

REVIEW OPEN ACCESS

Molecular Pharming: Advances, Applications, and Future Prospects in Biotechnology and Medicine

Md. Hridoy Ahmed^{1,2}  | Md. Mustak Khan³ | Shishir Dutta⁴ | Md. Foyzur Rahman⁵  | Mohammad Shariful Islam⁶ | Md. Najmul Hosen⁷ | Md. Saad Hossain⁸ | Md. Aftabur Rahman⁹ | Md. Sadman Hasan Sahil¹⁰ | Tanjuma Tasnim Hira¹¹ | Ifthesum¹² | Ashikur Rahaman¹ | Md. Afser Rabbi¹³ | Laila Khaleda¹

¹Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong, Bangladesh | ²BioPC – A Bioinformatics Lab of Research and Training, Chattogram, Bangladesh | ³Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong, Bangladesh | ⁴Department of Soil Science, University of Chittagong, Chittagong, Bangladesh | ⁵Department of Pharmacy, Dhaka International University, Dhaka, Bangladesh | ⁶Department of Zoology, University of Chittagong, Chittagong, Bangladesh | ⁷Department of Biochemistry and Biotechnology, University of Science and Technology, Chittagong, Bangladesh | ⁸Department of Genetic Engineering and Biotechnology, East West University, Dhaka, Bangladesh | ⁹Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka, Bangladesh | ¹⁰Faculty of Pharmacy, Jashore University of Science and Technology, Jashore, Bangladesh | ¹¹Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh | ¹²Department of Botany, University of Chittagong, Chittagong, Bangladesh | ¹³Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet, Bangladesh

Correspondence: Md. Hridoy Ahmed (Hridoy.geb.cu@gmail.com)

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ABSTRACT

Genetically engineered plants incorporate the use of a novel bioreactor known as molecular pharming, which has a transformative view on the pharmaceutical industry. The technique enables mass production, at a low cost, and reproducibly of a large number of different protein-based drugs, vaccines, and industrial enzymes. This review-based study outlines the chronological evolution of molecular pharming, investigates its essential principles and elective applications, and meticulously compares it with other methods, namely conventional biomanufacturing. We present the numerous host organisms employed, the leading-edge genetic engineering procedure, and the sophisticated approaches for protein purification and extraction. Additionally, we deliver in-depth analysis of the noteworthy advantages that take place in molecular pharming as a captivating substitute, in conjunction with obstinate challenges, including concerns of public insights, intricate regulatory frameworks, and consideration for economic sustainability. Finally, this comprehensive study explores the promising direction, evolving innovations, and essential areas that influence future research to fully reveal the extensive potential of plant-based biopharmaceutical production for industrial strains and global health.

1 | Introduction

Molecular pharming, which is known as biopharming, represents a groundbreaking biotechnological approach that leverages genetically engineered plants to produce high-value protein-based pharmaceuticals. These include essential vaccines, therapeutic antibodies, vital enzymes, and critical blood products

for both human and animal applications [1, 2]. This amazing technology harnesses the inherent cellular machinery of plants to express and assemble complex proteins, which offers a highly flexible and remarkably cost-effective platform for a diverse range of biotechnological and medical applications [3–6]. By tapping into the natural protein synthesis capabilities of plants, molecular pharming presents itself as a coercive and affordable alterna-

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tive to conventional drug production methods, promising more accessible medicines, particularly in resource-limited settings [7–9].

Molecular pharming began to take shape in the late 1980s and early 1990s, which is sparked by significant advancements in genetic engineering techniques, plant transformation methodologies, and downstream processing technologies, which leads to the production of plant-made antibodies, vaccines, and therapeutic proteins [10–12]. Initially, proving the concept's workability demonstrated the feasibility of the approach. Subsequent efforts have progressively aimed at scaling up production processes and substantially improving protein yields. Key milestones marking the field's evolution include the successful development of transgenic plants such as rice, potatoes, and tobacco, which demonstrated the capability to produce a variety of therapeutic proteins and vaccines. More recently, the global disruption caused by the COVID-19 pandemic significantly accelerated the development of this field [2, 13]. It underscored the critical need for rapid production techniques and vividly demonstrated the capacity of plants to swiftly and extensively manufacture therapeutic proteins and vaccines, thereby highlighting their pivotal role in transforming both biotechnology and global health strategies.

This review aims to provide a thorough and insightful overview of molecular pharming, encompassing its comprehensive historical context, the fundamental scientific principles that underpin its operation, its current and diverse applications, and its future prospects. Our objective is to capture and articulate the significant advancements, the inherent difficulties, and the potential trajectory of this dynamic field. We will place particular emphasis on the critical technologies that drive its progress, the expanded production techniques being developed, and the influential industry leaders contributing to its evolution.

2 | Fundamentals of Molecular Pharming

At its core, molecular pharming operates on the basic principles of genetic engineering, fundamentally transforming plants into efficient, living bioreactors. This intricate process involves the precise introduction and subsequent expression of foreign genes, typically recombinant DNA, within various plant cellular structures, including whole plant cells, differentiated tissues, or suspension cultures.

2.1 | Basic Principles and Mechanisms

The foundational mechanism involves the controlled integration and expression of foreign genes, which can be able to encode the desired therapeutic proteins, into the plant's genome. A widely adopted strategy directs target proteins to specific plant compartments, such as chloroplasts [14]. Targeted protein expression boosts overall yields and ensures stable transgene integration, often aided by maternal inheritance, which helps to reduce the risk of unintended gene transfer [15]. A noticeable advantage of employing plant systems is their intrinsic capacity to perform essential posttranslational modifications [16]. These cellular adjustments, vital for the proper folding, activity, and stability

of complex therapeutic proteins, are faithfully executed by the plant's cellular machinery. This capability is vividly demonstrated by the successful creation of monoclonal antibodies in plants that exhibit humanized N-glycosylation patterns, a critical feature for reducing immunogenicity and enhancing therapeutic efficacy [17]. Additionally, advances like AgroLux, a bioluminescent *Agrobacterium* strain, have emerged, utilizing luciferase enzymes to provide a real-time, noninvasive readout of gene expression during agroinfiltration experiments [18]. As illustrated in Figure 1, these innovations provide deeper insights into how gene expression unfolds over time and across plant tissues, giving researchers greater control over protein production.

2.2 | Genetic Engineering Techniques

To successfully implement molecular pharming, it heavily depends on a set of complex and advanced genetic engineering techniques that facilitate the precise insertion and effective expression of foreign genes within plant cells.

- ***Agrobacterium*-Mediated Gene Transfer:** This technique is most widely used and highly effective for the stable insertion of genes into plant genomes. The process initiates with identifying and isolating the specific gene that is required for insertion into the plant's genome, as shown in Figure 2. Then, this target gene is carefully cloned into a specialized plasmid vector; in this case, a tumor-inducing (Ti) plasmid from *Agrobacterium tumefaciens* or a root-inducing (Ri) plasmid from *Agrobacterium rhizogenes* is commonly used [19]. Critically, these plasmids are engineered to carry not only the foreign gene but also a selectable marker gene (e.g., encoding resistance to an herbicide or antibiotic). This marker gene enables researchers to easily identify and select plant cells or plants that have successfully incorporated the recombinant plasmid. The modified *Agrobacterium* containing the recombinant plasmid is then co-cultivated with various plant tissues, including leaf discs [20], stem segments [21], root explants [19], cotyledons [22], hypocotyls [23], embryos callus [24], tissue suspension cultures [25], petals and floral tissues [26], and seedlings [27]. These co-cultivation conditions are carefully optimized to facilitate the transfer of the bacterium's T-DNA (transfer DNA) into the plant cells. Following this, transformed plant cells are precisely selected using the integrated marker gene, and through advanced tissue culture methods, these cells are regenerated into entire, genetically modified plants. This approach is highly favored due to its robust transformation effectiveness, its capacity to produce stable transgenic lines, and its broad applicability across a wide spectrum of plant species [28]. However, despite these benefits, the *Agrobacterium*-mediated transformation technique faces many limitations. This method was found to be less efficient for many kinds of monocot plants, including wheat and corn, which require intensive optimization of the transformation procedure [29]. Moreover, the random insertion of T-DNA could result in interruption of endogenous genes along with possible position effects and silencing, which pose biosafety risks [30]. Additionally, the regeneration procedure was found to be laborious and this resistance gene marker system faces regulatory constraints [31]. A summary

