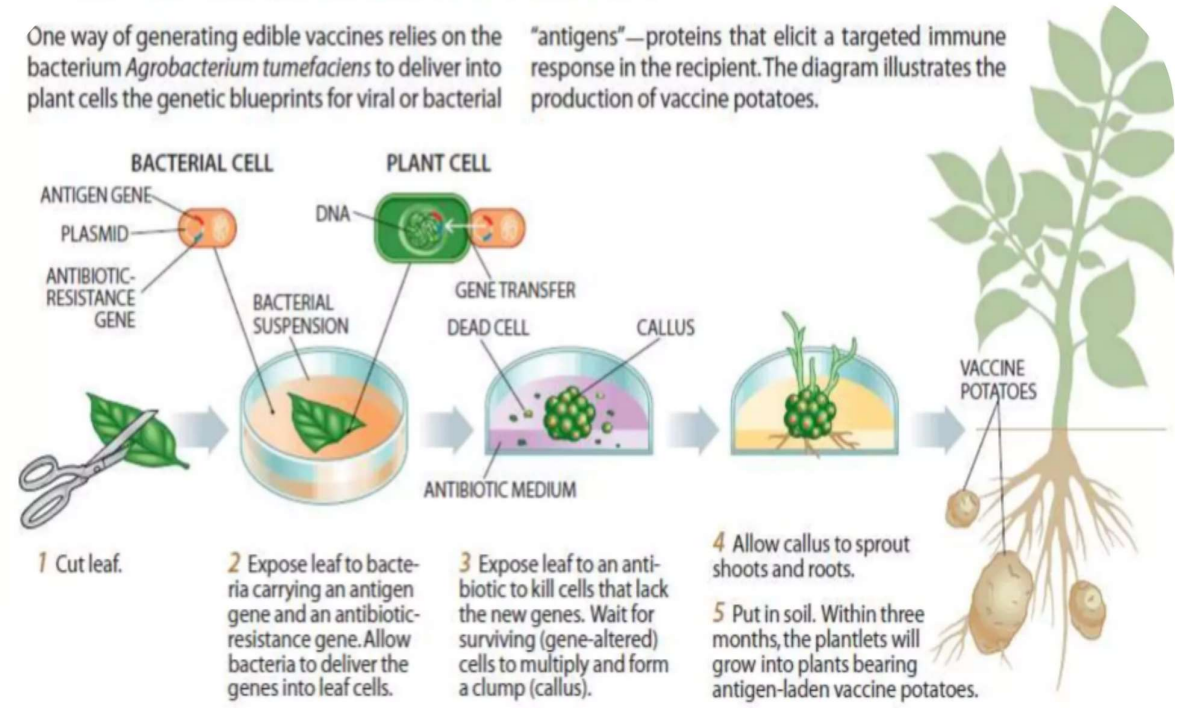
The selection of plants for biopharming takes into account the specific plant parts that are utilized for producing plant-made pharmaceuticals (PMPs), such as seeds, leaves, and tubers. Additionally, the choice of plants can be influenced by the soil composition prevalent in a particular country or region. One significant application of plant molecular farming is the development of edible vaccines. This approach involves introducing desired genes into fruits, vegetables, and/or cereals that are easily consumable by humans. By consuming these edible plant products, individuals can obtain the beneficial effects of the vaccine gene, potentially providing immunization against specific diseases. The use of edible plants for vaccine production offers several advantages **[4]**. It facilitates vaccine delivery, especially in areas where access to traditional vaccine administration methods may be limited. Edible vaccines have the potential to simplify distribution and administration, reduce the reliance on trained medical personnel, and increase acceptance, particularly among children. The versatility of plant molecular farming allows for the exploration of various plant species and plant parts to produce PMPs. The selection of suitable plant species and parts depends on factors such as the desired pharmaceutical product, yield potential, scalability of production, and practical considerations for downstream processing and formulation.

Edible Vaccines:

Researchers at Cornell University's Boyce Thompson Institute for Plant Research have achieved successful genetic modification of potatoes, banana trees, and tomato plants to produce vaccines within the fruits. Among these crops, bananas have garnered significant attention due to their widespread cultivation in impoverished nations and their popularity, especially among children. Bananas, being a commonly consumed fresh fruit, offer a convenient and accessible method of vaccine delivery. In addition to vaccine production, scientists are also placing emphasis on enhancing the

nutritional content of staple crops. For example, researchers are actively involved in developing genetically modified rice varieties that incorporate additional beta-carotene, vitamins, and other essential nutrients. This initiative aims to address nutritional deficiencies prevalent in populations heavily reliant on rice as a dietary staple **[5]**. The overarching goal of these endeavors is to leverage genetic engineering techniques to improve the nutritional value of commonly consumed crops. By enhancing the health benefits and addressing specific nutritional needs in vulnerable populations, these efforts hold promise for promoting better overall health and well-being.

