



Research Article

Molecular Pharming: Advances, Applications and Future Prospects

Sarla Rani^{1*} and Mahesh Kumar²

¹Dept of Biotechnology, Pt. CLS Govt PG College, Karnal-132001 (Haryana), India

²Dept of Biotechnology, IIHS, Kurukshetra University, Kurukshetra-136119 (Haryana), India

Article Information: Received on 19-11-2022 Accepted 27-12-2022 Available online 29-12-2022

Abstract

Molecular pharming, also referred to as plant molecular farming or biopharming, is the use of genetically engineered plants, plant cells, tissues or organelles as biological production systems for high-value recombinant proteins and other useful biomolecules. During the last four decades, the field has progressed from proof-of-concept expression of foreign proteins in transgenic plants to sophisticated transient-expression platforms, chloroplast engineering, glycol-engineering, synthetic biology and targeted genome editing. These developments have improved expression levels, product quality and manufacturing flexibility. Molecular pharming is now relevant to therapeutic proteins, monoclonal antibodies, vaccines, industrial enzymes, diagnostics, nutraceuticals and functional foods. Plant systems provide several potential advantages, including scalable biomass production, comparatively low infrastructure requirements, rapid adaptation of transient systems and a reduced risk of contamination with many human pathogens. Important milestones include plant-derived glucocerebrosidase, recombinant antibodies, vaccine antigens and the development of plant-produced virus-like particle vaccines. Nevertheless, product yield, batch consistency, downstream purification, plant-specific post-translational modifications, containment, regulatory approval and public acceptance remain important barriers. This review summarizes the historical development, principles, production techniques, major applications, representative examples, advances available, limitations and future prospects of molecular pharming. The molecular pharming can now be considered as a complementary biomanufacturing platform that can provide rapid, flexible and potentially economical production of selected high-value products.

Keywords: Molecular pharming; plant-made pharmaceuticals; recombinant proteins; chloroplast transformation; glycol-engineering; vaccines; synthetic biology.

Introduction

The biotechnology industry requires reliable systems for producing recombinant proteins, vaccines, antibodies, enzymes and other biologically active molecules. Bacteria, yeast, insect cells and mammalian cell cultures remain major production platforms, but each has technological and economic constraints. Molecular pharming provides an alternative in which plants are engineered to act as production hosts for high-value biomolecules. Plants can generate substantial biomass, can be cultivated under controlled or agricultural conditions and possess cellular machinery capable of folding and processing many complex eukaryotic proteins [1], [2].

The term molecular pharming generally emphasizes the production of pharmaceutical or medically relevant compounds in plants. The platform has been investigated using tobacco, *Nicotiana benthamiana*, cereals, legumes, vegetables and plant cell cultures. Stable nuclear transformation, chloroplast transformation and transient expression have each contributed to the development of the field [3].

A major change occurred with the emergence of rapid transient-expression systems. Rather than waiting for stable integration and regeneration of a new transgenic line, recombinant genes can be introduced into mature plant tissues using *Agrobacterium*-mediated agroinfiltration or viral-derived vectors. This approach has been particularly important for vaccine development and rapid response to emerging pathogens. Modern systems also use deconstructed viral vectors, optimized promoters, targeting signals and engineered host plants to

*Corresponding author: **Sarla Rani**

doi: <https://doi.org/10.54618/IJMAHS.2023312>

This is an open-access article, which permits the use and distribution of article provided that original author and source are credited

increase recombinant protein accumulation [4]. The literature available demonstrates that molecular pharming has progressed beyond a laboratory curiosity. Several plant-derived products have reached clinical or commercial development, while the technology has become increasingly integrated with synthetic biology, genome editing, proteomics and advanced downstream processing. At the same time, commercialization remains selective because technical performance alone does not guarantee regulatory or economic success [5].

Historical Development

The foundation of molecular pharming was established when researchers demonstrated stable expression of foreign genes in plant cells during the 1980s. One of the early landmark studies reported expression of human growth hormone in transformed plant tissue, demonstrating that plants could produce a mammalian protein [6]. Soon afterward, expression of immunoglobulin components in transgenic plants demonstrated that plants could be used for antibody production [7].