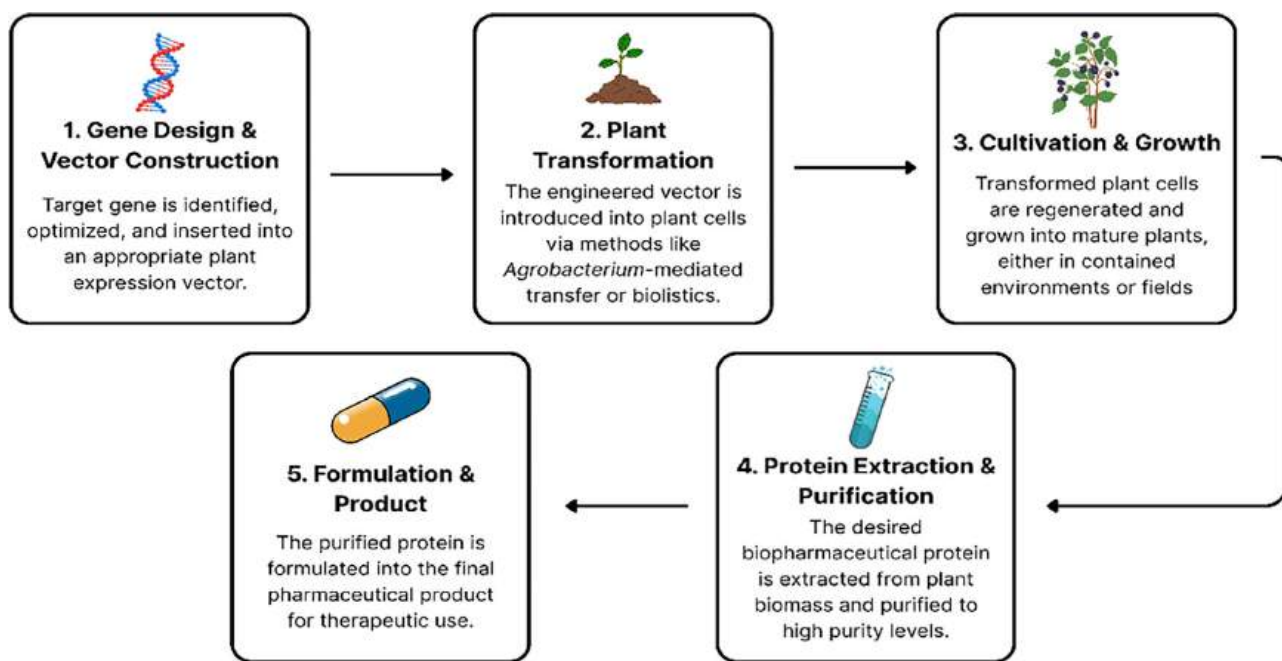


FIGURE 1 | A step-by-step overview of the main stages involved in making biopharmaceuticals using plant systems.

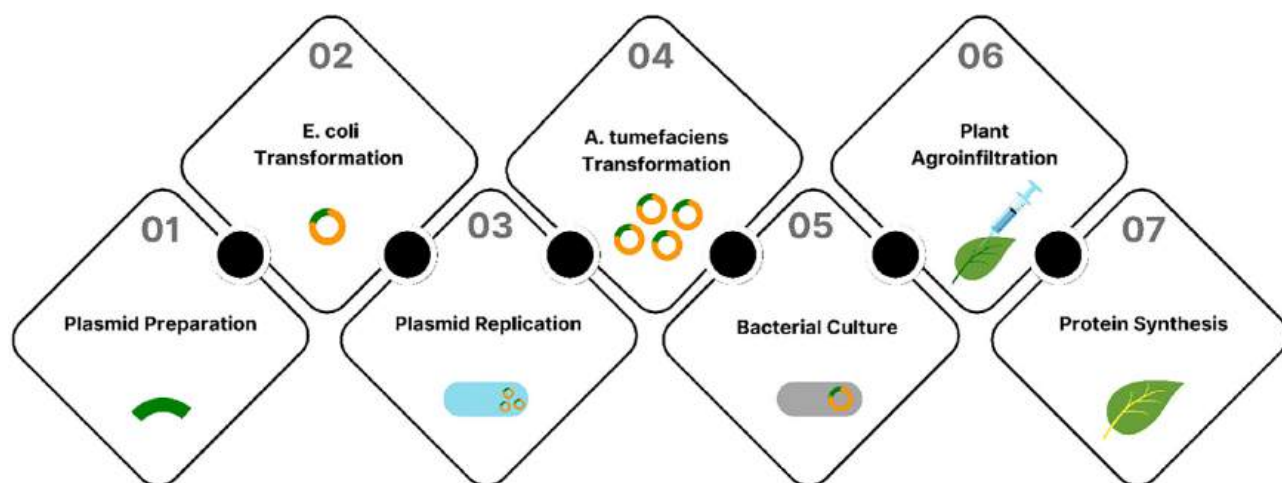


FIGURE 2 | *Agrobacterium*-mediated molecular pharming: An overview.

of *Agrobacterium*-mediated DNA transfer and its capabilities is shown in Table 1.

- **Particle Bombardment (Biolistics):** Also known as biolistics, this physical method directly delivers DNA into plant cells. In this process, microscopic gold or tungsten particles are coated with plasmid DNA, which encodes the desirable gene [32, 33]. These DNA-coated particles are then moved at high velocity into plant tissues or cells using a gene gun, a specialized device [34]. The particles penetrate the plant's cell walls and membranes physically; this allows the DNA to enter the cytosol. As the inserted foreign gene gets inside, it can now integrate with the plant's genome, which leads to its expression. The cells that transform are eventually selected and regenerated into complete plants. This method is particularly valuable as it can be applied to plant species that are not responsive to *Agrobacterium*-mediated gene transfer,

which makes it a versatile tool for both transient and stable genetic alteration in a wide range of tissues and cell types [35]. However, particle bombardment is also associated with some disadvantages. It is often prone to the occurrence of transgene insertion mutations, as it may generate several or broken transgene insertion mutations, leading to unstable transgene expression or transgene silencing [36]. Physical damage to the plant tissues may also reduce the cell viability as well as the efficiency of cell regeneration. Moreover, the use of the technique is hampered by the need to utilize expensive equipment [37].

- **CRISPR/Cas9 System:** A highly potential and precise gene-editing method for the accurate modification of specific genes within the plant genome [38]. In this process, RNA (gRNA), designed as a guide specifically targets a particular segment within the plant genome. Both the gRNA and the

TABLE 1 | *Agrobacterium* species and their DNA transfer capabilities.

Agrobacterium species	DNA transfer capability	Disease caused	Notes
<i>Agrobacterium tumefaciens</i>	High	Crown gall disease	Most widely studied and used for plant genetic engineering
<i>Agrobacterium rhizogenes</i>	High	Hairy root disease	Used for inducing hairy roots and genetic engineering
<i>Agrobacterium radiobacter</i>	High (similar to <i>A. tumefaciens</i>)	Crown gall disease (formerly)	Sometimes used interchangeably with <i>A. tumefaciens</i>
<i>Agrobacterium rubi</i>	Moderate	Cane gall disease	Less studied; potential DNA transfer capabilities
<i>Agrobacterium vitis</i>	Moderate	Crown gall disease in grapevines	Carries similar plasmids to <i>A. tumefaciens</i>
<i>Agrobacterium larrymoorei</i>	Suggested (needs further study)	Plant tumors	Genetically similar to <i>A. tumefaciens</i>
<i>Agrobacterium fabrum</i>	High (reclassified from <i>A. tumefaciens</i>)	Crown gall disease	Similar genetic profile to <i>A. tumefaciens</i>

Cas9 nuclease gene are carried by an expression vector in this technology. CRISPR/Cas9 vector is introduced to the targeted plant cells, through particle bombardment or *Agrobacterium*-mediated gene transfer methods. Once inside, the Cas9 nuclease enzyme, is guided by the gRNA, creates an accurate double-strand break at the target site of the genome. The plant's natural DNA repair mechanism fix this break, which leads to targeted gene insertion, deletion, or modification. The edited cells are then selected and eventually regenerated into whole plants. As this technology is highly precise and specific it is widely recommended, due to its ability to perform targeted gene removal, insertions, and modifications in a wide range of plant species [39]. Despite its advantages, CRISPR/Cas9 is not without limitations. Off-target mutations may occur if gRNA design is suboptimal, potentially resulting in unintended genetic alterations [40]. Efficient delivery of CRISPR components and regeneration of edited plants remain challenging in many species. In addition, regulatory uncertainties surrounding genome-edited crops in several countries may restrict its commercial application [41].

