

PLANT MADE PHARMACEUTICALS (PMP's)-A PROTEIN FACTORY:

AN OVERVIEW

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Summary

Plant Made Pharmaceuticals (PMPs) are the emerging innovation in the field of biotechnology. Plant-Made Pharmaceuticals (PMPs) are a category of therapeutic agents (pharmaceutical proteins) produced in live plants that could ultimately be used by the medical community to combat life-threatening illnesses. Medicines produced from plants represent one of the brightest new hopes in medicine. Plant produced medicines can become most popular way to treat the health disaster in future. Plants hold the potential for cost-effective, large-scale production of recombinant proteins, vaccines and antibodies for industrial and pharmaceutical uses. Over the last decade, plant based production of pharmaceuticals has made remarkable progress as the expression of a diverse set of proteins has been demonstrated and some plant made vaccines moved towards clinical testing. Therapeutic proteins produced by transgenic plants include antibodies, antigens, growth factors, hormones, enzymes, blood proteins and collagen. The advantages they offer in terms of production scale, economy, product safety, ease of storage and distribution cannot be matched by any current commercial system; they also provide the most promising opportunity to supply low-cost drugs and vaccines to the developing world.

Key words: - Plant Made Pharmaceuticals, Therapeutic proteins, Transgenic plants.

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Introduction:-

Plant-Made Pharmaceuticals (PMPs) are the result of an innovative application of biotechnology, whereby plants are genetically modified to produce new drugs and biologics that can prevent or treat diseases and save lives¹. In this process, plants themselves become “factories” that manufacture therapeutic proteins. These proteins are then extracted, refined, and used as active pharmaceutical ingredient (API) in many medicines. Plants and their products have been used for centuries to prevent and cure diseases. More than a quarter of all the medicines used in the world today contain ingredients derived from plants. However, recently it is only biotechnology that has been used to generate plants that produce specific therapeutic proteins, products that are traditionally synthesized using recombinant microbes or transformed mammalian cells². The first of these plant derived pharmaceutical proteins (PDPs) are now approaching commercial release^{3, 4}. Despite industry inertia and conservatism, plants have emerged as one of the most promising general production platforms for tomorrow’s biologics. Plants allow the cost-effective production of recombinant proteins on an agricultural scale, while eliminating risks of product contamination with endotoxins or human pathogens^{5, 6, 7, 8}. Another advantage of the use of plants in recombinant protein production is that vaccine candidates can be expressed in edible plant organs, allowing them to be administered as unprocessed or partially processed material⁹.

During the last two decades, approximately 95 biopharmaceutical products have been approved by one or more regulatory agencies for the treatment of various human diseases including diabetes mellitus, growth disorders, neurological and genetic maladies, inflammatory conditions, and blood dyscrasias¹⁰.

Why only the plants?

1. Plants are the cheapest, most abundant source of protein on the planet.
2. Plant provide low upstream production
Costs: low cost
3. Plants, as eukaryotes, can express and process most prokaryotic and eukaryotic proteins: stability
4. Plant provide scalable production capacity and flexibility: easy scale up
5. Plant seed permit stockpiling of inexpensive inventory: good storage
6. Plants are free from animal and human
Pathogens: safety
7. Using plants as factories to produce therapeutic proteins also enables researchers ability to

develop novel and complex molecular forms that could not otherwise be produced in mammalian cell cultures.

"Plants are the most efficient producers of proteins on earth."
Roger Beachy, Ph.D., president,
Donald Danforth Plant Science
Center

"Using plants to produce pharmaceuticals in the field could reduce the costs of goods by as much as 50 percent."
Peter Latham, president,
BioPharm. Services, Inc.

Transgenic Plants in the Biopharmaceutical Market:-

Plants that can be used for food crops are a natural choice for PMP production because researchers have extensive agricultural knowledge and familiarity of these plants, as well as experience with their growth. Scientists have a vast understanding of genetics, agronomics and the environmental impact these plants have, as well as their composition. Plants such as alfalfa, barley, corn, duckweed, rice, safflower and tobacco have received APHIS regulatory permits for field trials. These field trials are aimed at delivering the next generation of essential proteins for life-saving medicines.

