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Unlocking the Potential of Molecular Farming-A Comprehensive Overview

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SUMMARY

Recently, modern biotechnology has unveiled plants as a promising new reservoir of pharmaceutical proteins, encompassing vaccines, antibodies, blood substitutes, and various therapeutic agents. Diverging from mammalian-derived rDNA drugs, plant-derived counterparts offer distinct advantages by circumventing mammalian viral vectors and human pathogens. Plants present an array of benefits, including cost-effective cultivation, prolific biomass production, swift transition from gene manipulation to protein expression, minimal capital and operational expenses, scalable production, eukaryotic post-translational modifications, and a robust protein yield. This burgeoning field of molecular farming introduces a transformative avenue for molecular medicines, ranging from plasma proteins, enzymes, and growth factors to vaccines and recombinant antibodies, all comprehensively understood at a molecular level. Bio-pharming holds the promise of abundant and affordable reservoirs of pharmaceutical drugs, encompassing vaccines targeting infectious diseases and therapeutic proteins tailored for conditions such as cancer and cardiovascular ailments.

INTRODUCTION

Molecular farming, known as "agrobiotechnology" in some circles, encompasses the genetic alteration of agricultural commodities to generate proteins and chemicals for commercial and pharmaceutical use or this process involves the cultivation of plants to produce pharmaceutically significant and economically valuable proteins [4]. The significant expense of medical treatments in many developing nations necessitates the production of not only novel drugs but also more affordable alternatives to those already available. Molecular farming presents efficient solutions to address the burgeoning demand for biomedicines.

Plant molecular farming involves the cultivation of plants in agriculture not for food, feed, or fiber, but rather for the production of pharmaceutical or industrial compounds. The spectrum of possibilities spans from the creation of medical products like drugs and vaccines to the synthesis of items such as biodegradable plastics and industrial chemicals [18]. Within this realm, molecular farming in plants holds the potential to yield virtually limitless quantities of recombinant proteins, serving as invaluable diagnostic and therapeutic tools in healthcare and the life sciences. Leveraging the prolific biomass generated by plants, protein production can be amplified through techniques like plant suspension cell culture in fermenters or the cultivation of genetically modified plant lines in the field [9]. Moreover, transgenic plants can be engineered to yield organs abundant in specific recombinant proteins, facilitating their long-term storage [13]. This underscores the potential of utilizing transgenic plants as bioreactors for the molecular farming of recombinant therapeutics, encompassing vaccines, diagnostics like recombinant antibodies, plasma proteins, cytokines, and growth factors. Process of Agrobiotechnology entails modifying plant cells to produce valuable proteins, often with therapeutic applications in humans [18]. Human proteins are commonly targeted due to the risk of viral or prion contamination associated with proteins sourced from natural origins, a risk that is largely absent in plants. Illustrative Example of Agrobiotechnology An exemplar of agrobiotechnology is the production of hirudin from Brassica napus seeds.

Objectives and Scope of Molecular farming

Agrobiotechnology, or molecular farming, focuses to provide a safe and cost-effective approach for the large-scale production of recombinant pharmaceutical proteins. Its goal is to leverage agriculture's capabilities to cultivate and harvest plants for the synthesis of recombinant therapeutics, diagnostics, industrial enzymes, and environmentally friendly chemicals.

Biotechnology's Role in Molecular farming

Molecular farming represents an experimental application of biotechnology, involving genetic modification of crops to yield proteins and chemicals for medicinal and commercial purposes. The majority in the developing world faces challenges affording therapeutics produced through conventional methods. In summary, plants offer a cost-effective and straightforward means of producing valuable recombinant proteins on a large scale, boasting numerous advantages in terms of cost, safety, and applicability. While utilizing transgenic plants presents certain limitations and concerns, ongoing optimization efforts aim to address these issues. Molecular farming in plants has largely dominated the production of pharmaceutical proteins, as other systems such as bacteria, yeasts, animal cells, and transgenic animals have been supplanted due to their inherent limitations. The successful production of various recombinant proteins from plants underscores their potential as bioreactors, capable of synthesizing a wide array of pharmaceutical agents. This approach addresses the increasing demand for biomedicines, particularly in light of the inefficiencies and high costs associated with existing production systems.

Why plants are used as production system?

The utilization of plants for expressing recombinant proteins began in the late 1980s [6]. Since then, the plant expression platform has encountered numerous challenges. However, in 2012, Protalix Biotherapeutics commercialized the first plant-based product, "Elelyso," for treating Gaucher's disease [3]. The use of plants for producing high-value recombinant proteins, ranging from pharmaceutical therapeutics to non-pharmaceutical products such as antibodies, vaccine antigens, enzymes, growth factors, research or diagnostic reagents, and cosmetic ingredients [25], has steadily improved and advanced significantly over recent decades. This progress has triggered a substantial paradigm shift in the pharmaceutical sector.