- **Transient Expression Systems:** These systems are implemented for swift and temporary expression of foreign genes without their stable integration into the plant genome. In this process the desired gene is cloned into a plasmid vector provided with regulatory elements, necessary for expression. A common cloning vector named pUC19, known for its multiple cloning sites and high copy number is used for this process [42]. The plasmid is then incorporated into *Agrobacterium*, which is eventually infiltrated into plant leaves (e.g., *Nicotiana benthamiana*) using either a syringe or vacuum infiltration technique. Within a few days after the infiltration, the gene expression occurs rapidly, which facilitates immediate examination of gene function or protein synthesis within the infiltrated tissues. These methods are extremely valuable to produce therapeutic proteins, antibodies, enzymes, and vaccines, as they allow for a rapid target gene expression, reduce the time required for protein synthesis compared to stable integration, and are ideal for functional genomics research and high-throughput screening (HTS) [43]. However,

there are some limitations to transient expression systems as well. The expression is transient and unhereditary. Thus, their use is limited to short-term expression. Large-scale transient expression also requires special facilities and optimum conditions for infection rates to produce the protein on a large scale [44].

- **Protoplast Transformation:** This method involves the introduction of DNA into plant cells (protoplasts) that have had their rigid cell walls enzymatically removed. The procedure entails applying specific enzymes to plant tissues to break down the cell walls, thereby producing naked protoplasts. Plasmid DNA can then be directly introduced into these protoplasts either through electroporation or by incubation in the presence of polyethylene glycol (PEG), or by RNP complex, both of which aid in DNA uptake into the cells [45, 46]. The transformed protoplasts are subsequently cultivated under controlled conditions to regenerate their cell walls and eventually grow into entire, genetically modified plants. This method is appropriate for both transient and stable genetic alteration, making it applicable for gene editing and functional research, as it permits direct entry into plant cells, ensuring efficient DNA uptake [10]. Despite its effectiveness at the cellular level, protoplast transformation faces significant limitations. Plant regeneration from protoplasts is highly species-dependent and remains inefficient for many economically important crops. The technique is technically demanding and time-intensive, and regenerated plants may exhibit somaclonal variation, limiting its applicability for large-scale molecular pharming [47].
- **Viral Vectors:** Viral vectors are an effective and temporary means of expressing genes in plants. In this process, by cloning the desired gene into a chosen viral vector, a recombinant viral genome is created. For plant-based molecular pharming, the commonly used viral vectors are adenoviruses, lentiviruses, and adeno-associated viruses (AAV) for gene therapy, while tobacco mosaic virus (TMV) or potato virus X (PVX) are used in plant-based molecular pharmacology [48]. The viral genome is designed in a way that it can include necessary regulators such as promoters, enhancers, and polyadenylation

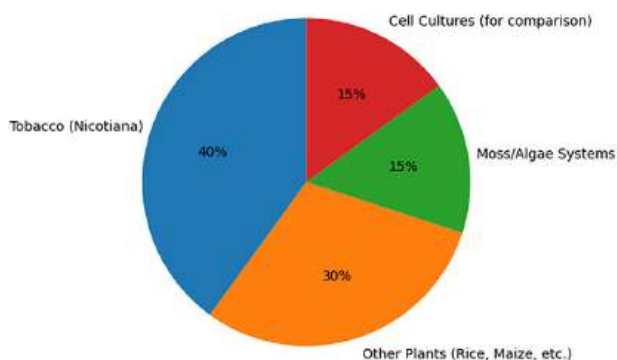


FIGURE 3 | Percentage of host organisms contributing to molecular pharming.

signals, along with the gene of interest [49]. This recombinant virus is then injected into plants, where it multiplies thoroughly and expresses the foreign gene throughout the plant. The target protein is rapidly produced and can be eventually extracted from the plant tissues. This method is suited for rapidly manufacturing large amounts of proteins and achieving uniform gene expression due to its rapid viral spread throughout the plant body along with having features such as high gene expression and delivery efficiency [50]. Nonetheless, viral vector systems cannot facilitate stable gene transfer and only permit transient gene expression. The capacity for foreign DNA will be limited by viral genome size. Biosafety issues pertaining to viral diffusion and control can be challenging [51, 52].

2.3 | Host Organisms for Molecular Pharming

Choosing a host organism can be pivotal to the success of molecular pharming. Several considerations contribute to this decision, such as the nature of the target protein (whether posttranslational modifications are necessary), the expected protein amount, and its ability to be improved. Figure 3 and Table 2 provide examples of frequently used host organisms in this area.

- **Plants:** Plants are generally considered good hosts for molecular pharming, which has the inherent facility of genetic manipulation, as well as it can be scaled up for large volume production and perform complex eukaryotic posttranslational processing. Their production in outdoor as well as indoor crops provides an increase in production scalability and lower costs. Furthermore, the low risk of contamination is another advantage because plants are devoid of human pathogens [53]. Commonly employed plant hosts include:
 - Tobacco (*Nicotiana tabacum* and *N. benthamiana*): *N. tabacum* has long been considered a prototypical plant for molecular pharming, mainly for its fast growth cycle, large biomass production, and robust transformation protocols. It has been widely used in the manufacture of a variety of therapeutic proteins, including vaccines and antibodies. On the contrary, a close relative of *N. benthamiana* is especially highly regarded for transient operation systems because of the very high viral vector and *Agrobacterium* infestation susceptibility, leading to really rapid expression of a protein [54].
 - Rice (*Oryza sativa*) and Maize (*Zea mays*): These two food crops are commonly used to express oral vaccines and therapeutic proteins, exploiting the extensive cultivation and processing infrastructure in place for each [55]. Rice has been used as the host plant to express human growth hormone and human serum albumin to demonstrate its ability to perform as an industrial scale-up medicinal protein. The advantages of maize. Further positive features of maize include its physiologic traits, Gene expression of recombinant proteins within seeds, and its GRAS (Generally Recognized As Safe) licensing status, as well as its existing agricultural feedstock.
 - Potatoes (*Solanum tuberosum*) and Tomatoes (*Solanum lycopersicum*): Potatoes and tomatoes are also used for biopharmaceuticals and edible vaccines, which can be delivered orally as a convenient route. The novel notion of “edible vaccines” exploits these plants as production and delivery vehicles [56].
 - Carrot (*Daucus carota*): Carrot constitutes an important biopharmaceutical production platform, due to its highly developed genetic transformation and the property of totipotency, which allows for the generation of complete plants from somatic cells [54]. The platform improves upon existing methods due to cost and a lack of human pathogens.
- **Non-Plants:** Transgenic animals can synthesize complex proteins that must undergo posttranslational modifications typical of mammals, which are frequently required for the therapeutic efficacy of these proteins. These proteins can be secreted by animals into easily accessible biological fluids such as milk or eggs, or accumulated in other tissues, which greatly facilitates large-scale production and purification [54, 55].
 - Goats (*Capra aegagrus hircus*): It is possible to create transgenic goats to generate therapeutic proteins in their milk. A notable example is ATryn, an antithrombin protein produced in the milk of transgenic goats, which is used to prevent blood clots in patients with genetic antithrombin deficiency [57].
 - Cow (*Bos taurus*): Transgenic cows can produce significant amounts of therapeutic proteins, including human antibodies, in their milk [58].
 - Chickens (*Gallus gallus domesticus*): Therapeutic proteins can be produced in the eggs of transgenic chickens, providing a scalable and noninvasive method of production, as eggs are practical bioreactors that are simple to gather and prepare [59].
 - Rabbits (*Oryctolagus cuniculus*): The milk of rabbits is utilized to produce lower amounts of therapeutic proteins [58]. Their short gestation time and speedy growth make them well-suited for shorter production cycles.
- Microorganisms, including bacteria and yeast, are characterized by their high protein expression levels, rapid growth rates, and the simplicity of their genetic modification [60]. These features render them particularly efficient in producing simple proteins and enzymes that require few complex posttranslational modifications.
 - *E. coli*: *Escherichia coli* is also a major bacterial host for recombinant protein expression. It has gained favor due to its fast growth rate, well-defined gene map, and the

TABLE 2 | List of common host categories and organisms for molecular pharming.