Table 1: Examples of Current Plant-Made Pharmaceutical Research

Alfalfa	Plasma proteins
Arabidopsis	Human intrinsic factor (vitamin B12 uptake)
Corn	Anti-HIV and anti-Herpes simplex antibodies Microbicides for pulmonary infection MABs for cancer, arthritis, and other auto-immune diseases like Crohn's disease Vaccines for Hepatitis B transmission and Traveler's disease TGEV for animal health Aprotinin for blood loss and heart surgery Vaccines and antibodies for animal disease prevention Antibodies
Lemma	Human plasminogen for peripheral arterial occlusion Alpha interferon
Moss	Factor IX for treatment of haemophilia B
Rice	Alternatives to antibiotics in poultry diets Lysozyme for gastrointestinal health, topical infections and inflammations
Safflower	Pharmaceuticals and oil-body-based products for oral and dermal delivery
Spinach	Protective antigen for vaccine against Bacillus anthracis
Tobacco	TGF- glucocerebrosidase for Gaucher's disease Cancer vaccine for non-Hodgkin's lymphoma Alpha galactosidase for enzyme replacement therapy IgGs for the prevention of dental decay, prevention of common cold, and neutralization of chemotherapeutic drug toxicity GAD 7 cytokines for type 1 Diabetes IL-10 for inflammatory bowel disease Glycoprotein B of human cytomegalovirus (hCMV)

Tobacco, corn	Gastric lipase for cystic fibrosis Lactoferrin for gastro-intestinal infection and dry eye syndrome
Tomato, potato, Potato tuber	Edible vaccines against E.coli, Norwalk virus, Hepatitis B Antimicrobial peptides

Production of PMPS:-

Advance in biotechnology have made it possible to genetically enhance plants to produce therapeutic proteins essential for the production of a wide range of pharmaceuticals such as monoclonal antibodies, enzymes and blood proteins. These plants are grown under highly regulated conditions in confined growing environments and are strictly regulated by the U.S. Department of Agriculture (USDA). After the plants are harvested, they go through a series of processing steps that extract, separate, purify and package the therapeutic proteins. The refined therapeutic properties are ultimately used as the API in many life saving medicines.



Figure 1: Steps involved in making a Plant Made Pharmaceuticals (PMPs)

Transgenic Plants as Protein Factories:-

Plants are one of many possible systems for producing protein, which also include microbial, fungal and animal cell cultures, and transgenic animals. Other plant systems include transgenic plant cell cultures^{11, 12} and non-transgenic plants inoculated with recombinant plant viruses^{13, 14, 15, 16}. Potentially, plants provide a cheap source of recombinant proteins¹⁷, such as industrial enzymes¹⁸, technical materials¹⁹ and biopharmaceuticals²⁰. Using the existing

infrastructure for crop cultivation, processing, and storage will reduce the amount of capital investment required for commercial production.

Table:-2 Examples of Pharmaceutical Proteins Expressed in Transgenic Plants

S.No.	Protein	Transformed species
1.	Trout growth factor	<i>N. tabacum</i> ²⁰
2.	Human α -interferon	<i>O. sativa</i> ²¹
3.	Hirudin	<i>N. tabacum</i> ²²
4.	Erythropoietin	<i>N. tabacum</i> ²³
5.	Glucocerebrosidase, human Protein C serum protease	<i>N. tabacum</i> ²⁴
6.	Human α and β hemoglobin	<i>N. tabacum</i> ²⁵
7.	Human muscarinic Cholinergic receptors	<i>N. tabacum</i> ²⁶
8.	Murine granulocyte-macrophage Colony stimulating factor	<i>N. tabacum</i> ²⁷
9.	Interleukin-2 and Interleukin-4 Suspension cell	<i>N. tabacum</i> ²⁸
10.	Human placental alkaline Phosphatase Rhizosecretion	<i>N. tabacum</i> ²⁹
11.	Human α 1-antitrypsin Suspension cells	<i>O. sativa</i> ³⁰
12.	Human growth hormone (Somatotrophin) seeds	<i>N. tabacum</i> ³¹
13.	Human growth hormone	<i>N. tabacum</i> ³²

Vaccines:-

Vaccines consist of any antigenic substance(s) (proteins, peptides, attenuated living or killed organisms) capable of eliciting an immune response that precludes infection or disease manifestation upon later challenge by a pathogenic organism.

Considerable developments have occurred since the idea for transgenic plant vaccines was first envisaged by Charles Arntzen in the early 1990s^{33, 34, 35}. The Children's Vaccine Initiative, established in 1992, aims to develop cheap edible vaccines for the developing world. Several transgenic plant vaccines, for both human and animal use, have been tested in animals against diseases such as hepatitis B³⁶ and foot-and-mouth³⁷.

