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Biopharming: In Plant Versus Microorganism Recombinant Biopharmaceuticals Production

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ABSTRACT

Biopharming is a relatively new field in biotechnology that uses transgenic organisms for the production of vaccines and therapeutic proteins for certain health-related conditions. Biopharming is grooming day by day as it is safe, less time-consuming, and less expensive as compared to commercialized drugs that are in use. Many plants and microorganisms are used in biopharming also known as molecular Pharming. These plants include alfalfa, barley, rice, banana, corn, tomato, potato, and many more. Recombinant vaccines are also produced in economically important microorganisms including some bacteria and yeast. Many of the biopharma drugs are approved by the Food and drug administration and many are under clinical trials. A much new technology implied in biopharming is edible vaccines, which cure disease by oral administration of antigens. This review intends to look at the food crops and microorganisms with a medically oriented new approach. Fundamental developments, discoveries, and advances in biopharming are discussed, and finally to look forward at the future biomedical potential of molecular Pharming.

Keywords: Biodrugs, Biomarkers, Edible vaccines, Molecular pharming, Pharmaceutical proteins

INTRODUCTION

Developments in plant molecular biology and biotechnology in recent years have ceased the plant breeding concept not only as a food source but also started to use as a bioreactor in therapeutically important recombinant protein production. Word Pharming is a portmanteau of pharmaceuticals and farming and is used to describe the application of genetic engineering in transgenic animals and plants for the development of useful pharmaceuticals. Pharming is also known as biopharming or molecular farming. Biopharming (so-called nonfood agriculture) products majorly include recombinant proteins and metabolic products. Plants can be referred to as potential biopharma factories as they can produce unlimited amounts of recombinant proteins economically and safely [1, 2].

Common crop plants are being programmed by recombinant DNA techniques to produce high-value-added pharmaceutical products (Figure 1). These plants are then harvested; the required material is extracted and then purified from the plant [3]. Many plants are being used for the production of vaccines for diseases like Hepatitis B, Cholera, HIV, etc. These plants include bananas, tomatoes, rice, carrot, and many more [4]. The market share of biologic-derived drugs is been increasing day by day due to their perceived safety and effectiveness [5].

Synthesis of insulin and somatotrophin played the role of mile stone in an earlier era of biopharming during the 1970s. After their synthesis many companies invested in the biotechnological stream of medicines [6]. Therapeutic uses of many plants and

animals can be dated back to ancient Egypt and the beginning of civilization. The same is the case with Hirudo medicinalis which is commonly known as leeches. This plant is used for the treatment of several bloodletting conditions including periorbital hematomas, severe macroglossia, and purpura fulminant. The first medicinal leech bio pharm was developed in the year 1981 in Swansea [7]. Recombinant proteins, also known as pharmaceuticals, antibodies, enzymes, nutritional additives, and food constituents, can act as valuable products for industry, research, and health care [8].

The statistics show that the entire population in China requires only 40 acres of land for the production of hepatitis B edible vaccines annually. As per this, 200 acres of the plot are required for the production of edible vaccines for all infants in the world [9]. Edible vaccines are composed of antigenic protein introduced into the plant cells which induce these altered plants to produce the encoded protein. The edible vaccine has no way of forming infection and safety is assured as it is only composed of antigenic protein and is devoid of pathogenic genes. Edible vaccines have a significant role in stimulating mucosal immunity as they come in contact with the digestive tract lining [10].

In recent years, additional studies have sought to overcome the limitations of conventional vaccines through the development of edible formulations. Since the inception of the idea, it has been evident that using plants to produce vaccines would have several advantages. However, unlike biomolecule production, edible vaccine formulations do not need processing or purification steps before administration, which serves to further lower production-associated costs [8, 9]. Indeed, another advantage of this strategy is that plant cells would provide antigen protection due to their rigid cell wall. This is also known as the encapsulation effect and could increase the bioavailability of antigenic molecules. This technology also offers advantages in terms of storage and cold chain-free delivery due to the high stability of the expressed antigens encapsulated within the plant and seed tissues. Moreover, plant materials can be stored at elevated temperatures for longer periods and grown where needed, making this strategy even more attractive for developing countries [11].