Host category	Organism	Key advantages/applications	Reference
Plants	<i>Nicotiana tabacum</i>	Fast growth, high biomass, robust transformation; widely used for vaccines and antibodies	[54]
	<i>Nicotiana benthamiana</i>	Highly susceptible to viral vectors and <i>Agrobacterium</i> ; ideal for transient expression systems	
	<i>Oryza sativa</i> (Rice)	Used for oral vaccines; expression of human growth hormone and serum albumin; scalable infrastructure	[69]
	<i>Zea mays</i> (Maize)	Seed-based protein expression; GRAS status; established agricultural feedstock; oral vaccines and therapeutics	[69]
	<i>Solanum tuberosum</i> (Potato)	Edible vaccines; oral delivery of biopharmaceuticals	[56]
	<i>Solanum lycopersicum</i> (Tomato)	Edible vaccines; oral delivery; convenient production and administration	[56]
	<i>Daucus carota</i> (Carrot)	Totipotency; efficient genetic transformation; low cost; lack of human pathogens	[54]
Animals	<i>Capra aegagrus hircus</i> (Goat)	Milk-based production of therapeutic proteins; ATryn antithrombin	[70]
	<i>Bos taurus</i> (Cow)	High-yield milk production of therapeutic proteins, including antibodies	[58]
	<i>Gallus gallus domesticus</i> (Chicken)	Therapeutic proteins in eggs; scalable, noninvasive bioreactor system	[59]
	<i>Oryctolagus cuniculus</i> (Rabbit)	Milk-based production; short gestation; rapid growth; small-scale output	[58]
Microorganisms	<i>Escherichia coli</i>	Rapid growth; high protein yield (up to 25% of cellular protein); limited posttranslational modification	[71]
	<i>Saccharomyces cerevisiae</i>	Eukaryotic posttranslational modification; insulin and hepatitis B vaccine production	[62]
	<i>Pichia pastoris</i>	High cell density growth; complex posttranslational modification; enzymes and monoclonal antibodies	[72]

possibility to produce a large amount of protein, up to 25% of total cellular protein. However, the inability of the organism to make sophisticated posttranslational modifications limit its usefulness for some therapeutic proteins [61].

- *Saccharomyces cerevisiae* (Yeast): This yeast species is extensively utilized to produce proteins that need to undergo eukaryotic posttranslational modifications. *Saccharomyces cerevisiae* is an excellent choice for the large-scale manufacturing of biopharmaceuticals such as insulin and the hepatitis B vaccine, owing to its rapid growth and the relative simplicity of its genetic modification [62].
- *Pichia pastoris* (Yeast): This specific yeast species is favored due to its capacity for sophisticated posttranslational alterations and its ability to achieve high cell density growth. It is used in the preparation of a variety of therapeutic proteins, such as enzymes and monoclonal antibodies [63].

In the context of microbial systems, the terms molecular pharming and microbial fermentation largely describe the same underlying production technologies, but they differ in conceptual emphasis rather than in practical implementation. Microbial fermentation traditionally refers to the industrial cultivation of genetically engineered bacteria or yeast in bioreactors for large-scale recombinant protein production, with a primary

focus on process optimization, yield, scalability, and cost efficiency [64, 65]. In contrast, molecular pharming is a broader, product-centered concept that frames microbes as programmable biological factories for the targeted synthesis of high-value biopharmaceuticals, such as therapeutic proteins and vaccines, and is more commonly used in discussions that compare microbial platforms with plant- and animal-based expression systems [66, 67]. From a technical standpoint, both approaches rely on the same core steps, including strain engineering, controlled bioreactor cultivation, induction of recombinant gene expression, and downstream purification. As a result, molecular pharming in microbes does not represent a distinct manufacturing strategy but rather a conceptual or semantic rebranding of recombinant microbial fermentation, with greater emphasis on pharmaceutical applications and platform comparison across biological production hosts [64, 68].

2.4 | Protein Extraction and Purification

Efficient recovery and purification of the protein extracts are the most crucial steps in molecular pharming for producing pure biopharmaceuticals for therapeutic studies. These methods result in careful detachment of the target protein from the cells of

the host organism, and the separation between the protein and undesirable contaminants, which may cause denaturation of the protein, and may even render such protein ineffectual.

- **Protein Extraction:** In this initial stage, the target protein is separated from the complex structure of the host organism's cells or tissues. The specific extraction method to be employed primarily depends on the unique characteristics of the target protein and the biological nature of the host organism.
 - Homogenization: The basic step which is employed for cell lines is physical disruption of the cells to release the intracellular contents of the cell, including the target protein related to homogenization. It is usually performed mechanically by using blenders, bead mills, or high-pressure homogenizers [73]. For plant tissues, homogenization is frequently performed in extraction buffers containing protease inhibitors [74]. These inhibitors are crucial in inhibiting the proteolytic breakdown of proteins, which can happen rapidly after cellular compartments are compromised.
 - Enzymatic Lysis: Enzyme lysis is a technique of destroying the harsh and stiff walls of the plant materials using special enzymes such as cellulase and pectinase. The enzyme helps in easing such structural barriers, thus making the release of intracellular proteins more efficient, as well as maintaining the integrity of the proteins, hence yielding [75].
 - Sonication: Sonication is the procedure that uses ultrasonic waves of high frequency to rupture cell membranes and release contents inside them. This technique is particularly applicable toward lysis of small cells, and sensitive plant tissues due to the formation of cavitation forces through the ultrasonic waves which effectively but tenderly disrupt the cellular structures without causing excessive amounts of heat which destroys proteins leading to their loss in functionality [76].
 - Buffer Selection: It is crucial to find the optimal extraction buffer for retaining protein solubility and stability during extraction. For example, non-ionic detergents, such as Tween 20 or Triton X-100, are added for solubilizing membrane proteins, and salts, such as sodium chloride, are included to maintain ionic strength and protein stability in solution. In general, buffers, such as PBS and Tris-HCl, are present in the compositions to preserve the pH of the compositions within a desired range that supports protein stability. Furthermore, inhibitors like EDTA (ethylenediaminetetraacetic acid) and PMSF (phenylmethylsulfonyl fluoride) are added to inhibit the proteolytic degradation of proteins during extraction [77].
 - Centrifugation: This method is employed to perform a separation of soluble proteins and cellular debris after they have been homogenized. First, low-speed centrifugation (e.g., 10,000 x g) is carried out to clear undissolved particles and whole cells. This is followed by high speed spinning (e.g., 100,000 x g) to collect the particles in smaller size that leaves the soluble proteins at the supernatant stage [43].
- **Protein Purification:** This phase involves meticulously isolating the target protein from the crude extract to obtain a high degree of purity and ensure its full functionality. A variety of chromatographic and nonchromatographic procedures are employed, selected based on the specific characteristics of the protein.
 - Affinity Chromatography: This is a highly selective technique that leverages the specific binding affinity between the target protein and a chosen ligand, which is immobilized on a chromatography matrix. For example, His-tagged proteins bind strongly to immobilized nickel or cobalt ions on a resin. Antibodies are purified using Protein A or G columns, which specifically bind to the Fc region of immunoglobulins. Glycoproteins are purified using lectin columns, which bind to particular protein-carbohydrate moieties [78]. Elution is typically achieved by changing buffer conditions, such as using low pH buffers or adding imidazole.
 - Ion Exchange Chromatography: This method separates proteins based on their net charge at a specific pH. Negatively charged proteins bind to positively charged resins (anion exchangers like DEAE cellulose), while positively charged proteins bind to negatively charged resins (cation exchangers like CM cellulose). Elution is achieved by elevating the salt concentration or adjusting the pH [79].
 - Size Exclusion Chromatography (Gel Filtering): Also known as gel filtration, this technique separates proteins according to their hydrodynamic dimensions. The protein mixture is passed through a column packed with porous beads. Larger proteins elute more quickly as they are excluded from the pores, while smaller proteins penetrate the pores and elute later. This technique is frequently used for protein desalting, buffer exchange, and final polishing steps [80].
 - Hydrophobic Interaction Chromatography: This method separates proteins based on their hydrophobicity. Proteins are loaded onto a column in high salt environments, which encourages hydrophobic interactions between the protein and the resin. These links are then dislocated by steadily dropping the salt content or by adding hydrophobic solvents, leading to the elution of the bound proteins [81].
- **Protein Analysis and Characterization:** Following purification, the target protein should be analyzed and characterized to establish its purity, integrity, and activity.
 - SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis is commonly used to determine the purity and molecular weight of proteins. Proteins are denatured and SDS coats them with a uniform negative charge; they are then sorted according to size during electrophoresis. Proteins separated on a gel are usually stained by Coomassie Brilliant Blue or silver stain [82].
 - Western Blotting: This method is used for the detection of certain proteins with antibodies. Proteins separated by SDS-PAGE are transferred to a membrane (PVDF, Nitrocellulose, and so forth). The membrane is subsequently incubated with primary antibodies against the target protein, and detected by a second antibody against IgG conjugated to enzymes or fluorescence [83].
 - Mass Spectrometry: Mass spectrometry gives a precise, thorough list of the protein's mass, structure, and presence of posttranslational modifications. Purified proteins are frequently digested with proteases such as trypsin to yield

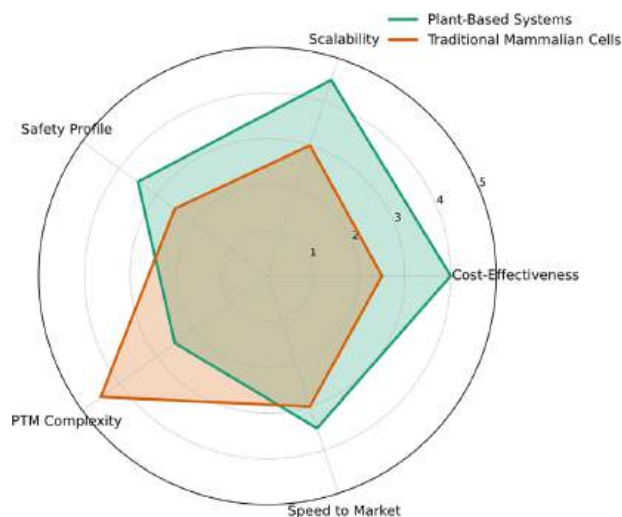


FIGURE 4 | Plant-based molecular pharming platforms offer several competitive advantages over traditional microbial fermentation and mammalian cell culture systems, particularly in terms of cost, scalability, and safety.