- Plants exhibit remarkable flexibility, capable of producing a wide array of proteins in significant quantities.
- Crop plants have the ability to synthesize diverse proteins devoid of mammalian toxins and pathogens.
- Crop plants yield large biomass volumes at low cost, making industrial-scale production feasible even in limited facilities.
- Functional proteins undergo post-synthesis modifications akin to eukaryotes.
- Proteins can be stored within compartments or seeds, remaining stable until required.
- Consequently, crops are highly suitable for producing safe, cost-effective therapeutic proteins and other goods.
- Simplification or omission of purification steps is feasible.

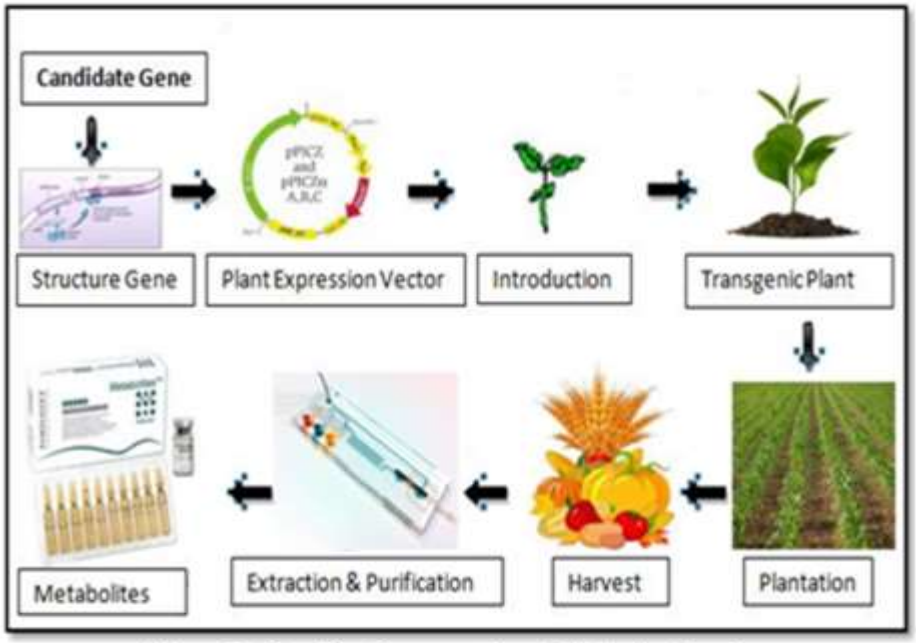

Figure 1: Simplified Representation of Molecular Farming

Source: [18]

Production of Biopharmaceuticals in Transgenic Plants

Biopharmaceuticals, which encompass proteins such as antibodies, produced within living systems for therapeutic, diagnostic, or dietary supplement purposes, have entered the realm of biotechnology-driven innovation. This approach, often termed "pharming," "bio pharming," or "molecular farming," has transitioned from theoretical speculation to practical experimentation, with trials underway in fields and greenhouses

nationwide [9]. Through genetic modification, plants have emerged as a promising avenue for the production of pharmaceutical proteins, marking a significant advancement in biotechnology. This recognition extends to the potential synthesis of vaccines, antibodies, blood substitutes, and various other therapeutic agents within plant systems (Table 1).

Table 1: Some examples of plants used for biopharmaceutical production

S.No	Category	Plants used
1	Model plants	Arabidopsis thaliana
2	Leafy crops	Tobacco, lettuce, alfalfa, clover
3	Cereals	Maize, rice, wheat, barley
4	Legumes	Soybean, pea, pigeon pea
5	Fruits and vegetables	Potato, carrot, tomato, banana
6	Oil crops Oilseed	Rape, Camelina sativa
		Source: [18]

Plant-Derived Recombinant Proteins

The idea of leveraging plants for producing foreign proteins, including pharmaceutical and non-pharmaceutical variants, has been extensively researched and documented. Numerous studies have demonstrated the efficacy of in vivo and in vitro plant systems in generating vaccine candidates for veterinary and human applications. Additionally, these studies have shown that plant-produced antigens can elicit robust immune responses in animal models and confer protection in challenge experiments. Table 2 & Table 3 illustrate examples of the diverse pharmaceutical and non-pharmaceutical proteins expressed in plant systems.

Table-2 Selected list of vaccine candidates and antibodies synthesized in plants targeting diverse diseases.