During the 1990s, research expanded to vaccine antigens, antibodies and industrial proteins. The idea of edible vaccines attracted considerable attention because food crops could potentially deliver antigens through oral administration. Although edible-vaccine concepts revealed important biological and regulatory difficulties, they helped establish plants as versatile recombinant-protein hosts [8].

The use of tobacco and related species became widespread because these plants produce substantial biomass and are amenable to transformation. Plant cell cultures were also explored as contained production systems along with the improved vectors, subcellular targeting and process development.

Later around 2010s rapid expansion of transient expression was emerged as an efficient technique. *N. benthamiana* became a leading production host because its leaves can be efficiently infiltrated with *Agrobacterium*. Viral replicons and deconstructed viral vectors enabled high-level expression without the lengthy development of stable transgenic lines. This transition was particularly important for vaccines and emergency biologics [9], [10]. And by the period 2020–2022, the field was increasingly focused on industrial translation. Researchers were combining transient expression with genome editing, humanized glycosylation, VLP technologies, proteomics and improved purification. Plant-made glucocerebrosidase became a prominent pharmaceutical example, demonstrating that a plant-derived recombinant enzyme could progress to an approved therapeutic product. COVID-19 vaccine research further demonstrated the speed with which plant platforms could generate candidate antigens and VLPs [11].

Principles of Molecular Pharming

The basic molecular pharming workflow involves selection of a target molecule, design of an expression vector, introduction of the construct into a suitable plant system, expression and accumulation of the recombinant product, harvesting of biomass, extraction and purification and analytical characterization. The production strategy is selected according to the target protein, required quantity, desired post-translational modifications, time available and regulatory pathway.

Expression constructs commonly contain a promoter, coding sequence, regulatory untranslated regions and a terminator. Signal peptides may direct recombinant proteins to the secretory pathway, chloroplasts, vacuoles or apoplast. Codon optimization and removal of unfavorable sequence elements can further improve the expression.

After expression, the product must be separated from plant proteins, pigments, nucleic acids, cell debris and other host components. Consequently, downstream processing can strongly influence the economics of molecular pharming. Modern research increasingly treats upstream expression and downstream purification as an integrated manufacturing problem rather than independent steps [12], [3].

Major Techniques

Stable Nuclear Transformation

Stable nuclear transformation introduces recombinant DNA into the plant nuclear genome. *Agrobacterium*-mediated transformation is widely used, while particle bombardment can be useful for selected species and tissues. Stable transformation permits inheritance of the recombinant trait and is suitable when a long-term production line or seed-based accumulation is desired. However, generating and characterizing stable lines can take considerable time and expression may vary because of insertion position, transgene silencing and copy-number effects [13], [2].

Agrobacterium-Mediated Transient Expression

Agroinfiltration introduces recombinant constructs into leaf tissue using *Agrobacterium*. The bacterium transfers T-DNA into plant cells, where the recombinant gene is expressed without necessarily becoming a stable inherited transgene. This approach can generate protein rapidly and is therefore particularly useful for research, vaccine candidates and short development cycles. *N. benthamiana* is the principal host used in many transient-expression platforms.

Plant Viral Vectors

Plant viruses can be adapted as vectors to increase

recombinant nucleic acid replication and protein accumulation. Modern deconstructed viral vectors remove unnecessary viral functions while retaining strong expression characteristics. Viral vector technology has supported production of vaccine antigens, antibodies, VLPs and other proteins. The principal advantages are high expression and rapid production, whereas biological containment and vector design remain important considerations [14].

Chloroplast Transformation

Chloroplasts possess multiple copies of their genome, allowing potentially high expression of selected recombinant proteins. Chloroplast transformation generally uses homologous recombination and can permit coordinated expression of several genes. Because chloroplast inheritance is frequently maternal in plants, chloroplast engineering has also been investigated as a strategy for reducing pollen-mediated transgene movement. Species-specific transformation efficiency and protein-folding limitations remain challenges [15].