TABLE 3 | Comparative data of different host organisms for molecular pharming.

Feature	Microbial	Mammalian	Plant-Based
Cost	Low	High	Moderate
Scale	High	Moderate	High
Posttranslational modifications	Poor	Excellent	Good (improving)
Pathogen risk	Low	High	Very Low
Regulatory acceptance	High	High	Emerging

peptides. These peptides are ionized and analyzed in a mass spectrometer to determine the mass-to-charge ratio and sequence [84].

3 | Comparison With Traditional Pharming and Other Biotechnological Methods

Molecular pharming offers distinct advantages compared to traditional pharmaceutical production methods, which primarily rely on microbial fermentation or mammalian cell cultures, depicted in Figure 4 and Table 3.

- **Cost-Effectiveness and Scalability:** Plant-based systems provide a uniquely cost-effective and highly scalable platform for producing pharmaceuticals, as impressively demonstrated by the commercial-scale biotherapeutics manufacturing facilities that have been developed for plant-made pharmaceuticals [14]. This method reduces overall production costs and makes medications more widely available, particularly in impoverished areas, and notably enables long-term storage without refrigeration [1, 85, 86]. In stark contrast to established microbial and mammalian cell production technologies, plant-

based systems offer significant economic benefits. Producing therapeutics from plants is 4–5 times cheaper than using mammalian cell culture methods [87].

- **Plant-based molecular pharming offers a cost-efficient and scalable platform for biopharmaceutical production,** as demonstrated by the establishment of commercial-scale facilities for plant-made therapeutics [88]. Cost reductions arise primarily from lower capital investment, reduced energy requirements, and the elimination of expensive sterile bioreactors and animal-derived culture media. In addition, several plant-produced pharmaceuticals exhibit improved stability, enabling storage and distribution without continuous cold-chain infrastructure, which is particularly advantageous for low-resource settings [85, 89].
- **Economic analyses have estimated that recombinant protein production in plants can be approximately 4 to 5-fold less expensive than production in mammalian cell culture systems,** largely due to lower upstream and facility-related costs [90]. However, these estimates are context-dependent and vary with protein complexity, purification requirements, and production scale. Compared with microbial fermentation systems, plant-based platforms generally have higher production times but offer advantages in producing complex proteins requiring posttranslational modifications that are difficult to achieve in bacterial hosts. While microbial systems remain the most cost-effective option for simple proteins and enzymes, plant-based systems provide a favorable balance between cost, scalability, and protein quality for complex biopharmaceuticals.
- **Safety and Posttranslational Modifications:** A critical advantage of plant-based systems is their inherent safety; they eliminate the risk of human pathogens, which can be a concern with mammalian cell cultures [91]. Furthermore, plants offer the unique benefit of producing proteins with human-like glycosylation patterns, which is essential for the proper biological activity and reduced immunogenicity of many therapeutic proteins [92]. Moss bioreactors, for instance, exemplify this by providing a less hazardous, more affordable, and less contaminated option that can be designed to produce glycan structures resembling those of humans, thereby reducing immunogenic reactions [5, 93].
- **Simplified Production and Oral Delivery:** The innovative use of plant cells for bioencapsulation of protein drugs offers a low-cost delivery method with significant potential applications in oral tolerance induction [94]. Specifically, plant molecular pharmaceuticals streamlines the process by removing the need for expensive purification procedures, simplifying vaccine production and administration [95]. Furthermore, oral administration of microalgae-based vaccinations has a lot of advantages, including enhancing patient compliance and negating the need for trained medical professionals to administer them [96].
- **Rapid Response and Monitoring:** ArgoLux is a recently developed monitoring approach in plant-based molecular pharming that enables real-time, nondestructive assessment of recombinant protein expression in living plant tissues [18]. Unlike conventional pharming systems such as microbial or mammalian cell cultures, which typically require

destructive sampling and downstream processing to evaluate protein production, ArgoLux allows continuous and dynamic monitoring without interfering with plant growth or protein accumulation. This rapid-response capability provides a realistic representation of in planta protein expression and offers a significant advantage for optimizing production timelines, particularly during early-stage screening and scale-up.

- **Versatility:** Plant molecular pharming stands notable versatility, particularly in its ability to perform complex posttranslational modifications that are essential for the structural integrity and biological activity of many recombinant proteins [97]. In comparison, microbial and cell-free expression systems often lack the cellular machinery required for these modifications, limiting their suitability for certain therapeutic proteins. Chloroplast genetic engineering and cell-free platforms, however, can achieve high protein yields and are considered environmentally friendly and cost-effective, especially for producing enzymes and selected secondary metabolites [96, 98]. Microbial hosts remain highly valuable for pathway engineering and enzyme modification, enabling the biosynthesis of pharmacologically important alkaloids and other small-molecule compounds [99]. Collectively, these systems highlight that while alternative platforms excel in speed, yield, or metabolic engineering, plant-based molecular pharming uniquely combines scalability, complex protein processing, and sustainability.
- **Sustainability and Novelty:** This method presents a potentially safer and more cost-effective substitute for conventional techniques in the production of therapeutic proteins, leveraging plants as factories for medicinal compounds [1, 85, 86]. Over the past 25 years, the discipline of molecular pharming, which aims to produce vaccines and biopharmaceuticals from crops has developed, with carrots being a major contributor to this progress [95, 100]. CRISPR/Cas9 molecular pharming offers a more focused method of altering plant glycosylation pathways than conventional pharming techniques, enabling tailored alterations for recombinant proteins with appropriate glycosylation patterns [13, 101]

Despite these compelling advantages, molecular pharming faces persistent obstacles. These include challenges related to achieving consistently high protein yields in transgenic plants, limitations in capital for large-scale production and clinical development, complex and evolving regulatory concerns, and the ongoing need for technology adaptation to meet the rigorous standards required for vaccines and therapeutics [14]. Nevertheless, ongoing research and development efforts continue to refine expression technologies and explore new approaches to enhance the efficacy of plant-based pharmaceutical production. The main impact of molecular pharming is therefore likely to be the provision of novel strategies for medical intervention that remove some of the constraints of conventional manufacturing processes, rather than the wholesale replacement of current manufacturing infrastructure [102].

4 | Applications of Molecular Pharming

The field of molecular pharming has transitioned from a niche research area to a significant contributor to the biopharma-

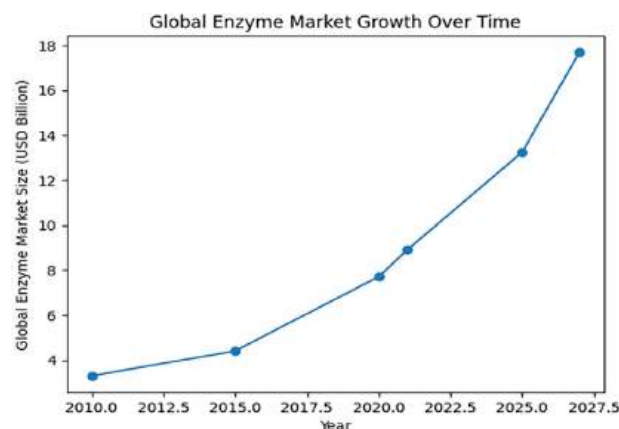


FIGURE 5 | Projected annual market value for plant-derived pharmaceuticals in the coming years (Illustrative based on trends).

ceutical industry, with lead candidate molecules progressing into later-stage clinical trials and the establishment of pilot and commercial-scale facilities [14]. This indicates a growing acceptance of plant-based technologies in the biopharmaceutical sector.