In 1999, at a conference, Professor Potrykus declared success in developing iron-rich rice. This achievement is significant because more than two billion people worldwide suffer from iron deficiency. The basic process of biopharming, which involves genetically modifying plants to produce pharmaceuticals or other beneficial substances, is presented in **Picture 2**.



Picture 2

Examples:

The selection of specific plants for biopharming purposes is based on their suitability for producing vaccines or other pharmaceuticals against particular diseases.

Here are some examples,

- **Potatoes** (*Solanum tuberosum*) are a preferred model for producing vaccines against diseases such as Tetanus,

Diphtheria, Hepatitis B, and Norwalk virus.

- **Banana**s (*Musa sapientum*) have been engineered to express HBsAg, an antigen for Hepatitis B. The leaf of the banana plant contains the antigen. However, one drawback is that bananas take 2-3 years to mature and spoil quickly after ripening.
- **Golden rice** (*Oryza sativa*) **[6]** is a genetically modified rice variety enriched with beta-carotene, which provides both color and improved nutrition to the grains.
- **Tomatoes** (*Solanum lycopersicum*) have been developed as an effective genetically engineered plant for producing vaccines against Acute Respiratory Syndrome (SARS) caused by the Corona virus.
- **Spinach** (*Spinacia oleracea*) shows promise for scalable production of glycoproteins and nucleoproteins that can act against Rabies virus.
- 6. **Carrots** (*Daucus carota*) are being studied for the development of an edible vaccine against Enterotoxigenic *E. coli* (ETEC).

These examples demonstrate the versatility of plant molecular farming in utilizing different plant species for the production of specific vaccines or pharmaceuticals.

To mitigate the risk of pollen-mediated gene flow and prevent contamination of food or feed crops by pharmaceutical plants, various strategies can be implemented. These methods aim to maintain the integrity of neighboring crops, protect consumer health, and adhere to regulatory requirements. Here are some commonly employed approaches **[7]**:

1. **Buffer Zones**: Establishing physical separation through the creation of buffer zones between pharmaceutical crops and neighboring food or feed crops. These buffer zones help reduce the chances of pollen transfer and gene flow.

2. **Isolation Distances**: Implementing appropriate isolation distances between pharmaceutical crops and other crops to minimize the likelihood of cross-pollination and gene transfer. The specific isolation distances may vary depending on the crop species and regulatory guidelines.

3. **Pollination Control**: Implementing measures to control pollination, such as using self-pollinating varieties or employing techniques like bagging or covering flowers to prevent pollen dispersal.

4. **Flowering Time Management**: Carefully managing the flowering time of pharmaceutical crops to minimize overlap with neighboring crops' flowering periods. This can help reduce the chances of pollen transfer and gene flow.

5. **Genetic Sterility**: Utilizing genetic modifications to render the pharmaceutical crops sterile, preventing the production of

viable pollen and reducing the risk of gene flow.

6. **Field Management Practices**: Implementing appropriate field management practices, such as removing male flowers or implementing strict control measures for seed production and harvesting, to minimize the chances of pollen contamination.

7. **Communication and Cooperation**: Promoting open communication and collaboration between pharmaceutical crop growers and neighboring food or feed crop producers to ensure awareness of planting schedules, potential risks, and mitigation measures.

It is crucial to conduct thorough risk assessments and adhere to local regulations and guidelines specific to each crop and region. By employing these strategies on a case-by-case basis, it is possible to minimize the risk of pollen-mediated gene flow and ensure the safety and integrity of neighboring food or feed crops.

1. Pharmaceutical plants may generate pollen that fertilises adjacent food or feed crops of the same species. If this happens, the pharmaceutical might show up in the neighbouring crop's seed, which could have an adverse effect on both the marketplaces for crops and the people or animals who use the seed **[8]**.

Methods to minimize this risk include-

- ✓ Spatial and Temporal isolation, the use of male sterility (i.e., plants that don't produce viable pollen) and
- ✓ Detasseling (removing tassels before they shed pollen) in the case of corn.