Plant Cell Suspension Cultures

Plant suspension cultures provide a contained production environment and avoid some concerns associated with field-grown transgenic crops. Cells can be maintained in controlled bioreactors and harvested under standardized conditions. The approach is particularly relevant when environmental containment and batch control are priorities. However, growth kinetics and volumetric productivity must be competitive with microbial or mammalian systems.

Glycoengineering

Therapeutic glycoproteins require appropriate glycan structures because glycosylation can influence stability, half-life, receptor binding and immunological activity. Native plant glycosylation differs from human glycosylation. Glycoengineering therefore modifies endogenous pathways to produce more human-compatible glycan profiles. This technology is a key advance for antibodies and other therapeutic glycoproteins [16].

Genome Editing and Synthetic Biology

CRISPR/Cas genome editing permits targeted modification of genes involved in protein degradation, glycosylation, metabolism and plant defense. Synthetic biology adds modular promoters, regulatory elements, genetic circuits and pathway design. Together, these technologies can transform the plant from a passive expression host into an engineered production chassis. Many current studies emphasized targeted editing, deconstructed vectors and improved glycosylation as major advances [4].

Host Plants

N. benthamiana is the dominant transient-expression host because of its susceptibility to *Agrobacterium* and its capacity for high recombinant protein accumulation. Tobacco is another major platform, particularly for stable transformation and biomass production. Cereals such as rice and maize have attracted interest because seeds and endosperm can provide stable accumulation and storage. Legumes and other crops have also been investigated. Host selection depends on the target product and manufacturing objective. A host that is excellent for transient antibody production may not be optimal for long-term seed storage or production of a metabolite. Thus, molecular pharming is increasingly viewed as a collection of complementary platforms rather than a single universal system.

Applications

Therapeutic Proteins

Plants can produce enzymes, hormones, cytokines and other therapeutic proteins. Taliglucerase alfa, a recombinant glucocerebrosidase produced in carrot cell culture, is an important example of a plant-derived pharmaceutical that reached regulatory approval. Its development demonstrated that plant cell culture can support a clinically useful enzyme product and provided an important milestone for the field.

Monoclonal Antibodies

Plants can co-express antibody heavy and light chains and assemble functional immunoglobulins. Plant-made antibodies have been studied against infectious diseases, cancer targets and other conditions. Glycoengineering can modify Fc-associated glycans to improve compatibility with human therapeutic requirements. The rapidity of transient production also makes plants attractive for experimental antibody development [7], [17].

Vaccines

Molecular pharming is particularly promising for subunit vaccines and VLPs. Plants can produce viral antigens without requiring propagation of the human pathogen. Plant-derived vaccine candidates have been investigated for hepatitis B, hepatitis C, influenza, HPV, HIV, Zika and SARS-CoV-2. VLPs are attractive because their repetitive structures can stimulate immune responses while lacking a complete infectious viral genome [9], [10].

COVID-19 and Emerging Infectious Diseases

The COVID-19 pandemic provided a strong demonstration of the rapid-response potential of plant

molecular pharming. Plant-produced SARS-CoV-2 VLPs and recombinant antigens were developed using transient expression platforms. The ability to redesign constructs and produce material rapidly is particularly relevant when pathogens evolve or new outbreaks occur [11].

Industrial Enzymes

Molecular pharming can produce enzymes for food processing, animal feed, diagnostics, textiles, paper, detergents and chemical biotechnology. For industrial products that do not require complex mammalian post-translational modifications, plants can provide an alternative source of recombinant protein. The economic value depends strongly on yield, extraction efficiency and purification requirements.

Diagnostics and Research Reagents

Recombinant antigens, antibodies and enzymes produced in plants can serve as diagnostic reagents and research tools. Rapid transient expression is particularly useful when a new antigen or antibody is required within a short period. Plant systems can therefore complement bacterial, yeast and mammalian expression technologies.