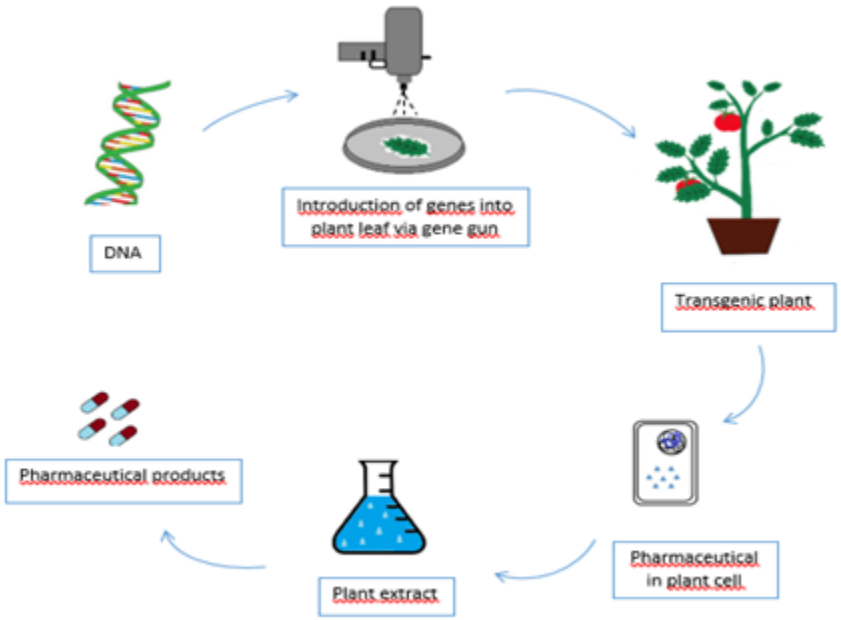

Figure 1. Biopharming by Recombinant DNA Technologies

MATERIALS AND METHODS

2.1. Techniques used

Large molecule therapeutics cannot be chemically synthesized, traditionally produced using microbial fermentation or mammalian cell culture. But current techniques used to prepare drugs are unable to meet the demands [5]. Genetic engineering implies many techniques for pharmaceutical development. Transgenic plants are important alternatives in the production of recombinant pharmaceutical proteins. The vaccines can be produced in plants by stable genetic transformation and transient expression systems. In the stable genetic transformation, a gene is introduced in the nuclear or chloroplast genome of plants whereas in transient expression, plant viral technology is used [9]. Transient gene expression is a quick, safe, economical, and

reproducible approach for the production of plant protein therapeutics [10]. Since the use of recombinant DNA technology, a lot has been achieved in the field of therapeutic protein production [11].

2.1.1. Polymerase Chain Reaction (PCR)

With PCR, DNA isolation, gene sequencing for particular proteins, replication, and production of multiple copies, resulting in the production of large quantities of particular proteins are possible [5].

2.1.2. Gene Delivery:

There are 2 types of gene delivery methods; direct and indirect gene delivery method [11]. Gene insertion in plants requires targeted delivery through a plant vector. Many approaches are being used for gene delivery and insertion. These approaches include a biolistic gun method, particle bombardment, DNA uptake, Agrobacterium tumefaciens, electroporation, and sonication, etc. [3]. The 2 types of antigen expression methods used are nuclear transformation and chloroplast transformation. Both of them can be done by a biolistic method. Between the two, the chloroplast transformation method is more commonly used for the production of edible vaccines. Cholera, tetanus, rotavirus, canine poliovirus, and plague are all examples produced by the biolistic method [12].

2.1.3. Tissue Culture

The plants having foreign genes are known as transgenic plants. These plants are first transformed into callus and then seed-producing plants using tissue technique. The tissue culture technique involves the growth of plants in nutrient media in the absence of soil. Fertile plants are grown in a greenhouse or field. After the harvesting, a particular protein is extracted and isolated from the leaves or seed material of plants [5]. The pluripotency of somatic cells is a promising approach for the production of transgenic plants and their application in biopharmaceuticals [12].

2.1.4. Genetically Engineered Plant Virus

This method redesigns a suitable plant virus to generate a chimeric gene for viral coat protein. Thus it's a vector that delivers genetic elements into the plant cell. This method leads to transient antigen expression in plants. The recombinant virus generated is a product of viral replication present throughout viral infection in plants which expresses the required protein or peptide. Moreover, synthesis and accumulation of vaccine epitopes can be done by changing viral capsid protein. However, the products of viral reproduction must be purified first from infected plants and then vaccinated [9, 10].

2.2. BIOPHARMED PLANTS

Plants can be used for producing pharmacologically active ingredients including blood product substitutes, drugs, hormones, mammalian antibodies, proteins, therapeutic agents, and vaccines. Various plants such as banana, corn, tomato, wheat, rice, lettuce, carrot, Arabidopsis thaliana, and tobacco (Figure 2) have been used in vaccine production for diseases such as hepatitis B, HIV, and cholera [13]. Biopharming deals with the cultivation of crops for pharmaceutical purposes and enhancing their ability to produce intended therapeutic proteins, which can be extracted and purified for the production of protein-based drugs [14]. Viral proteins expressed in plants may find use as potential vaccines or diagnostic tools. Recombinant Agrobacterium can harbour a gene of interest, when infiltrated into the Nicotiana benthamiana plants, and produces rapid results in 2 to 7 days [15].

Figure 2. Some plants used as edible vaccines

2.2.1. Alfalfa

eBRV4 is a VP4 protein of the bovine rotavirus and is an immunorelevant peptide. eBRV4 epitope can be expressed efficiently in transgenic alfalfa as a translational fusion protein. The reporter enzyme in this case is β-glucuronidase. The eBRV4 produced in alfalfa is potent in promoting anti-rotavirus antibody response in adult female mice. The results show that it is feasible to induce lactogenic immunity against a pathogen using an edible vaccine produced in transgenics [16]. Codon-optimized gene sVP6 encoding human group A rotavirus VP6 protein is added into the alfalfa genome by the use of the Agrobacterium-mediated transformation method. After harvesting, transgenic alfalfa extract was administered in Balb/c mice. Immunized mice were observed to produce high titers of anti-VP6 serum IgG and mucosal IgA. The antibodies generated are also transferred to offspring by passive immunization and the offspring are observed to develop less severe diarrhea after challenge with simian rotavirus SA-11. This proves that transgenic alfalfa protects children and animals from diarrhea caused by rotavirus [17].