There are, however, some limitations associated with the use of transgenic plants for vaccine production³⁸. A major limitation of the expression of recombinant antigens in transgenic plants is obtaining a protein concentration adequate to confer total immunity, given varying protein expression among and within the various plant species. Tight control of expression yields will likely be necessary to reduce variability and assure consistent, effective immunization³⁸.

During the last decade, nearly a dozen vaccine antigens have been expressed in plants (Table 3). Transgenic potatoes can produce antigens of enterotoxigenic *E. coli* heat labile enterotoxin B subunit, and is effective in immunizing against viruses and bacteria that cause diarrhoea. Still other 'edible vaccines' are under development for rabies, foot and mouth disease (veterinary), cholera, and autoimmune diabetes. Transgenic lupin and lettuce plants can express hepatitis B surface antigen. Efforts are underway to develop an 'edible vaccine' against the measles virus using the tobacco plant. A plant based oral subunit vaccine for the respiratory syncytial virus (RSV) using either the apple or the tomato is under development³⁹.

Table 3:- Recombinant Vaccines Expressed in Plants

S. No.	Vaccine antigen	Transformed species
1.	Escherichia coli heat-labile enterotoxin	<i>N. tabacum</i> , <i>S. tuberosum</i> ⁴⁰
2.	Norwalk virus capsid protein	<i>N. tabacum</i> , <i>S. tuberosum</i> ⁴¹
3.	Diabetes-associated autoantigen	<i>N. tabacum</i> , <i>S. tuberosum</i> ⁴²
4.	Hepatitis B surface proteins	<i>S. tuberosum</i> ⁴³
5.	Foot and mouth disease VP1 structural protein	Virus <i>A.thaliana</i> ⁴⁴
6.	<i>E. coli</i> heat-labile Endotoxins	<i>S. tuberosum</i> ⁴⁵
7.	Rabies virus	Virus particle ⁴⁶
8.	Human insulin-Cholera toxin B Subunit fusion protein	<i>S. tuberosum</i> ⁴⁷
9.	Foot and mouth disease VP1 structural protein	<i>Medicago sativa</i> ⁴⁸
10.	Hepatitis B virus surface antigen	<i>Lupinus luteus</i> <i>Lactucsativa</i> ⁴⁹
11.	Human cytomegalovirus glycoprotein B	<i>N. tabacum</i> ⁵⁰
12.	Diabetes-associated autoantigen	<i>N. tabacum</i> , <i>D. carota</i> ⁵¹