Functional Foods and Nutraceuticals

Plant molecular pharming has expanded into functional foods and nutraceuticals. Plants can be engineered to accumulate proteins, vitamins, carotenoids, peptides and other bioactive compounds. Rice, wheat, maize and soybean are particularly relevant because of their importance as staple crops. A recent report highlighted the potential of plant molecular farming for functional-food development while noting the need for safety assessment, consumer acceptance and regulatory clarity [18].

Some important representative examples include human growth hormone expression in transformed plants, plant-made immunoglobulins, hepatitis B surface antigen, plant-derived industrial proteins, taliglucerase alfa, plant-produced monoclonal antibodies and vaccine VLPs. These examples collectively demonstrate the transition from simple recombinant-protein expression toward products requiring complex assembly, glycosylation and quality control. The development of plant-produced monoclonal antibodies also illustrates the importance of product quality. A recombinant antibody is not defined only by its amino-acid sequence; folding, assembly, glycosylation, aggregation and biological activity must all be controlled. This requirement has driven improvements in host engineering and analytical characterization.

Advantages

- Potentially scalable biomass production using greenhouse, controlled-environment or agricultural systems.
- Rapid production through transient expression, avoiding lengthy development of stable lines for every new product.
- Plants can synthesize many complex eukaryotic proteins and support post-translational processing.
- Plants do not naturally harbor many of the human pathogens associated with animal-cell manufacturing.
- Different plant tissues can be selected for accumulation, secretion or storage of the target product.
- Genetic engineering, genome editing and synthetic biology can be combined to optimize the production host.
- Plant platforms may provide flexible regional manufacturing for selected vaccines and biologics.

Limitations and Challenges

The major technical limitation is variability in recombinant protein accumulation. Expression can be influenced by plant genotype, environmental conditions, construct design, plant age and endogenous defense responses. Proteolysis and aggregation can reduce both yield and product quality. Modern host engineering and optimized vectors can address some of these problems but cannot eliminate them completely [4].

Post-translational modifications represent another important challenge. Plant glycosylation differs from mammalian glycosylation, although glycoengineering has greatly reduced this limitation. Some proteins also require specific processing or chaperone interactions that are not reproduced efficiently in plant cells.

Downstream processing can account for a large part of total manufacturing cost. As the plant biomass contains abundant host proteins, pigments, nucleic acids and structural components, hence the efficient extraction, clarification, concentration and chromatography becomes essential. Further, some strategies such as secretion into the apoplast, tissue-specific accumulation and purification tags can also help.

Environmental containment and biosafety must also be considered. Stable genetically modified plants may create risks of transgene movement through pollen or seed. Physical containment, biological containment and appropriate cultivation practices are important [19]. Regulatory approval remains a significant barrier. Regulators need evidence of identity, purity, potency, consistency, safety and environmental control. Public acceptance of genetically modified organisms can also affect commercialization. Consequently, successful molecular pharming requires coordination between molecular biology, process engineering, analytical science, regulatory science and economics.

Advances

First, transient expression became a mature platform for rapid recombinant-protein production. Second, humanized glycosylation improved the suitability of plants for complex therapeutic glycoproteins. Third, deconstructed viral vectors enabled strong expression without maintaining complete viral genomes. Fourth, genome editing provided tools for redesigning host pathways and reducing biological bottlenecks [4]. Proteomics and mass spectrometry also became valuable for understanding host responses, product heterogeneity and process consistency. These analytical approaches can help identify proteases, stress responses and unwanted product variants and therefore support development of more reproducible manufacturing processes [20].

Plant molecular pharming also became increasingly connected with nanotechnology. Plant viruses and VLPs can function as both production platforms and nanoscale scaffolds for antigen presentation. Such integration expands the scope of molecular pharming from simple protein expression to engineered biomaterials and vaccine technologies.