Purified anti-human IgG can be produced by its gene expression of perennial transgenic alfalfa. Transgenic plants expressing heavy and light chains encoding mRNAs are produced and crossed to express fully assembled C5-1. The antibody is observed to be stable in drying hay as in extracts made in pure water. A large number of monoclonal antibodies can be purified from transgenic alfalfa and can be used for the determination of antibodies that do not cause agglutination [18]. Alfalfa plants were bio-pharmed to express Eeg95-EgA31 of *Echinococcusganulosus.*

2.2.2. Banana

Embryonic cells of bananas have been transformed with the S gene of hepatitis B virus surface antigen via Agrobacterium-mediated transformation. Transgenic plants were grown till maturity in the greenhouse and confirmation of HBsAg expression was done by real-time PCR. Transgenic banana plants were multiplied in vitro using floral apex cultures. An enhancement of expression of HBsAg is observed in wounded transgenic banana fruit [19]. The leaf contains antigens.

2.2.3. Barley

Barley, which is the fourth most used grain in the world today, is one of the basic products of agriculture. Barley is proved successful as a promising bioreactor for biopharming and is considered an ancient but rediscovered product in molecular farming [20]. Barley seeds can be used for antibodies, diagnostic immune reagents, pharmaceutical proteins, and vaccine production. Antibody fusion proteins are prepared in transgenic barley seeds for their direct use in medical diagnostic assays. Many people around the world are affected by HIV and related infections. Biopharming has been preferred in recent years for the production of HIV-detecting proteins, and preventive and therapeutic vaccines [21]. Barley gene constructs were used with suitable promoters and a high-level expression of anti-glycophorin single-chain antibody fused to HIV epitope was obtained. This fusion protein can be used for determining the HIV-1 antibodies asset in human blood. These fusion proteins can also be produced in tobacco and potato but barley grains are observed to be the most favorable bioreactor for this purpose as they are economical to produce and harvest [22]. Collagen and gelatin-related proteins are also expressed in barley seeds. It is observed that barley seeds are very potent bioreactors for recombinant full-length collagen type 1α1 and related 45KD-a rCla1 fragment production. These seeds accumulate both proteins in large amounts. The highest accumulation of rCla1 is obtained in the gluten promoter and the lowest accumulation is obtained in the alpha-amylase promoter [23]. Transformation of barley is usually done by microprojectile bombardment and by direct gene transfer to protoplasts [24]. Barley grains are also being used for the production of growth factors that do not contain endotoxin [25]. Barley transformation has certain limiting factors which include strong genotypic addiction to barley transformation protocols, transformation performance, transgene stability, transgene insertion sites, somaclonal variation, and public acceptance [26, 27].

2.2.4. Nicotiana benthamiana

A major aspect that has brought N. benthamiana to such light is its ability to rapidly and highly express transgenes or gene-silencing signals placed within the borders of a T-DNA plasmid and delivered into the leaf by the very simple method of Agrobacterium infiltration. This method has its basis in plant virus agro infection and was changed to agroinfiltration after it was shown to have worked for N.benthamiana transgenes in 1997 [28]. N. benthamiana plant systems are used for producing heterologous proteins due to benefits including proper folding and processing of proteins, low cost, large production, and easier scalability of the process (28). A candidate therapeutic vaccine, LALF32-51-E7, has been prepared for cervical cancer caused by High-risk human papillomavirus [29] in the N. benthamiana plants. The vaccine is now under the steps of further purification and research as a protein body-inducing peptide [30]. Also, mosquito-borne Rift Valley fever virus (RVFV) is a virus that causes potential miscarriages in humans and there is no commercial vaccine for them. Chimeric RVFV virus-like particle production was carried out in N. benthamiana using Agrobacterium by Mbewana et al. [31]. Plant biotechnology inc. (Hayward, CA, USA) created an immunoadhesin

(DPP4-Fc) in biopharma tobacco. A strong binding is exhibited by DPP4-Fc towards MERS-CoV which prevents lung cell infection by the virus. ZMapp is the most advanced immunotherapy against Ebola Virus Disease (EVD). It is a drug that consists of humanized monoclonal antibodies (mAbs) which are capable of neutralizing the ebola virus. Production of this experimental drug opened the gates to visibility in molecular farming.

2.2.5. Potato

Potato tubers have been used for the expression of anti glycophorin single chain antibody combined with HIV epitope. This fusion protein can be used as a diagnostic reagent alternative to SimpliRED. It can be used for the detection of HIV-1 antibodies in human blood. Transgenic potatoes have also been improved to express varied antibody and vaccine nominees and human β-amyloid peptides [32]. Potato leaves are used to express xenoproteins and potato tubers are used for the production of human serum albumin. cDNA of mature human serum albumin is expressed in Solanum tuberosum under the transcriptional control of S. tuberosum in B33 promoter and potato proteinase inhibitor II terminator. Produced recombinant albumin is accumulated in tubers of plants [33]. Adiponectin is an anti-diabetic protein of 30kDa, secreted from adipocytes. cDNA for mouse adiponectin can be expressed in sweet potatoes and can be used as a pharmaceutic medicine for diabetes therapy [34]. VP1 protein from foot and mouth disease virus has been expressed in transgenic potatoes. These potatoes contain the VP1 gene cloned under the regulatory activity of a single or double copy of the S35 cauliflower mosaic virus (CMV) promoter, which results in increased expression [35]. Potato is a suitable model for vaccines against hepatitis B, tetanus, Norwalk virus, and diphtheria. The first edible potato vaccine was for e.coli causing enteritis. It also played a role in the oral strengthening of the hepatitis B vaccine in humans. The benefit of the potato edible vaccine is its ease of transformation and propagation. It also doesn't need to be refrigerated. Yet its main disadvantage is, if cooked it denatures the antigen.