Vaccine Candidates					
Recombinant Protein	**Pathogen/Disease**	**Expression System**	**Transformation Method**	**Expression Level**	**Reference**
Hepatitis B surface antigen	Hepatitis B virus	Tobacco (Nicotiana tabacum)	Agrobacterium mediated (Stable expression /Nucleus)	66 ng/mg of soluble protein	[14]
Structural protein VP60	Rabbit hemorrhagic disease virus (RHDV)	Potato (Solanum tuberosum)	Agrobacterium mediated (Stable expression /Nucleus)	0.3% of total soluble protein	[2]
Spike (S) protein of transmissible gastroenteritis virus	Transmissible gastroenteritis virus (TGEV)	Tobacco (Nicotiana tabacum)	Agrobacterium mediated (Stable expression /Nucleus)	0.1–0.2% of total soluble protein	[26]
Hemagglutinin protein of rinderpest virus	Rinderpest virus (RPV)	Peanut (Arachis hypogea L.)	Agrobacterium mediated (Stable expression /Nucleus)	0.2–1.3% of total soluble protein	[10]
Spike (S) protein of transmissible gastroenteritis virus	Transmissible gastroenteritis virus (TGEV)	Corn (Zea mays)	Agrobacterium mediated (Stable expression /Nucleus)	13 mg/kg FW	[12]
Hepatitis B virus surface antigen	Hepatitis B virus (HBV)	Potato (Solanum tuberosum)	Agrobacterium mediated (Stable expression /Nucleus)	8.5 g/g FW	[24]
F4 fimbrial	Enterotoxigenic E.	Alfalfa	Agrobacterium	1.0% of total	[8]

adhesin FaeG	col	(Medicago sativaL.)	mediated (Stable expression/ Chloroplast)	Soluble protein	
L1 capsid protein gene	Cottontail rabbit papillomavirus	Tobacco (*Nicotiana tabacum*)	Agrobacterium mediated (Stable expression /Nucleus)	0.4–1mg/kg of total leaf mass	[11]
Structural protein VP2	Infectious bursal disease virus (IBDV)	Rice	Agrobacterium mediated (Stable expression /Nucleus)	40.21 g/g FW	[29]
Hepatitis B virus surface antigen	Hepatitis B virus (HBV)	Tobacco (*Nicotiana benthamiana*)	Agrobacterium mediated (Transient expression)	295 g/g FW	[7]
Heat-labile toxin B subunit (LTB)	Enterotoxigenic E. coli	Carrot (Daucus carota)	Agrobacterium mediated (Stable expression /Nucleus)	0.3% of total soluble protein	[17]
Structural protein VP1	Foot-and-mouth diseasevirus (FMDV)	Legume (Stylosanthes guianensis)	Agrobacterium mediated (Stable expression /Nucleus)	0.1–0.5% of total soluble protein	[28]

Table-3 Selected list of various non-pharmaceutical proteins produced in plants.

Recombinant Protein	Expression System	Transformation Method	Expression Level	Reference
Human serum albumin	Potato (*Solanum tuberosum*)	Agrobacterium mediated (Stable expression/Nucleus)	0.25 g/mg (0.02% of total soluble protein)	[20]
Erythropoietin	Tobacco (*Nicotiana tabacum*)	Agrobacterium mediated (Stable expression/Nucleus)	26 pg/mg total protein	[15]
Alfa 1-antitrypsin	Rice (*Japonica* rice)	Biolistic method (Stable expression/Nucleus)	4.6–5.7mg/gdry cell	[23]
Aprotinin	Corn	Biolistic method (Stable expression/Nucleus)	0.069% of total extractable seed protein	[32]
Human basic fibroblast growth factor(bFGF)	Rice (*Oryza sativa*)	Agrobacterium mediated (Stable expression/Nucleus)	185.66mg/kg	[1]
Lumbrokinase	Sunflower (*Helianthus annuus* L.)	Agrobacterium mediated (Stable expression/Nucleus)	5.1g/kgseeds	[5]
Human acidic fibroblast growth factor1(FGF-1)	*Salvia miltiorrhiza*	Agrobacterium mediated (Stable expression/Nucleus)	272ng/gFW	[21]

Approaches Employed for Recombinant Protein Production in Plants

The methods utilized for expressing recombinant proteins in plants can be categorized as stable or transient expression. PMF employs the following strategies for expressing vaccine candidates: stable nuclear transformation, stable chloroplast transformation, or transient expression through the use of plant viral vectors. Additionally, stable transformation of hydroponically cultivated plants enables the recovery of recombinant plant proteins from the growth medium [16] (Figure 2).