Future Prospects

Future development is likely to focus on engineering the entire production chassis. CRISPR/Cas systems can modify glycosylation, proteolysis, metabolic flux and defense pathways, while synthetic biology can create standardized genetic parts and regulatory circuits. The objective will increasingly be predictable, reproducible and high-yield production rather than simply successful expression. Greenhouses and vertical systems can provide standardized light, temperature, humidity and nutrition. For high-value pharmaceuticals, the additional infrastructure may be justified by improved containment and batch reproducibility. Automation, imaging and data-driven cultivation could further reduce variation.

Molecular pharming may also contribute to space biotechnology. Plants can potentially serve as biological factories during long-duration missions, where continuous resupply of pharmaceutical products is difficult. The concept of plant-based 'space foundries' has been proposed as a future application for on-demand production of medical countermeasures [21].

Functional foods and nutraceuticals may become another major application. Engineering staple crops to produce beneficial proteins or metabolites could combine agricultural production with health-related biotechnology. Nevertheless, food safety, dose control, environmental containment and consumer acceptance will determine whether such approaches achieve broad use [18]. Ultimately, molecular pharming is likely to coexist with established microbial and mammalian platforms. Its greatest value will arise where plants offer a combination of speed, scalability,

complex protein-processing capability, flexibility or lower infrastructure requirements that fits the target product.

Conclusion

Molecular pharming has developed from early demonstrations of foreign-gene expression into a sophisticated plant-based biomanufacturing technology. Stable transformation, transient agroinfiltration, viral vectors, chloroplast engineering, glycoengineering, genome editing and synthetic biology have progressively improved the ability of plants to produce valuable recombinant molecules. Applications include therapeutic enzymes, antibodies, vaccines, VLPs, industrial proteins, diagnostic reagents and functional-food components.

The field nevertheless faces challenges involving yield, product consistency, post-translational modification, downstream processing, containment, regulation and public acceptance. Continued integration of plant synthetic biology, CRISPR-based engineering, high-throughput analytics, controlled-environment cultivation and improved purification should address many of these limitations. Many other studies also supports molecular pharming as a flexible complementary platform with particular promise for rapid-response vaccines, selected biopharmaceuticals, specialized proteins and future distributed biomanufacturing.

References

- [1] R. Fischer and J. F. Buyel, "Molecular farming—The slope of enlightenment," *Biotechnol. Adv.*, vol. 40, Art. no. 107519, 2020.
- [2] S. Schillberg, N. Raven, R. Fischer, R. M. Twyman, and A. Schiermeyer, "Molecular farming of pharmaceutical proteins in plants: Moving from laboratory to market," *Curr. Opin. Biotechnol.*, vol. 61, pp. 174–182, 2019.
- [3] J. F. Buyel, "Process engineering of plant molecular farming for biopharmaceutical production," *Biotechnol. Adv.*, vol. 37, pp. 1–20, 2019.
- [4] H. Liu and M. P. Timko, "Improving protein quantity and quality—The next level of plant molecular farming," *Int. J. Mol. Sci.*, vol. 23, Art. no. 1326, 2022.
- [5] J. Saxena et al., "Plant molecular farming for the production of valuable proteins—Critical evaluation of achievements and future challenges," *J. Plant Physiol.*, vol. 258–259, Art. no. 153359, 2021.
- [6] A. Barta, K. Sommergruber, D. Thompson, K. Hartmuth, M. A. Matzke, and A. J. M. Matzke, "The expression of a nopaline synthase–human growth hormone gene fusion in transformed tobacco and sunflower callus," *Plant Mol. Biol.*, vol. 6, pp. 347–357, 1986.
- [7] A. Hiatt, R. Cafferkey, and K. Bowdish, "Production of antibodies in transgenic plants," *Nature*, vol. 342, pp. 76–78, 1989.
- [8] A. M. Walmsley and C. J. Arntzen, "Plants for delivery of edible vaccines," *Curr. Opin. Biotechnol.*, vol. 11, pp. 126–129, 2000.
- [9] E. P. Rybicki, "Plant-made vaccines for humans and animals," *Plant Biotechnol. J.*, vol. 8, pp. 620–637, 2010.