2.2.6. Rice

Rice is consumed as one of the staple food in the majority of the world. Rice seeds are observed to provide high recombinant protein accumulation and long-term storage. Daily consumption of transgenic rice grains is expected to provide an easy, secure, and stable oral delivery system that will benefit human health [36]. Transgenic rice accumulating T cell epitope peptides of Cry j 1 and Cry j 2 of Japanese cedar was fed to mice that were prone to cedar pollen allergy. It is observed that when they are exposed to cedar pollen, allergen-specific IgE, IgG, and CD4 T cells proliferative reactions are inhibited. It is also observed that allergen-specific CD4 T cell-derived allergy-associated T helper 2 cytokines of IL-4, IL-5, and IL-13 and histamine release levels in serum are also noticeably reduced. Hence it is proved that a plant-based edible vaccine for Japanese Cedar pollinosis is a potent therapeutic [37]. Oral administration of human T cell epitope (7Crp) of Japanese cedar in monkeys revealed that there are no adverse effects for this therapy at high or low doses [38]. Recombinant human lactoferrin (rhLF) can be stably expressed in brown rice and can be extracted from brown rice flour with 95% purity. It is seen that the rhLF expressed in brown rice is similar to the human counterpart other than its glycosylation. Hence it is one of the important parameters showing success in plant-based medicines [39]. Immunoreactivity glycoprotein (G protein) B is also continuously expressed in more than three generations in transgenic rice. It is also seen that this glycoprotein is stable for 27 months after extraction [40]. Lactostain which is used as a therapeutic for Hypercholestorelomia can be expressed in transgenic rice with increased stability [41].

2.2.7. Tomato

Tomato represents one of the most economically important fruits worldwide. It is an incredible source of nutrients, which have positive impacts on human health, including vitamin C, lycopene, and beta-carotene [42]. Transgenic tomatoes are produced which can express rabies virus glycoprotein, synthetic hepatitis C and HIV vaccine nominees and immunologically active cholera toxin B protein [32]. Tomato plants are engineered to express the gene for the G protein which covers the rabies virus's outer surface. The recombinant constructs contain G protein genes from ERA strains of rabies virus which include signal peptide under the control of 35S promoter from CMV. PCR was performed to select transgenic plants expressing the gene. Oral administration of this gene with tomato elicits protective immunity in animals against the rabies virus. These transgenic tomatoes can be a potent antirabies edible vaccine [43]. Today, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an important pandemic that causes a large number of deaths. The development of diagnostic kits, vaccines, and antiviral drugs is of great importance to reduce and control the spread of the disease. It is thought to be ideal for these to be produced by plants through molecular farming, which is a fast, reliable, and inexpensive method [44]. Tomato is the plant in which the vaccine against acute respiratory syndrome SARS was first developed. For its development S-spike protein (S-protein) and its translucent fragment are the best choice considered. The tomato genome is inserted with the N-terminal of the SARS-CoV protein (S1) for the development of effective and inexpensive and safe vaccines. When given to mice they showed a spike in SARS-CoV specific IgA as detected by ELISA and Western Blot technique.

2.2.8. Lettuce

This is a very valuable plant that can be used as an edible vaccine. This model is effective against enteric diseases caused by E.coli in humans and animals. It has beneficial effects against Hepatitis B and is used in its raw form.

2.2.9. Papaya

In 2007, a papaya-based vaccine expressed synthetic peptides of Taenia solium which causes cysticercosis. These peptides were expressed in 19 biopharma papaya clones. The vaccine was tested in rats and 90% of them showed an immune response. Thus this edible vaccine could provide relief for the two main disease carriers i.e., pigs and humans [44].

2.2.10. Quinoa

An edible vaccine for poultry veterinary medicine was developed in 2012, which expressed the VP2 antigen of infectious bursitis virus in the plant of quinoa (Chenopodium quinoa).

2.3. BIOPHARMED MICROORGANISMS

Microbial systems for the production of biopharmaceuticals are well studied and their genetic manipulations let microbial metabolism optimization for common biopharmaceuticals production. Microorganisms as a production house of drugs should be identified for limiting steps and pathway regulation. The study on microorganism-derived biopharmaceuticals involves the designing of strategies to limit by-products with high flux to intended biosynthetic pathways [45].