4.1 | Production of Pharmaceuticals

Molecular pharming can produce a wide variety of proteins that are free of mammalian toxins and pathogens. Molecular pharming represents a novel source of molecular medicines, such as plasma proteins, enzymes, growth factors, vaccines, and recombinant antibodies, whose medical applications are understood at a molecular level [12]. Projected annual market value for plant-derived pharmaceuticals in the coming years is shown in Figure 5. Also, molecular pharming's versatility allows for the production of a wide range of biopharmaceuticals, catering to diverse medical needs and industrial applications shown in Figure 6. This breadth of application is a key driver of its market potential.

- **Recombinant Proteins and Therapeutics:** This category includes parental therapeutics and pharmaceutical intermediates. A significant breakthrough occurred in 2012 when the first molecular pharming product was approved for use in humans: the enzyme taliglucerase alfa, a recombinant form of human glucocerebrosidase developed by Protalix Biotherapeutics for the treatment of the lysosomal storage disorder Gaucher's disease [102]. Furthermore, two clinical trial applications for plant-derived pharmaceuticals were approved in the European Union (EU): one for insulin produced in safflower (developed by SemBioSys Genetics) and another for an HIV-neutralizing monoclonal antibody produced in tobacco, notable because it was developed by a publicly funded consortium [103]. Plants are capable of producing complex and large proteins that are challenging to make chemically or by microbial fermentation [53]. They offer a flexible option by combining the expensive and scalable nature of microbial systems with the intricate protein processing capabilities of animal cells [85, 104].
- **Monoclonal Antibodies (mAbs):** The production of recombinant monoclonal antibodies (mAbs) with the ability to

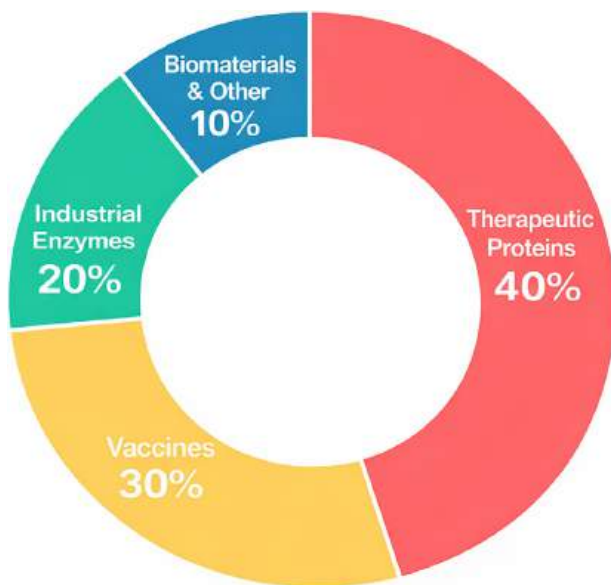


FIGURE 6 | Molecular pharming's versatility allows for the production of a wide range of biopharmaceuticals, catering to diverse medical needs and industrial applications.

neutralize viruses or toxins is a significant application of molecular pharming [2]. While many different molecular pharming systems have been described, commercial platforms are consolidating around three main areas: transgenic plants, cell and tissue cultures, and transient expression systems [105]. These biopharmaceuticals, particularly mAbs, are becoming increasingly valued as treatment options due to their high specificity and limited adverse effects [53]. For instance, mAbs can stimulate the host immune system against a target cancer cell, can inhibit enzymes or inactivate other proteins, and can mimic a signaling ligand or present an antigen [53].

- **Industrial Proteins:** This category specifically includes industrial enzymes. The enzyme industry is in an excellent position to contribute to a cleaner environment, offering industries and consumers an opportunity to replace processes using aggressive chemicals with mild, nontoxic enzyme processes [106, 107]. Enzymes can replace chemicals or processes that present safety or environmental issues; for example, they can replace acids in starch processing, reduce sulfide use in tanneries, and replace pumice stones for stonewashing jeans [106]. The global market for industrial enzymes was estimated to reach \$3.3 billion in 2010 and was expected to reach \$4.4 billion by 2015 [106]. The global enzyme market expanded markedly from USD 7.70 billion in 2020 to USD 8.9 billion in 2021, reflecting a compound annual growth rate (CAGR) of approximately 14%. Market analyses comparing 2019 and 2020 reported a temporary decline of about 1% in 2020. Despite this short-term setback, the market has shown sustained growth and is projected to reach USD 13.25 billion by 2025, maintaining a similar annual growth rate. Enzyme consumption is further expected to increase substantially, potentially reaching USD 17.7 billion by 2027, although future growth may be influenced by disruptions such as additional waves of coronavirus infections [108]. These enzymes are produced by fermentation using microorganisms like *Aspergillus* species,

Trichoderma species, *Bacillus* species, and *Kluyveromyces* species under carefully controlled conditions [109]. Genetic engineering is routinely used to enhance enzyme production and performance in host strains [106]. Enzymes can be categorized into detergent, technical, food, and feed enzymes [109]. The food and feed sector consumes over one-third of enzyme production, with applications in starch conversion (amylases, glucoamylase), improving bread quality, juice clarification, cheese making (chymosin), and lactose-free dairy products (lactase) [107, 109, 110]. Detergent enzymes (proteases, lipases, cellulases) are the largest single market, used to eliminate stains and improve fabric cleaning [106]. Enzymes also find applications in pulp and paper (xylanases, lipases, cellulases, laccases), pharmaceuticals (glucose oxidase in sensor strips), and increasingly in biofuel production from lignocellulosic biomass [106].

4.2 | Production of Vaccines

Advances in genetic engineering have led to the development of recombinant subunit vaccines, representing soluble immunogenic antigens or virus-like particles (VLPs), and recombinant monoclonal antibodies (mAbs) capable of neutralizing viruses or toxins [111]. These recombinant plant systems may be used as an economic alternative to produce animal and human vaccines.

- **Oral Delivery:** Oral delivery of recombinant plant-produced vaccines is conceptually desirable due to its simplicity, safety, and low cost, allowing for local production [111]. Transgenic plants that express antigens in their edible tissue can serve as an inexpensive oral-vaccine production and delivery system; therefore, immunization might be possible simply through consumption of an "edible vaccine" [112]. For instance, efforts have focused on antigens that assemble into ordered structures, such as virus-like particles (VLP), with the hope that they will be more resistant to digestion, more likely to reach the gut-associated lymphoid tissue (GALT) and, thus, more likely to be perceived as a foreign antigen [113].
- **Scalability and Production Hosts:** The primary advantage of plant-based vaccines is the potential for very large-scale production, particularly if open field-grown plants can be used [112]. While tobacco plants were initially convenient for testing recombinant antigen production, concerns about toxic alkaloids necessitate purification for human consumption [113]. Researchers are now investigating expressing abundant proteins in banana fruit for human delivery systems [113]. Microalgae could be poised to become the next candidate in recombinant subunit vaccine production, offering advantages over terrestrial crop plant-based platforms, including scalable and contained growth, rapid transformation, and stable cell lines [96].
- **Examples of Plant-derived Vaccines:** A plant-based vaccine candidate based on the heat-labile enterotoxin B subunit (LT-B) of ETEC has been successfully produced in transgenic potato. This candidate vaccine has demonstrated immunogenicity and conferred protection against challenge in animal studies and a Phase 1 clinical study [111]. A vaccine against Norwalk virus (NV), noroVAXX, was expressed in transgenic potatoes and contains recombinant VLPs formed by the major

capsid protein (CP) of NV [111]. A hepatitis B vaccine candidate has been developed by introducing the gene of hepatitis B surface antigen (HBsAg) into tobacco and potato, inducing both cellular and humoral immune responses in mice [111]. More recently, recombinant monovalent vaccine candidates for influenza (H5N1 and H1N1 strains) were designed and produced in *N. benthamiana* plants using an agro-viral launch vector-based transient expression system [111].

4.3 | Nutraceuticals and Functional Foods

Molecular pharming can significantly contribute to the growing field of nutraceuticals and functional foods, which are recognized for providing health benefits beyond basic nutrition and are increasingly popular in treating various health disorders.