2. When it comes to cross-pollinated products like maize, the danger of gene flow via pollen drift is higher.
3. There may be commingling of PMP products with food or feed products.
4. The newly imported gene or its products could have an adverse effect on the ecosystem.
5. By absorbing substances from plants through their skin, taking in pollen, or inhaling dust during collecting, workers on farms may be exposed to biopharmaceutical amounts which are harmful.

Regulations for Biopharming in USA

Pharmaceutical products produced through plant molecular farming, also known as biopharming, are subject to regulation by the U.S. Food and Drug Administration (FDA). The FDA's primary responsibility is to ensure the safety, appropriate dosing, and purity of medical products, including those produced through pharm farming. Prior to the industrial manufacturing of plant-made pharmaceuticals (PMPs), the FDA conducts a thorough evaluation of the product's safety and effectiveness. This determination is made before the commercial production of PMPs begins.In order to assess the risks

associated with pharmaceuticals, comprehensive testing is carried out in accordance with Good Laboratory Practice (GLP) guidelines, similar to the requirements set by the Environmental Protection Agency (EPA) for pesticide certification. These GLP guidelines ensure that data is audited, guard against fraud or deception, and enable the thorough reconstruction of the work submitted.

The FDA's role in regulating pharmaceuticals extends throughout the entire manufacturing process, including waste streams. To regulate production procedures in the industrial setting, the FDA has established Good Manufacturing Practices (GMPs) **[9]**. GMPs ensure that manufacturing processes are consistent and that products are safe, pure, and effective. With the inclusion of plant molecular farming, GMPs now cover procedures in all phases of PMP production, including production in open fields. By implementing GLPs and GMPs, the FDA aims to guarantee the safety, quality, and efficacy of plant-made pharmaceuticals in both laboratory and industrial settings. This comprehensive regulatory approach is designed to safeguard public health and the environment.

The Coordinated Framework

The Regulation of Biotechnology, established as a federal policy in 1986, serves as the framework for evaluating products derived from modern biotechnology in the United States. Three key federal organizations, namely the Animal and Plant Health Inspection Service (APHIS), the U.S. Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) under the U.S. Department of Health and Human Services, play crucial roles in regulating the safe use of genetically modified (GM) crops. APHIS operates the Biotechnology Regulatory Services (BRS) program, which oversees the import of genetically engineered organisms that may pose risks to plant health. Their responsibilities include assessing and granting permits for specific genetically modified organisms created through genetic engineering. Alongside APHIS, the EPA is involved in the regulation of GM crops by evaluating and approving the use of genetically modified organisms in agriculture, particularly with regard to their potential impacts on the environment. They focus on assessing the potential risks associated with pesticides and other substances used in conjunction with GM crops. The FDA, as part of its mandate, is responsible for ensuring the safety and proper labeling of food products derived from biotechnology. They conduct rigorous assessments of GM crops to evaluate their safety for human consumption.

These three federal organizations collaborate to establish comprehensive

regulations and guidelines to ensure the safe development, use, and commercialization of genetically modified crops in the United States.

USDA-APHIS:

(US Department of agriculture - Animal and Plant Health Inspection Service)

The FDA and APHIS play crucial roles in overseeing the manufacturing and regulation of Plant-Made Pharmaceuticals (PMPs). APHIS, specifically through its Biotechnology Regulatory Services (BRS) program, grants permits for the cultivation of PMPs during both the manufacturing and research phases. The USDA, which houses the BRS, focuses on safeguarding agriculture and the environment, and it regulates genetically modified (GE) plants, including their importation, interstate transportation, field testing, and cultivation **[10]**.

The federal Plant Protection Act (PPA) of 2000 consolidated the responsibilities previously covered by various statutes like the Plant Quarantine Act, Federal Plant Pest Act (FPPA), and Federal Noxious Weed Act. Under the PPA, the USDA has the legislative authority to regulate GE plants. Each transformation event involving GE plants is subject to separate regulation by the USDA. This is because the site of gene insertion can vary between transformation events, potentially leading to different gene expression patterns, levels of gene products, and other effects such as the elimination of endogenous genes **[11]**. The USDA's BRS division within APHIS administers resources and handles the notification process for field trials of GE plants. This notification approach is typically used for basic or well-known GE plants and involves submitting a letter to BRS outlining how the proposed GE facility meets the specified performance and characteristic standards, as well as six factors.