2.3.1. Escherichia coli

Approximately one-third of the approved recombinant proteins are produced in E. coli. distinguished by their genetic, high yield, and rapid growth properties. Comprehensive tools are also developed for protein expression in E. coli. These tools contain expression vectors, protein folding, production strains, and fermentation technologies. Full-length glycosylated antibodies, bacterial N- glycosylation, novel strain engineering, and cell-free systems recommended that complex proteins can be expressed in E. coli [46]. Bovine somatotrophin (bST) can be produced in E. coli under the tryptophan promoter control. Plasmid used for this purpose contained two cistron systems and altered codon usage for higher expression of bovine somatotrophin. It is observed that intracellular concentration for bST decreases at high cell concentrations, which is due to an increase in degradation by proteases. This degradation can be decreased by exponentially feeding yeast extract as an organic nitrogen source [47]. Genetically engineered E. coli K12 BMH-710-18 can be used for expressing hum interferon alpha 1. Production of human interferon alpha 1 in E. coli is based on growth dynamics and glucose consumption model. The cells are continuously supplied with nutrients during the cultivation. The cell density is observed to increase from 58 g/l to 80 g/l and following it, is the increased interferon production [48]. Beta-galactosidase is an enzyme that is produced by single-stage fed-batch bioprocess in E. coli. XL-1 blue strain of E. coli is used for this purpose. This strain can harbor multi-number foreign plasmid PT. The optimized fed-batch process is observed to maintain an exponential growth phase for 50 hours and produce a cell density of 51 g/L dry cell weight or beta-galactosidase activity of 990 U/mL [49]. Genetically engineered E. coli can express and accumulate interleukin- 2 (IL-2) intracellularly in regulated temperatures under the control of phage lambda pL promoter. Interleukin- 2 accumulation is seen to decrease with an increase in cell density at induction. Acetate induction in the medium may be a major limiting factor for interleukin- 2 expressions in high-density cultures [50].

2.3.2. Yeast:

Yeast expression systems are feasible bioreactors as they are easy to grow and manipulate. Yeast systems have been a staple system for producing large amounts of proteins for industrial and biopharmaceutical uses for many years. Proteins secreted by yeast are overly glycosylated thus the proteins which are expressed in yeast are the ones whose functions are not affected by any sort of post-translational changes. A few yeast systems which have been used for recombinant protein production are Saccharomyces, Scizosaccharomyces pombe, Pichia pastoris, and Hansanuela polymorphic [51]. Yeast expression systems are expected to produce a high amount of recombinant serpins, as many yeast species' genetics are well understood and can be manipulated to stimulate targeted extreme production of many serpins. Certain yeast strains are protease deficient and few species are also capable of carrying out post-translational modifications resembling those of humans [52]. Yeast Yarrowia lipolytica can stimulate the overproduction of citric and isocitric acid at different percentages. This yeast is also able to produce alpha ketoglutaric acid at high proportions by changing cultivation conditions [53].

2.4. COMPARISON BETWEEN PLANT AND MICROORGANISMS DERIVED RECOMBINANT BIOPHARMACEUTICALS

High-value pharmaceutical peptide production in transgenic plants is an appealing, preferable and economically probable technique as compared to its alternative cell lines, yeast and bacteria due to many reasons which are explained in the following text [36]. Vaccines against many diseases are on sale, but most are expensive. Many underdeveloped or developing countries

are unable to maintain disease control with these vaccines due to their cost. Therefore many efforts are being made for the production of edible vaccines which are the least costly as compared to commercialized vaccines [54]. For example, Africa plans to benefit from molecular biofarming in the next few years [55]. Plant-derived biopharmaceuticals are cheap as compared to their alternate drugs which are commercially available [56]. It is calculated that the cost of production of therapeutics from plants is four to five times lower as compared to the cost of the same amount of therapeutic drug using mammalian cell culture or microorganisms-derived drugs [5]. However, Escherichia coli is a source of production of the least expensive, easiest, and quickest proteins [57].

2.4.1. Post-translational modifications

Only those proteins can be expressed in microorganisms that do not require post-translational modifications or whose functions are not affected by any sort of post-translational modifications. The proteins which are rich in disulfide bonding are also not expressed preferably in microorganisms and especially bacteria. However, transgenic plants are preferred in the production of such peptides. Major experiments are being carried out on A. thaliana for recombinant protein production which wants post-translational modifications [57].

2.4.2. Production capacity

Biopharm drugs are simple to increase for mass production. Recombinant antibody for rabies prophylaxis has been produced in plants at a large scale. Plant-derived recombinant antibodies can be massively produced as they can be extracted from the leaves of the plants and thus are a potent source for rabies prophylaxis [1]. Plants are used as a very charming hypothesis for many biological molecules as they are produced in a wide range and mass production of drugs is hence obtained [58]. However, biopharmaceutical production in microorganisms is limited by certain factors and is not as massively produced as in plants [57]. The high level of recombinant protein expression in plants is one of the significant parameters for the accomplishment of herbal medicines [39].

2.4.3. Administration method

The most common route of administration for plant-derived biopharmaceuticals is oral administration. These drugs are produced in edible plants and hence can be eaten. It is observed that oral administration of these drugs and proteins yields the maximum required response. Due to their easier route of administration, these drugs are preferable to their alternatives [40]. On the other hand, microorganism-derived drugs are preferred on a large scale but there are certain limitations which include their usual administration via intravenous or intramuscular route. These drugs are directly injected into the blood which may result in some of the toxic effects of these drugs hence they are less safe as compared to plant-derived drugs [56].