- [10] B. J. Ward, P. Gobeil, A. Seguin *et al.*, "Efficacy, immunogenicity and safety of a plant-derived virus-like particle vaccine for COVID-19," *medRxiv*, 2021.
- [11] Y. H. Chung, D. Church, E. C. Koellhoffer *et al.*, "Integrating plant molecular farming and materials research for next-generation vaccines," *Nat. Rev. Mater.*, vol. 7, pp. 372–388, 2022.
- [12] L. R. Wilken and Z. L. Nikolov, "Recovery and purification of recombinant proteins from plant-derived biomass," *Biotechnol. Adv.*, vol. 30, pp. 419–433, 2012.
- [13] R. M. Twyman, E. Stoger, S. Schillberg, P. Christou, and R. Fischer, "Molecular farming in plants: Host systems and expression technology," *Trends Biotechnol.*, vol. 21, pp. 570–578, 2003.
- [14] G. P. Lomonosoff and M. A. D'Aoust, "Plant-produced biopharmaceuticals: A case of more than molecular farming," *Plant Biotechnol. J.*, vol. 14, pp. 1329–1335, 2016.
- [15] P. Maliga, "Progress towards commercialization of plastid transformation technology," *Trends Biotechnol.*, vol. 21, pp. 20–28, 2003.
- [16] R. Strasser, "Plant protein glycosylation," *Glycobiology*, vol. 26, pp. 926–939, 2016.
- [17] R. Fischer, S. Schillberg, S. Hellwig, R. M. Twyman, and J. Drossard, "GMP-compatible production and purification of a monoclonal antibody in plants," *Biotechnol. Bioeng.*, vol. 109, pp. 298–306, 2012.
- [18] Y. Long *et al.*, "Plant molecular farming, a tool for functional food production," *J. Agric. Food Chem.*, vol. 70, pp. 2108–2116, 2022.
- [19] A. L. Klocko, "Genetic containment for molecular farming," *Plants*, vol. 11, Art. no. 2436, 2022.
- [20] González-Moro *et al.*, 2022. [Full reference details not provided in the original manuscript — please add.]
- [21] K. Hoshikawa *et al.*, "Molecular pharming to support human life on the moon, Mars and beyond," *Front. Plant Sci.*, vol. 12, Art. no. 668580, 2021.
- [22] V. Yusibov, S. J. Streatfield, and N. Kushnir, "Clinical development of plant-produced recombinant pharmaceuticals: Vaccines, antibodies and beyond," *Hum. Vaccines*, vol. 7, pp. 313–321, 2011.
- [23] Q. Chen and K. R. Davis, "The potential of plants as a system for the development and production of human biologics," *F1000Research*, vol. 5, Art. no. 912, 2016.
- [24] A. K. Sharma *et al.*, "Plant molecular farming: Current status and future prospects," *Plant Cell Rep.*, vol. 38, pp. 1–15, 2019.
- [25] J. Yao, Y. Weng, A. Dickey, and K. Y. Wang, "Plants as factories for monoclonal antibody production," *Plant Cell Tissue Organ Cult.*, vol. 123, pp. 1–15, 2015.
- [26] S. Ma *et al.*, "Expression of therapeutic proteins in plants: Recent advances and future prospects," *Plant Biotechnol. J.*, 2015.
- [27] T. V. Komarova, T. V. Gasanova, A. S. Komarov, and Y. L. Dorokhov, "Endoplasmic reticulum stress in plants and molecular farming," *Front. Plant Sci.*, vol. 5, Art. no. 60, 2014.
- [28] J. Xu, X. Ge, and M. C. Dolan, "Towards high-yield production of pharmaceutical proteins with plant cell culture," *Biotechnol. Adv.*, vol. 29, pp. 278–299, 2011.
- [29] L. Faye and V. Gomord, "Success stories in molecular farming—A brief overview," *Plant Cell Tissue Organ Cult.*, vol. 65, pp. 173–180, 2001.