- **Nutraceuticals:** Agricultural by-products are now recognized as valuable sources of functional ingredients like antioxidants and dietary fibers, broadly termed “nutraceuticals” [114]. This market represents a global value of approximately USD 3.7 trillion annually [115]. Scientists have found evidence that nutraceuticals are effective in treating endocrine disorders and certain cancers [115]. Proper delivery is crucial for nutraceutical compounds, and encapsulation (micro- and nano-scale) is used to establish stable transport and controlled diffusion of active ingredients [115]. The production process typically involves macroscopic pretreatment, macro- and micro-molecule separation, extraction, purification, and final nutraceutical formation [115]. Molecular pharming can enhance the synthesis of naturally occurring substances like taxol and artemisinin, which are found in plants but often in minimal quantities [116]. It can also improve the creation of anticancer medications like vinblastine and vincristine, along with other bioactive chemicals with medicinal properties [117].
- **Functional Foods:** A functional food is defined as a conventional food consumed as part of a normal diet that provides physiological benefits and/or reduces the risk of chronic disease beyond basic nutrition [118]. Examples include iodized salt, vitamin A and D fortified milk, yogurt, folic acid-enriched bread, tomatoes, broccoli, soy products, blueberries, cranberries, garlic, wheat bran, and oats [118]. “Super-fortified foods,” containing over 100% of the recommended daily intake or added botanicals, also fall into this category, such as orange juice with Echinacea [119]. A challenge for functional food producers is ensuring sensory acceptability, which is not as critical for nutraceutical or pharmaceutical products. Omega-3 oils, critical for health promotion and chronic disease prevention, can be incorporated into foods or supplements, with microencapsulation techniques protecting them from oxidation and off-flavor development. Omega-3 concentrates are even used as prescription drugs for reducing blood pressure and triacylglycerols, demonstrating their dual role as nutraceuticals and pharmaceuticals [118].

4.4 | Biomaterials and Others

- **Biomaterials:** Biomaterials are defined as materials anticipated to interface with biological systems to augment, treat,

or replace any tissue, organ, or body function [120]. They are classified based on material properties (polymeric, ceramic, metallic) and further categorized into synthetic (metals, ceramics, nonbiodegradable/biodegradable polymers) and naturally derived biomaterials [121]. While synthetic biomaterials like metal hip implants and Dacron are commercialized, they often lack structural similarity to native tissues and have low biocompatibility [121]. Naturally derived biomaterials, including protein-based (collagen, gelatin, silk, fibrin) and polysaccharide-based (cellulose, chitin/chitosan) materials, are gaining interest due to their superior biocompatibility, biodegradability, and remodeling capabilities [121]. They effectively support cell adhesion, migration, proliferation, and differentiation, promoting tissue repair. Applications include drug delivery systems and medical devices like surgical sutures. Biosilica and biocalcite, for example, are promising candidates for biomaterials in regenerative medicine due to their anabolic action in bone formation [122].

5 | Advantages and Challenges

Molecular pharming presents several compelling advantages, positioning it as a transformative technology in biopharmaceutical production. However, it also faces significant challenges that must be addressed for its widespread adoption and commercial success.

5.1 | Regulatory and Ethical Considerations in Molecular Pharming

Robust regulatory oversight is essential to ensure the safety, efficacy, and quality of products derived from molecular pharming [123]. The molecular pharming sector has advocated stricter regulatory approval procedures for pharmaceutical crops [124]. Regulatory agencies such as the USDA and European authorities have issued guidelines for field testing genetically modified (GM) organisms for industrial and pharmaceutical use [125]. Animal studies supporting biopharmaceutical development are conducted under approved protocols to ensure ethical treatment and regulatory compliance [126–128]. Regulatory requirements for product approval vary across countries [100].

Field production of pharmaceutical crops requires permits and containment plans. In the United States, USDA-APHIS regulates genetically engineered crops and mandates confinement during production, transport, and handling [87]. Since 2004, over 3358 hectares and 13 host species have been approved for GM field trials [129]. APHIS has issued permits for plant-made insecticides since 2003 [130]. Comparable regulatory frameworks operate in Europe and Canada, where field testing also requires preapproved permits. It is anticipated that the regulation of plant-made pharmaceuticals will be as strict as that for conventional pharmaceuticals [131].

Gene flow and environmental containment remain central regulatory concerns [11, 132]. Recommended strategies include physical containment, elimination of unnecessary genetic elements, tissue-specific expression, site-specific recombination, host-species selection, and strict separation from food or feed

crops [132]. Transgenic rice exhibits low pollen-mediated gene flow (0.04%–0.80%), which can be reduced using spatial isolation [133–135]. Maize offers additional advantages, including GRAS status, seed-based protein expression, and established infrastructure [136].

Strict compliance with regulatory protocols for seed production, pollination, harvest, and containment is required to prevent contamination [87, 129, 130]. Pharmaceutical crops must follow good manufacturing practice standards to ensure product consistency, purity, and potency [87]. Regulatory systems should be science-based, applied on a case-by-case basis, and globally harmonized to reduce inconsistencies and trade barriers [11, 131, 132].

Biosafety and risk assessment frameworks address food-chain contamination and environmental release [11, 137]. Existing pharmaceutical and cosmetic regulations must adapt to plant-based systems [100]. Complete segregation of GM and non-GM crops is difficult, even with strict confinement [138]. The EU permits up to 0.5% GM presence in non-GM crops where unavoidable, while a 0.9% threshold applies to nonpharmaceutical plant-made products [139, 140].

Ethical issues parallel regulatory challenges and require careful evaluation before widespread adoption [102]. Major concerns include environmental protection, public safety, food security, and equitable access to benefits [123, 141, 142]. Preventing pharmaceutical proteins from entering the food supply is a primary ethical obligation [100, 143]. Risk mitigation strategies include the use of non-food crops such as tobacco, flax, jute, and algae [141, 144]. Algae-based vaccines are viewed more favorably because *Chlamydomonas reinhardtii* is GRAS-certified [145].

Ethical governance must also address intellectual property, transparency, and public engagement [116, 123, 146]. Socially responsible licensing can reduce inequitable access to biopharmaceutical innovations [123]. Public acceptance is hindered by misinformation and cultural or religious objections to transgenic plants [142, 146]. Transparency, labeling, and education are therefore essential for trust building [116, 140].

Overall, ensuring stringent regulation to prevent food-chain contamination and unintended environmental or health effects remains the central regulatory and ethical priority in molecular pharming [143, 147]. The long-term success of plant-made pharmaceuticals depends on strong, evidence-based regulation, ethical governance, and continuous dialogue among scientists, regulators, and the public [123].

5.2 | Challenges and Limitations

Despite these compelling advantages, molecular pharming faces several significant challenges that require ongoing research and innovative solutions.

- **Low Protein Yields and Production Time:** A notable disadvantage in molecular pharming is the often-low yield of protein products [148]. Product accumulation levels are frequently much lower than 1% of total soluble protein

(TSP), typically ranging from 0.01% to 0.1% TSP or less [149, 150]. These low accumulation levels significantly limit the commercial exploitation of recombinant plant systems. The time required for producing transgenic plants is also a considerable drawback [67]. To address this, numerous studies focus on improving therapeutic protein production by reengineering expressed genes and proteins at both upstream and downstream processing stages [149].

- **Protein Degradation:** The breakdown of genetically engineered target molecules, often complex proteins with attached sugar groups, presents a major obstacle. This degradation can occur in intercellular spaces or during transit between the ER and Golgi apparatus, affecting harvesting, extraction, and downstream purification [151, 152]. Strategies such as RNA interference, organelle-specific targeting, protein-stabilizing protease inhibitors, and continuous recovery of recombinant proteins in small amounts have been developed to simplify purification and minimize proteolysis [153, 154].
- **Glycosylation Differences and Immunogenicity:** A common concern is whether plant-produced proteins substantially differ from their original counterparts and if they are safe. While basic eukaryotic protein synthesis is conserved, differences exist in glycosylation mechanisms between plants and native environments [151]. Plant-specific glycosylation patterns can potentially elicit a strong immune response, potentially causing allergic reactions in patients [155, 156].
- **Regulatory Hurdles and Public Acceptance:** Regulatory uncertainties significantly hinder the widespread adoption of molecular pharming [157]. Public opposition to GM crops, particularly in regions like continental Europe, has effectively halted field cultivation of molecular pharming crops [100]. Concerns over GM plants' potential hazards to human health and the environment, as well as cultural and religious objections to eating transgenic plants containing animal genes, are ethical considerations [142]. Ensuring public acceptance requires addressing misinformation and stigma associated with genetically engineered plants [158].
- **Gene Flow and Containment:** Preventing the unintentional spread of transgenes from cultivated plants to related crops and wild species is a crucial containment challenge [132, 139]. This is primarily through pollen dispersal, leading to hybrid seeds [159]. Crop contamination incidents, like the Prodigene case in 2002 involving pharmaceutical corn volunteers in a soybean field, highlight these risks [87]. Physical containment (greenhouses, isolated plots) and biological methods (male sterility, chloroplast transformation) are employed to limit gene flow.
- **Economic Viability:** While initial concerns about noncompetitive yields exist, limited capital for scale-up and clinical development also poses a barrier [14]. The complex regulations for cisgenic crops also affect their marketability, particularly globally [160, 161]. For certain products like recombinant staphylokinase (SAK) in *Arabidopsis*, low protein accumulation and purification challenges can render the method economically unfeasible [162]. The cost structure of pharmaceutical crops is primarily driven by risk minimization, requiring sophisticated risk management, identity preservation, and stringent quality control procedures [87].