2.4.4. Safety

Plant-derived biopharmaceuticals are observed to be safer at high doses as compared to animal and microorganism-derived drugs [59]. The plant system offers flower, leaf, root, seed, stem and whole plant-based production [60]. Safe and consistent expression of drugs is obtained as the expression of the protein is only targeted to a special organ or tissue and it does not affect the whole plant. Moreover, these drugs are taken orally or intravenously thus reducing their action on non-targeted cells of the body which makes them safer [58]. Plant-based pharmaceuticals do not transport potentially harmful animal or human viruses to the drug hence they are inherently safer. Yeast expression systems are also accepted as a safe source for drug development but over-glycosylation of drugs in yeast can lead to ruin its bioactivity, safety, and potency [5].

2.4.5. Storage and transport capacities

With their low water content, poor protease activity, high protein storage ability, and stability in environmental conditions, seeds are long-term storage agents for vaccine antigens, antibodies, and other therapeutic proteins. Furthermore, they have easy and cheap transport [61].

2.4.6. Gene flow

The intrusion of biopharma crops into the human food chain can be avoided only with the meticulous distinction of food and non-food varieties of the same plant species using a series of biological and physical methods. A more secure distinction can be done by the use of in situ cultures [32]. Pollen from bio pharm crops can fertilize food crops and may produce a generation that has this gene for the expression of biopharma protein or drug. Escape of such kind of pollen in food crops can prove to have disastrous effects in the future [5]. Gene flow in transgenic plants is a potential concern for the conservation of genetic diversity. Due to gene flow, the domesticated gene pool becomes an important factor affecting the genetic diversity of the wild gene pool. It is also observed that the elite crop gene pool of some crops has been reduced due to transgenic crops [62].

2.4.7. Environmental impact

Biopharm crops may pose a risk to the environment including potential safety problems due to contamination with plant toxic metabolites. Another environmental aspect lies in the contamination of wild plants with an altered plant containing potent drugs. But not all bio pharm drugs are harmful as they are proteins with minor or no biological activity when swallowed by a human being [5].

2.5. FDA-APPROVED BIOPHARM DRUGS

The platforms which can be used for biopharmaceutical production include prokaryotes, yeast, fungi, insect cells, mammalian cells, transgenic plants, and transgenic animals. Recombinant human insulin stands as the first biopharma drug, approved by FDA in 1982. Since then, more than 200 biopharmaceuticals have been brought to market [23].

Many biopharm drugs and pharmaceuticals are approved by FDA and are commercially available in the market. Some of these pharmaceuticals include lactoferrin and lysozyme produced in rice, the Cholera vaccine, human serum albumin and interferon for hepatitis C expressed in tobacco plants, and hemoglobin produced in alfalfa. Transgenic corn, tobacco, and alfalfa are being used for the production and expression of hemoglobin, gastric lipase, albumin, and cancer therapeutic antibodies, potato, and rapeseed are used for the production of antibodies for detecting food and water pathogens, safflower is used for the production of anti-obesity peptides and somatotrophin. Pharmaceuticals produced from corn include monoclonal antibodies and EPI-19 which is used for treating bronchiolitis and pneumonia in infants. Patient-specific cancer vaccines, vaccines for B cell Hodgkin lymphoma, and alpha-galactosidase A are expressed and purified from tobacco. Potatoes have been used for the production of human growth hormone, hemoglobin factor VIII, and an edible vaccine for hepatitis B [5]. Some of the Food and Drug Administration (FDA) approved vaccines and proteins are discussed in detail and a summary of recombinant drugs is mentioned in Table 1. The US FDA has approved several biomedicines and drugs derived from plant origins. One of these drugs is Elelyso, which is produced in carrot cells and has been recommended by the FDA in 2012 for the treatment of type 1 Gaucher's disease. Another drug known as ZMapp has been produced by molecular farming and is observed to possess potent immunological activity for Ebola patients. Chen and Davis 2016 conducted research for the production of a cocktail of three monoclonal antibodies in Nicotiana tabacum [63].

2.5.1. Insulin

Human insulin derived from bacteria is the first recombinant product that is approved by FDA. Approval of recombinant insulin dates back to 1982. This recombinant insulin is produced by recombination between two Escherichia coli strains. One strain contains the gene for the insulin A chain and the other strain contains the gene for the insulin B chain. Insulin chains act as a tail to the protein beta-galactosidase. Both strains of bacteria are mixed and then incubated for a long time. Insulin is then purified [64]. Goeddel and colleagues [65] cloned human insulin A and B chains in plasmid pBR322. These synthetic genes were then fused to the E. coli beta-galactosidase gene which gives effective translation and transcription and produces a stable precursor protein. Insulin peptides were then cleaved from beta-galactosidase protein and were detected by radioimmunoassay. Recombinant insulin can also be produced in plants. Human insulin is transgenically expressed in plant seeds. The insulin accumulation level in seeds is observed to be exceeding 0.1% of total cellular protein. This insulin is beneficial as it can be transported and stored within the seeds and gets activated as soon as it is extracted from the seeds. Another advantage may include the amount of biomass produced is very low as compared to previously commercialized methods for insulin extraction. Biomass subjected to extraction is also limited as seeds have low water content [66].