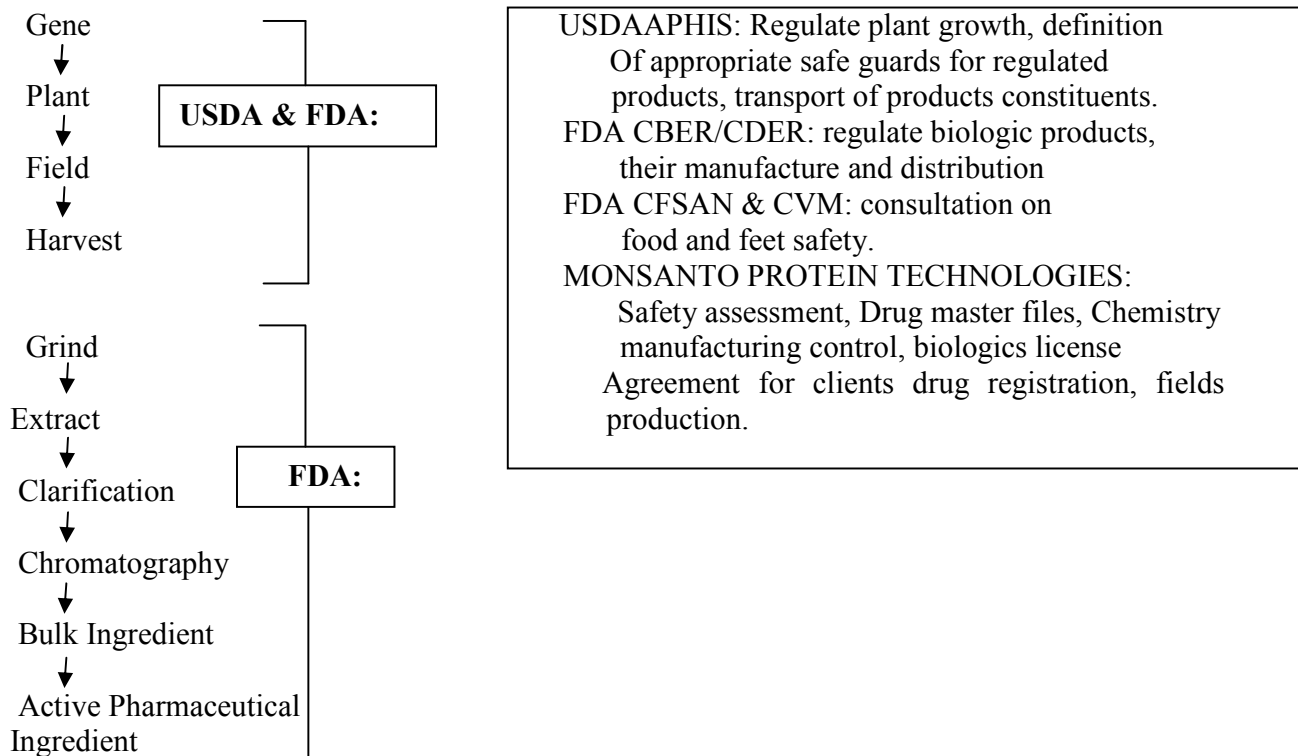
Antibodies:-

Antibodies are essential tools for the diagnosis, management and treatment of contagious diseases and cancers^{52, 53, 54, 55}. Compared with monoclonal antibodies (mAbs), recombinant antibodies (rAbs) have reduced immunogenicity, enhanced biological activity and are easier to detect^{54, 55}. Various functional rAbs have been produced in plants ('plantibodies') and have been successfully stored in dried leaves, seeds and potatoes, which demonstrates the potential for long-term storage of both the proteins and their production system (e.g.^{53, 54, 56, 57}). The first reported clinical trials of plant-produced antibodies were carried out by Planet Biotechnology, Inc (Mountain View, CA). Their drug CaroRx™ is based on tobacco-produced secretory IgA antibodies against Streptococcus mutants, a causal agent of human tooth decay^{52,56}. Plantibody research has highlighted the potential use of transient expression systems, including biolistic delivery of naked DNA using a particle gun, infection with transgenic viruses, and agroinfiltration^{52,53,58}.

Table: 4:- Recombinant Antibodies Expressed in Transgenic Plants

S.No.	Antibody format	Antigen	Plant organ	Cellular location	Transformed species
1.	IgG1	Phosphonate Ester	Leaf	ER	N. tabacum ⁵⁹
2.	IgM	NP hapten	Leaf chloroplast	ER	N. tabacum ⁶⁰
3.	scFv	Phytochrome	Leaf	Cytosol	N. tabacum ⁶¹
4.	scFv	Phytochrome	Leaf	Apoplast	N. tabacum ⁶²
5.	scFv	AMCV	Leaf	Cytosol	N. benthamiana ⁶³
6.	IgG	TMV	Leaf	Apoplast	N. tabacum ⁶⁴
7.	scFv	Cutinase	Leaf	ER	N. tabacum ⁶⁵
8.	scFv	BNYVV	Leaf	Apoplast	N. benthamiana ⁶⁶
9.	scFv	Abscisic acid	Leaf	ER	N. tabacum ⁶⁷
10.	scFv	Abscisic acid	Seed	ER	N. tabacum ⁶⁸
11.	scFv	Oxazolone	Tuber	ER	S. tuberosum ⁶⁹
12.	IgG	Human IgG	Plant	Apoplast	Medicago sativa ⁷⁰
13.	scFv	TMV	Leaf	Apoplast	N. tabacum ⁷¹