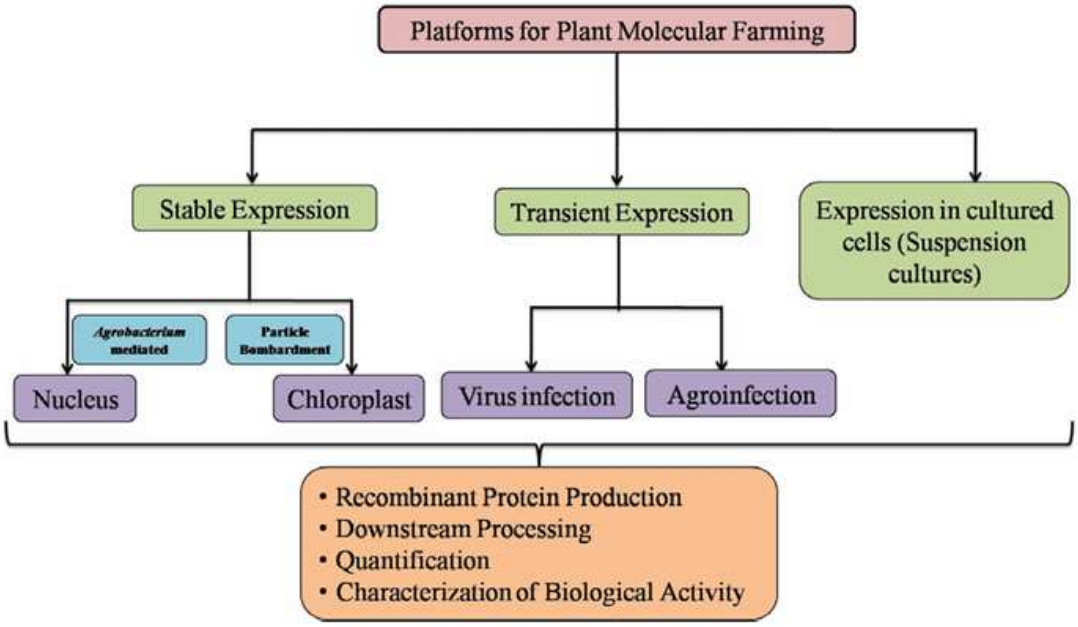

Figure 2. Overview of plant transformation approaches employed for the production of recombinant pharmaceutical and non-pharmaceutical proteins in plants. Source [18]

Stable transformation in plants demands considerable time and labor-intensive efforts, often resulting in insufficient protein expression to meet industrial-scale production requirements. Nonetheless, antigen expression in stable transgenic lines holds promise for developing oral vaccines, potentially reducing costs associated with protein purification [27,31]. Alternatively, transient expression methods such as agroinfiltration or virus-based vectors have been devised to complement transgenic plant systems, offering rapid and high-level protein expression within a few days. This approach is deemed a convenient platform, particularly for producing vaccine antigens or monoclonal antibodies targeting infectious diseases. Cell suspensions derived from undifferentiated callus cultures grown in liquid medium can be upscaled in bioreactors for large-scale protein production under sterile conditions. Notably, the first USDA-approved poultry vaccine and the inaugural FDA-approved recombinant pharmaceutical protein produced in plants, known as "Elelyso," were cultivated in tobacco and carrot cell suspension cultures, respectively. These milestones underscore the significance and competitiveness of plant suspension culture in the high-value protein production landscape of the biopharmaceutical industry [19,22,30,31].

Advantages of Molecular farming

Plant molecular farming (PMF) mitigates risks of batch loss and cross-contamination. Plant cells are impervious to human pathogens, and vice versa, reducing the risk of batch contamination. Additionally, plants serve as single-use disposable bioreactors, minimizing the risk of product cross-contamination.

Significance of Molecular farming

The core objective of agrobiotechnology is to manufacture ample quantities of reliable pharmaceutical proteins at reduced costs. Recent advancements in biotechnology have greatly enhanced gene transfer techniques in plants, making this approach increasingly feasible.

CONCLUSION

Plants present numerous advantages over traditional systems in the molecular farming of pharmaceutical proteins. These advantages include cost-effectiveness, rapid scalability, absence of human pathogens, and the accurate folding and assembly of complex proteins. It is conceivable that plants may eventually surpass other production systems due to their economic and safety benefits, potentially enabling widespread access to affordable pharmaceuticals for those in need. For the biotechnology and pharmaceutical industries, bio-pharming holds the promise of both economic and health advantages once products reach the commercialization phase. Achieving the full potential of plant molecular farming necessitates a combination of robust regulatory oversight and technological innovations. It is essential to recognize plants as a viable option among various manufacturing methods for therapeutic proteins. Attention is now shifting from foundational research towards commercial utilization, signalling that molecular farming is poised to rival established production technologies utilizing bacteria, yeast, and cultured mammalian cells.

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