By complying with FDA and APHIS regulations, the manufacturing and release of PMPs can proceed in a controlled manner, ensuring the safety, effectiveness, and environmental compatibility of these genetically modified pharmaceutical products[9]. The requirements include elements like the GE plant not coming from a variety of toxic vegetation and not being altered with human or animal pathogenic sequences. Plant-made pharmaceuticals (PMPs) and plant-made industrial products are excluded from the notification process. (PMIPs). The notice can be used to import and transport specific GE plants into the United States as well as to approve field trials (for more information on the notification procedure's criteria, see 7 CFR 340.3) **[12]**.

FDA

(Food and Drugs, Administration)

Pharm farming-produced products are indeed subject to FDA regulation, ensuring

their safety, purity, and appropriate dosing. Prior to the industrial manufacturing of Plant-Made Pharmaceuticals (PMPs), the FDA evaluates the safety and effectiveness of the pharmaceutical product and issues a determination. All pharmaceutical risk assessment tests associated with PMPs must adhere to Good Laboratory Practice (GLP) guidelines, similar to the tests required by the EPA for pesticide registration. GLP standards ensure data integrity, prevent fraud, and enable the replication of studies. As the first stage of the manufacturing process takes place on the farm, the FDA's role complements that of APHIS. The FDA also regulates the complete production of pharmaceuticals, from production to waste streams. In addition to GLPs, the FDA has established Good Manufacturing Practices (GMPs) to regulate production procedures, ensuring consistency and the safety, purity, and effectiveness of products. GMPs are applicable not only within manufacturing facilities but also in open fields, ensuring proper procedures throughout all phases of product production.

The FDA is responsible for ensuring the safety and security of both livestock feed and food for humans. The Center for Food Safety and Nutrition and the Center for Veterinary Medicine assess new genetically engineered (GE) foods and feeds, examining their composition, potential allergens, toxicants, and changes in nutrient levels **[11]**. The FDA has extensive experience with GMOs, having approved the first commercially available GM product, Genentech's HumulinTM, in 1982.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA), the FDA has the authority to regulate novel foods and feeds regardless of their manufacturing process, as stated in a 1992 policy statement. The FDA evaluates foods and feeds based on objective changes in product composition, rather than solely focusing on the process of genetic engineering. The FDA considers GM foods and feeds that are compositionally similar or nearly identical to their conventional counterparts as not adulterated and not requiring mandatory regulatory review. Although the FDA consultation process for GM foods and feeds is not mandatory, developers voluntarily submit dossiers containing compositional information for evaluation. FDA scientists assess the composition, including inserted gene products, genetic stability, nutritional assessment, allergenicity, and toxicology studies. The FDA published instructions in 1997 to guide developers in compiling the dossier. This consultation process provides assurance to consumers, allows independent evaluation by the FDA, and keeps the agency informed about new foods and feeds in development **[13]**. While the FDA consultation process is not compulsory, most developers of GM foods and feeds in

the US voluntarily undergo this process as it is straightforward, prudent, and provides reassurance to stakeholders. However, some individuals have called for mandatory FDA regulation. Nonetheless, in practice, most developers already complete the FDA consultation voluntarily.

EPA

(Environmental Protection Agency)

The Environmental Protection Agency (EPA) plays a significant role in agricultural regulation, particularly in regard to the control and regulation of pesticides. When it comes to Plant-Made Pharmaceuticals (PMPs), the EPA's involvement primarily focuses on regulatory monitoring if the plants possess traits related to pest-protection or herbicide tolerance, such as the Bt protein. In addition to its oversight of pesticides, the EPA is also responsible for upholding environmental protection through various laws. The Toxic Substances Control Act, Clean Air Act, and Clean Water Act impose obligations on the EPA to ensure the safeguarding of the environment from the potential adverse effects of manufacturing operations **[14]**.

Regulations for Biopharming in India

Biopharming in plants is an emerging field in India, currently at an experimental stage. The Review Committee on Genetic Manipulation (RCGM), under the governance of the Department of Biotechnology, Ministry of Science and Technology, has recently updated several guidelines and regulations related to the safety and research in biopharming. These include the "Recombinant DNA Safety Guidelines, 1990," "Revised Guidelines for Safety in Biotechnology," and "Revised Guidelines for Research in Transgenic Plants, 1998." These updates align with the Rules, 1989 of the Environment (Protection) Act, 1986 and incorporate the "Regulations and Guidelines on Biosafety of Recombinant DNA."