2.5.2. Hepatitis B virus vaccine

Hepatitis B viral vaccine is composed of hepatitis B virus surface antigen (HBsAg) and is produced in yeast Saccharomyces cerevisae by the use of recombinant DNA technology. This vaccine is commercialized with the name Engerix B® [Hep- B (Eng)]. This vaccine is a suitable alternate and effective to other vaccines used for hepatitis B prophylaxis. Engerix B is administered intramuscularly. The vaccine was approved in 1986. This vaccine is the first in its kind vaccine which is used against viral infection [67, 68].

2.5.3. Interferon

Recombinant human leukocyte interferon is synthesized in Escherichia coli. This recombinant interferon is shown to possess anti-proliferative activity and antiviral activity. This synthesized interferon has the same structure and function as that of human leukocyte interferon gamma 2 [69]. Recombinant interferon produced in E. coli has been approved by FDA and USDA. The promoter or regulator region from the yeast suppressible acid phosphatase gene is used to create vectors in the regulated expression of cloned genes. Genes for human leukocyte interferon are inserted into this vector. These transformed yeast cells then express interferon in significant amounts in a culture medium that lacks inorganic phosphate [70]. Methylotrophic yeasts

are being used as an expression system for recombinant interferon production. These yeast systems include Hansenula polymorpha, Pichia pastoris, and Candida boidinii [71]. Human interferon-gamma cDNA has been directly expressed in yeast S. cerevisae under the 3- phosphoglycerate kinase promoter transcriptional control [72].

2.5.4. Gastric lipase

Recombinant gastric lipase has been approved by FDA and is shipped in wet ice and stored at a temperature of -20º centigrade. These gastric lipases are used in the treatment of cystic fibrosis [73]. Gastric lipases are produced massively in transgenic tobacco plants. Recombinant dog gastric lipases which are similar in function to that of human pancreatic lipases have been produced in large amounts in transgenic tobaccos. These enzymes are similar to naive enzymes having acidic optimum pH and additional acid resistance. The dog's gastric lipase expression levels in tobacco are 5% to 7% [74]. Transgenic corn seeds are also been used for the expression of gastric lipase [75]. Human gastric lipases are also produced in fission yeasts S. pombe. This yeast expresses cDNA encoding human gastric lipases on multi-copy plasmids [76]. Successful transient expression of human gastric lipases in N. benthamiana leaves using CPMV-HT expression systems. Large quantities of gastric lipases are obtained by this method and are used for several applications [77].

2.5.5. Serum albumin

Serum albumin that is produced in transgenic potatoes and tobacco is the first recombinant plant-derived pharmaceutical ever produced. Its first production is in 1990. Direct expression of chimeric serum albumin genes was obtained in tobacco and potato plants by use of a modified CaMV 35S promoter. For the secretion of protein pre and pro sequence from extracellular tobacco protein PR-S was used. Human serum albumin is observed to be secreted in both cell suspensions and leaf extractions [78]. This protein was approved by US Agriculture Department. Complementary DNA of human serum albumin is expressed in the transcriptional control of the potato proteinase inhibitor II terminator and the patatin B33 promoter in potato tubers. Accumulation of 0.2% of human serum albumin has been observed in total soluble tuber protein [33].

2.6. EDIBLE VACCINES

A very interesting application of biopharming lies in edible vaccines. Edible vaccines are transgenic plants expressing recombinant vaccine proteins and peptides. Edible vaccines have gained considerable attention in the recent past. It is a novel technique for the administration of vaccines. Plant-induced antigenic vaccines delayed or prevented the starting of many diseases in animals and have been proven safe in many clinical trials [79]. In the simplest of words, the vaccines generated in plants and animals are edible. Edible vaccines are low-cost, easy to administer, and simple to store, especially for developing countries. These vaccines were primarily formed for infectious diseases but now research has been performed for their preventive role in birth control, cancer therapy, and autoimmune diseases. Currently, these vaccines are developed for multiple human and animal diseases [80]. Edible vaccines are a potent and economical alternative to fermentation systems. Genes coding antiviral and antibacterial agents are easily expressed in edible parts of transgenic plants and can be easily administered orally to patients who are diseased or are at high risk of disease [81]. The Hepatitis B virus vaccine is being prepared in potatoes by expressing hepatitis B surface antigen. This oral vaccine is very potent as it has a high shelf life and can be transported easily [82]. Similarly, allergen-specific T cell epitopes are prepared in rice for treatment and prophylaxis from type I allergy. This recombinant vaccine is produced in rice grains and can be administered orally [83].

2.6.1. Mechanism of action of edible vaccines

Edible vaccines induce the activation of the immune response system in the mucosa. (MIS). Figure below. Both innate and adaptive immunity (T and B cells) are activated by this configuration. And an effective vaccine will activate both the immunities which will also serve as a long-lasting memory against actual infection.