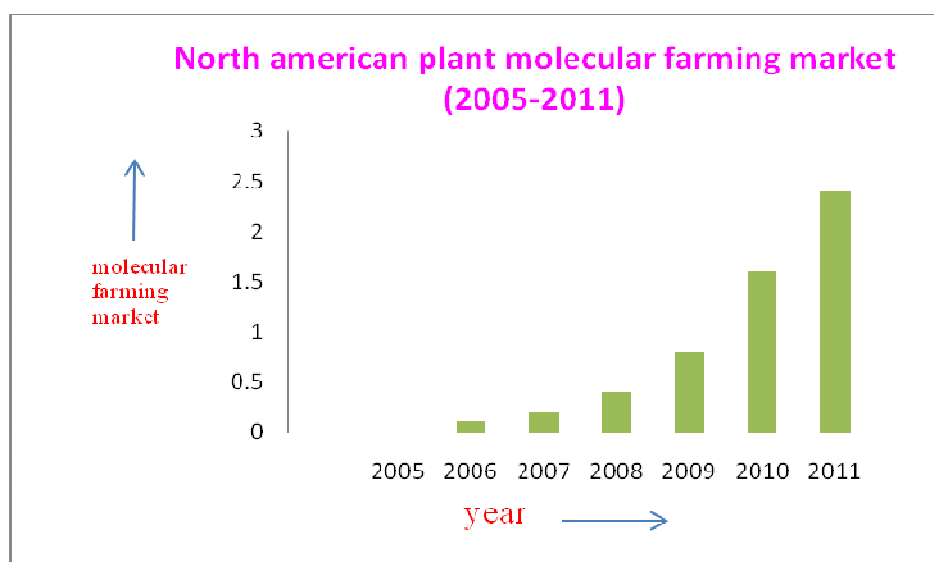
Regulation of Plant-Made Pharmaceuticals:-



Government approval and regulation of plant-made pharmaceuticals is even more extensive than current oversight of traditional ethical pharmaceuticals. In addition to the FDA, plant-made pharmaceutical research, development, testing and production also will be reviewed and regulated by the U.S. Department of Agriculture (USDA) under the 1986 Biotechnology Coordinated Framework. USDA and FDA regulations regarding development, testing, production, transportation and commercialization of plant- made pharmaceuticals are expected to adapt as the technology advances. Currently, a USDA field permit is required to plant crops that produce plant-made pharmaceuticals, with permits granted on a case-by-case basis. Prior to granting such a permit, USDA reviews all plans for seed production, timing of pollination, harvest, crop destruction, shipment and confinement. A USDA permit also is required to ship viable seed from modified plants that produce plant made pharmaceuticals, and double containment is required. All field trials are inspected by APHIS. There are criminal penalties for any violation of the regulations. According to the Biotechnology Industry Organization (BIO) — the primary trade association for companies that produce bi tech products—its member companies operate under strict quality assurance systems and standardized operating procedures that cover every aspect of plant-made pharmaceutical development.

Transgenic Plant Market Analysis:-⁷²

Transgenic crops have been commercially available for almost 10 years, and the market consists of two main sectors – crops conferring herbicide tolerance and those conferring insect resistance. The US is the biggest grower of transgenic crops, with 42 million hectares grown in 2003, making up 63% of the entire sector and generating revenues of \$1,500 million annually. No therapeutics are anticipated for launch before 2006, but the revenues generated from these products have the potential to drive the market to an estimated \$2,200 million by 2011, at a rate of 104.3% with this high growth rate indicating that the market effectively starting from zero. Market expansion will be highest around 2007-2008 as revenues from products released early in the development phase are realised. From 2011 onwards, the market is expected to continue expanding as drugs released late in the forecast period generate further revenues, and new products enter the development pipeline. The anticipated adoption rate of new products is likely to be fuelled by the relatively lower cost of products manufactured from biopharming compared with conventionally manufactured biopharmaceuticals.



However, the market for biopharmed products is still at the emerging stage and to date three products manufactured in transgenic plants have been commercialised for use as industrial enzymes. Prodigene Inc (College Station, manufactures aprotinin (AproliZean) from corn plants, while the Large Scale Biology Corporation (LSBC) (Vacaville, California) also manufactures aprotinin from tobacco plants. The closest product to market is CaroRx for the treatment of dental caries, which is manufactured by Planet Biotechnology Inc (Hayward, California) and is in Phase III clinical trials. Planet specialises in the expression of secretory Ig A antibodies in tobacco plants, which are more resistant to proteolytic degradation than conventional Ig G variants, and can be expressed at higher levels. Planet is collaborating with LSBC concerning the extraction of the CaroRx product, which could potentially reach the market in 2006. The company also has two other early stage products in clinical trials: RhinoRx for the treatment of the common cold, and DoxoRx for the treatment of chemotherapy-induced alopecia.

Conclusion

Above data suggests that plant based pharmaceuticals has paved the way to evergreen pharmaceutical industry. The widespread acceptance of derivatives of the transgenic plants in the field of biotechnology resulted in therapeutic agents to combat various diseases. Due to its easier and cost effective production has lead to new emerging biotechnology market. There are many plant derived pharmaceuticals which are in pipe line and some are at the experimental stages in laboratory.

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