The Rules, 1989 have a broad scope encompassing the handling of hazardous microorganisms, genetically engineered organisms or cells, and their byproducts on a large scale. To ensure nationwide compliance with these rules, six competent authorities have been designated, each with specific functions and responsibilities, as outlined in **Table 1 [15]**. These authorities are currently focused on genetic engineering of plants for biopharming purposes, involving the production of bioactive compounds such as vaccines in genetically engineered plants.

Table 1

IBSC Institutional bio safety committee	**Regulatory / Approval**
RCGM Review committee on genetic manipulaton	
GEAC genetic engineering and appraisal committee	
RDAC recombinant DNA advisory committee	**Advisory**

Plant Bio-safety level facilities:

In India, the primary objective of plant bio-safety is to ensure the safe conduct of experiments on genetically engineered plants in order to achieve the desired outcomes. The bio-safety measures for plants consist of four levels of facilities, which include structures like greenhouses, screen houses, and flexible film plastic structures. To conduct studies involving plants containing recombinant DNA (also referred to as genetically engineered or GE plants) and plants associated with GE microbes, small animals, or arthropods, specific physical confinement and work procedures are recommended. These recommendations outline the appropriate protocols for handling viroids, virusoids, bacteria, fungi, protozoa, and algae that interact benignly or beneficially with plants, such as certain Rhizobium species **[16]**. Additionally, these guidelines address microorganisms known to cause plant diseases. Furthermore, the recommendations also cover small animals such as plant pests, plant pollinators, nematodes, and other organisms that transmit plant diseases. These guidelines emphasize the necessity of using plants to assess the biological properties of these small animals.

[1] Plant Bio-safety Level 1 (PBSL-1)

a. Experiments on plants involving RG 1 organism. For example, experiments on plants with non-pathogenic nitrogen fixing bacteria and Agrobacterium spp.
b. Studies using model plants like Arabidopsis, Tobacco, and Chlamydomonas that have a history of safe eating as well as RG 1 organisms that do not code for any recognized toxins or allergies.
c. combination of RG 1 microbes and GE plants modified with genes from other plants that don't have any known invasive traits.

[2] Plant Bio-safety Level 2 (PBSL-2)

a. Experiments with RG 2 organisms on plants. Organisms needing PBSL-2 provide the greatest exposure risks when they enter the body by ingestion, inoculation, or mucosal membranes **[17]**.
b. Experiments using plant associated transgenic insects or small animals as long as they pose no threat to managed or natural ecosystems.

[3] Plant Bio-safety Level 3 (PBSL-3)

a. Growing genetically modified plants containing genes from microorganisms that fall under RG 3.
b. experiments on microbial diseases that affect insects or other small organisms that are connected to plants, if the organism is known to have the potential to have a major and negative influence on managed or unmanaged ecosystems.

[4] Plant Bio-safety Level 4 (PBSL-4)

a. Experiments involving specific uncommon, easily spreadable infectious organisms or potentially dangerous diseases of important Indian crops are carried out in the presence of their arthropod vectors.
b. Genetically modified plants of Category III and above that are used in bio pharming operations to create bio active chemicals (such as vaccines).

CONCLUSION

A pioneering biotechnological approach offers a viable pathway for the economical, scalable, and sustainable production of medicines and therapeutic proteins. Despite facing obstacles and ethical concerns, the field continues to progress thanks to ongoing advancements and a better understanding of plant-based manufacturing processes. Biopharming has the potential to revolutionize healthcare and improve lives on a global scale as it continues to develop. The prospect of broader access to more affordable drugs is an appealing one, but not at any expense. Current biotechnology practices that rely on indoor labs and cell cultures are unable to meet the growing demands due to high manufacturing costs and financial limitations. By utilizing biopharming, there is a possibility of producing vital medications in a more cost-effective and accessible manner, thereby reducing drug prices and enhancing medicine availability **[18-27]**. To ensure the responsible implementation of biopharming, it is crucial to establish clear, transparent, science-based regulations and implement a robust surveillance system. These measures will help maintain safety standards, address potential risks, and build public trust in the technology. In conclusion, plant molecular farming (PMF) appears to be a persistent force in the biotechnology landscape and is likely to expand regardless of differing opinions. Whether one fully supports, strongly opposes, or holds a more nuanced stance towards this new technology, its impact is expected to continue shaping the future of medicine.

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