6 | Future Directions and Prospects

Despite the significant challenges, molecular pharming is poised for transformative advancements, promising to revolutionize healthcare and various industries by offering innovative and sustainable solutions.

6.1 | Potential Future Developments

Future developments in plant-based production systems for biologics and biopharmaceuticals are expected to significantly impact the pharmaceutical and healthcare industries. Efforts in this field are likely to focus on enhancing protein expression levels, stability, and quality in plants, with the potential to make therapeutic proteins more accessible and cost-effective. Strategies to improve yield include advanced subcellular targeting techniques, protein body induction, and optimizing purification methods, which could make plant systems a feasible alternative to traditional production platforms [130]. Some areas are discussed below:

- **Enhanced Protein Expression and Quality:** Future efforts in plant-based production systems for biologics and biopharmaceuticals are expected to focus on enhancing protein expression levels, stability, and quality in plants [163]. Strategies to improve yield include advanced subcellular targeting techniques, protein body induction, and optimizing purification methods, which could make plant systems a feasible alternative to traditional production platforms [163]. A key avenue involves improving the structural and functional stability of plant-derived proteins through novel fusion strategies and exploring posttranslational modifications. By tailoring protein production to specific therapeutic needs, plant-based platforms could efficiently produce complex proteins, potentially transforming the production of pharmaceuticals [17].
- **Advanced Genetic Engineering:** New tools like AgroLux offer exciting possibilities for high-throughput analysis of *Agrobacterium* activity within various plant species, enabling more precise monitoring of gene expression and optimizing transient protein expression [18]. The use of advanced genetic engineering techniques, such as CRISPR/Cas9 and TALENs, is expected to drive the future of molecular pharming by enabling precise control over gene expression and enhancing the production of complex therapeutic proteins [101, 164]. Synthetic biology approaches could also support the development of tailored expression systems, enhancing the production of high-value proteins, including multi-subunit vaccines and human-like therapeutic proteins with complex posttranslational modifications [2].
- **Novel Plant Platforms and Bioreactors:** Research is expanding into alternative plant platforms for pharmaceutical production, such as using carrot-based systems for vaccine development, and cannabis biotechnology for optimized cannabinoid production [165, 166]. Innovations in transplastomic technology and root-specific promoters could increase production efficiency and offer new options for generating bioactive compounds in plants. Furthermore, the integration of -omics technologies, such as metabolomics and transcriptomics, will help identify genes essential for bioactive compound produc-

tion, accelerating the development of specialized cultivation practices for therapeutic plants [166]. Bioreactor-based plant cell suspension cultures, using host systems such as *Lemna minor* and *Chlamydomonas reinhardtii*, present another pathway for advancing molecular pharming [167].

- **Personalized Medicine and Space Applications:** As synthetic biology continues to evolve, it may enable plants to function as biofactories capable of producing specific biologics, including personalized medicines and vaccines. This technology could be particularly valuable in space exploration, where plants engineered to produce complex drugs on demand would reduce the need to transport pharmaceuticals [104]. Similarly, the adaptation of VLP expression systems may drive advancements in vaccine development, allowing for tailored virus nanoparticle (VNP) production that enhances immune response and therapeutic potential [168].
- **Global Health and Accessibility:** In low- and middle-income countries (LMICs), molecular pharming holds potential for producing affordable medicines tailored to local health needs [169]. Efforts to establish scalable biopharming facilities and training programs could build local capacity and support global health objectives [170]. By fostering collaborations between LMIC researchers and international institutions, molecular pharming can address region-specific diseases while promoting self-reliance in pharmaceutical production. Establishing scalable production systems, particularly in Africa, could enable rapid responses to emerging infectious diseases, enhancing local capacity for vaccine and therapeutic production.

6.2 | Areas Requiring Further Research

To fully realize the potential of molecular pharming, several critical areas demand continued and intensified research efforts across technical, economic, and societal dimensions.

- **Cost-Effectiveness and Scalability:** Comparative studies between plant, mammalian, and bacterial systems are essential to accurately determine the relative efficacy and true cost-efficiency of producing therapeutic proteins like recombinant hBMP2 [163]. Research must focus on optimizing plant cell culture systems, refining extraction methods, and developing more robust protein protection mechanisms within plant cells to significantly enhance production efficiency for large-scale protein manufacturing [14, 17]. Optimizing large-scale production facilities for plant-based systems is vital to overcoming scalability and efficiency challenges [85]. This includes improving bioreactor designs and cultivation conditions for hairy root systems and other plant-based platforms to ensure commercial viability [171].
- **Immunogenicity and Long-Term Effects:** A critical priority is the thorough investigation of the long-term effects and potential complications of plant-derived proteins in clinical applications to ensure patient safety and treatment efficacy [163]. Understanding the immunogenicity of plant-derived glycoproteins, their interactions with human immune systems, and their posttranslational modifications compared to mammalian systems will help develop competitive biophar-

maceuticals [93]. More studies are needed to test how stable and effective these plant-produced VLPs and other recombinant proteins are in diverse populations and environmental conditions is necessary [168, 172].

- **Regulatory Frameworks and Public Acceptance:** A strong and harmonized regulatory framework must be developed to effectively address concerns about contamination, environmental impacts, and unintended consequences for human health and ecosystems [173, 174]. For instance, robust physical and biological containment strategies are necessary to prevent genetically modified crops from inadvertently entering the human food chain [100, 124, 143]. Moreover, regulators will need to adjust to the distinctive ways these medicines are made from plant-based pharmaceuticals. Public perception of biopharming technologies and food safety concerns must be actively addressed through transparent communication and education to foster acceptance and trust [124, 175]. Streamlining regulatory pathways and ensuring robust quality control measures are essential for commercialization [85].
- **Protein Stability and Posttranslational Modifications:** Research needs to delve deeper into the molecular mechanisms of RNA silencing and codon optimization to improve protein expression across various plant species [9, 176]. Investigating posttranslational modifications, protein folding, and glycosylation patterns will enhance the stability, functionality, and efficacy of plant-derived therapeutics [177]. Glycoengineering in plant systems presents another frontier for research, requiring investigation into its implications on plant physiology, recombinant protein yield, and therapeutic glycoprotein functionality [13].
- **Advanced Biocontainment:** Studies should evaluate the long-term ecological impacts of transgenic plants, optimize containment strategies to minimize gene flow, and mitigate contamination risks [160]. Research into the risks of horizontal gene transfer, contamination in food and feed chains, and ecological impacts of biopharming will ensure environmental safety and societal acceptance [173, 174].
- **Specific Applications and Bottlenecks:** The potential of *Cannabis* trichomes as phytochemical factories requires exploration, particularly in identifying transcription factors that regulate trichome formation and metabolite production [166]. The optimization of transplastomic expression in non-green tissues, such as carrot roots, remains an underexplored area [165]. Additionally, addressing technical and economic bottlenecks in PMP production, such as stability, efficacy, and immunogenicity in clinical contexts, is essential for commercialization [178]. Research into bioreactor designs, postharvest processing, and metabolic pathway optimization will enhance the economic viability of molecular pharming [179].

7 | Conclusion

Molecular pharming represents a transformative approach to biopharmaceutical production, offering a potent blend of scalability, cost-effectiveness, and sustainability. This positions it as a key player in addressing critical global health disparities and advancing biotechnological innovations [9, 180]. While signif-

icant progress has undeniably been made, effectively tackling challenges related to optimizing protein yields, overcoming glycosylation differences, navigating complex regulatory landscapes, and fostering broader public acceptance remains paramount for its widespread commercialization and full societal impact.

Looking ahead, future research will intensely focus on further enhancing protein expression and quality through cutting-edge genetic engineering techniques, exploring novel plant systems and bioreactors, and meticulously developing tailored solutions for pressing global health needs, particularly in resource-limited settings. By fostering robust interdisciplinary collaborations among scientists, industry leaders, and policymakers, and by consistently prioritizing transparent communication with the public, molecular pharming can fully realize its transformative potential. This will lead to a future characterized by improved healthcare accessibility worldwide, a reduced environmental footprint from pharmaceutical production, and sustained economic growth driven by biotechnological advancements.

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Consent

Written consent was obtained from all the parents who provided additional demographic data.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used and/or analyzed during the current study are also available from the corresponding author upon reasonable request.

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