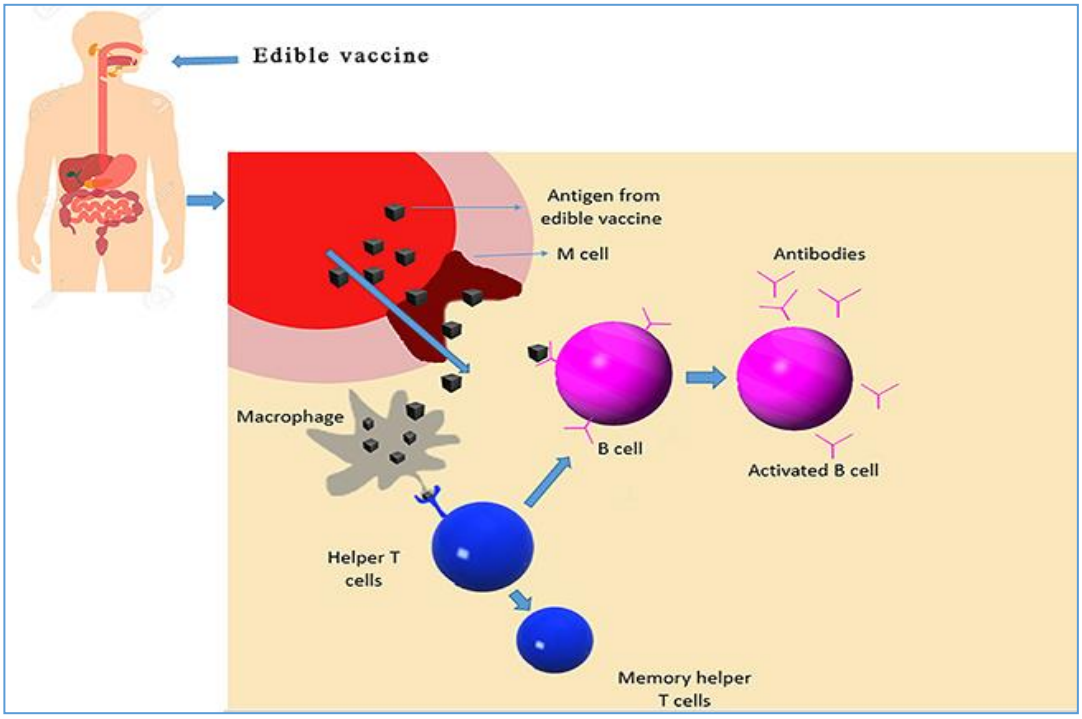

Figure 3. Mechanism of action of edible vaccines.

Conclusion

As biopharming is a new industry so it has a large number of technical and regulatory barriers to deal with. But experience to date is very successful and it seems that this field of biotechnology and health care is ready for rapid growth and profitableness [84]. The future of biopharming is expected to be largely dependent on the ability of this sector to sustain public trust with a more thoughtful approach to the environment and the safety of plant production systems [32]. Biopharming is expected to have a huge contribution to global vaccine programs and might have a significant effect on health care especially in underdeveloped and developing countries [85]. In the future, there will be a driving force toward higher expression levels and improvement in biopharma plants and crops. Biopharming is a controversial topic these days but in the future, it will be known to solve many problems on the planet. Further research is required on the possible toxic effects of recombinant drugs produced in transgenic organisms in the long run. Biopharmaceuticals promise a better and non-disease future on the planet Earth. In the future biopharming may be proved as a useful tool for the production of different proteins and vaccines, which are essential for human beings [86]. Using biopharming, cheaper and more efficient biologically active compounds can be produced [14]. Biopharmaceuticals are undergoing many innovations, which include biosimilars, bio drugs, bio-betters, and diagnostics biomarkers such as lab-on-chip technology and drug therapy [87].

Although there are several obstacles to commercializing plant-produced drugs, including detailed examination of plant varieties by regulatory agencies, research is going on and these drugs are expected to be on the market soon [63]. Some of the major obstacles in the usage of these technologies include low protein capacity and post-translational modifications, which can be overcome by researching expression forms, appropriate plant host systems, and glycol-engineering of proteins for designing high-expression strategies Table-1 [10].

Table 1. Food and Drug Administration (FDA) approved vaccines, proteins and a summary of transgenic recombinant vaccines.

Protein or vaccines	Transgenic source plant or microorganism	Disease or condition	Nature of vaccine
Hepatitis B viral vaccine	Banana: Yeast	Hepatitis B	Prophylactic
Insulin	Potato: *E. coli*	Diabetes	Prophylactic
Cholera vaccine	Bananas Tomatoes, Potato tubers	Cholera	Prophylactic
Rabies vaccine	Tomato, Spinach	Rabies	Prophylactic
Foot and mouth disease virus VP1 protein	*Arabidopsis:*Potatoes	Foot and mouth disease	Prophylactic
VP4 bovine rotavirus	Alfalfa	Rotavirus induced diarrhea	Therapeutic
Adiponectin	Sweet Potatoes	Diabetes	Prophylactic
T cell epitope peptides of Cryj1 and Cryj2	Rice	Type 1 allergy (Japanese cedar pollinosis)	Therapeutic
Lacto statin	Rice	Hypercholesterolemia	Therapeutic
Interferon alpha	*E. coli:* yeast	Antiviral	Therapeutic and prophylactic
Elelyso (teliglucerase alfa)	carrot cells	Type 1 Gaucher's disease	Therapeutic

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CONFLICT OF INTEREST

N/A

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