REVIEW ARTICLE

Plant-Based Vaccine Production Through Transient Expression System: A Narrative Review

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SUMMARY

Transient expression systems involve the temporary expression of a foreign gene in plant tissues to enable efficient, high-yield vaccine production. Plant-based systems minimize contamination risks because the introduced gene does not integrate into the host plant genome. Cultivation of host plants (e.g., *Nicotiana benthamiana*) does not require expensive bioreactors and enables efficient production of vaccines within 3–7 days. Plants can synthesise structurally complex proteins with correct folding and glycosylation, including virus-like particles, which are important for eliciting effective immune responses**.** A commonly employed method for transferring foreign genes into plant cells to achieve transient expression is Agrobacterium tumefaciens–mediated infiltration. These vaccines target a broad range of viral diseases, such as rabies, COVID-19, Human immunodeficiency virus, influenza, and Newcastle disease virus, as well as non-communicable diseases such as type 1 diabetes and certain cancers. Among various host plants, *Nicotiana benthamiana* is most widely used due to its high protein yield and ease of transformation. Despite these benefits, gene silencing, limited yields in certain plants, and viral mutations still hinder the effective production of vaccines. Alongside technical and scientific limitations, the approval process is delayed by public mistrust and a lack of unified global biosafety guidelines. This review presents the current status of plant-based vaccine development, covering types and delivery strategies, real-world case studies, regulatory and biosafety considerations, and the challenges and limitations associated with their production. It also outlines future perspectives to address existing hurdles and promote broader development and adoption of plant-based vaccines.

Keywords: *Agrobacterium, COVID-19, Nicotiana, Plants, Vaccines.*

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Introduction

All living organisms rely on their immune systems for protection against pathogens. However, with time, the body's defense mechanisms gradually deteriorate, reducing their effectiveness in combating infections. To enhance this system, vaccines play a crucial role in biological defence therapeutics. Detrimental pathogens are introduced into the vaccine in either a deactivated or an attenuated form. Upon administration, these pathogens are identified as antigens. This mechanism activates the immune system to respond and generate memory cells that retain information concerning specific pathogens. The predominant method employed in vaccine production involves the utilization of live attenuated pathogens, the application of inactivated microorganisms, and the incorporation of non-harmful components thereof. These methodologies present numerous challenges, including elevated production costs, extended production timelines, a sophisticated purification

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system, and a required cold-chain storage system.[1] Due to the challenges, the researchers explore a modern approach to vaccine production: a plant-based vaccine produced using a transient expression system. The transient expression system is used to produce an antigen in the plant. Transient expression systems represent a critical concept in this context. A transient expression system is a process where the gene of interest is temporarily introduced and expressed without integrating into the plant genome. For this process, mostly a bacterium known as Agrobacterium *tumefaciens* is used. A. tumefaciens uses a cellular machinery to transfer its DNA into plant tissue, a process called Agrobacterium *tumefaciens*-mediated infiltration. Vaccines produced through this process are more affordable, faster, and safer. An important question is why plant-based vaccines are considered safer. Because plants do not carry human or animal pathogens, this lowers the risk of contamination.[2,3] (Table 1).

This section highlights the stages involved in developing plant-based vaccines. The development of plant-based vaccines involves several stages. In this approach, a gene encoding a pathogen-associated protein is introduced into the plant genome, leading to expression of the corresponding antigen. This antigen is then exploited as the target for rapid vaccine development. After this, the protein is harvested and purified to produce a vaccine, and this technique is termed “molecular farming”. The purification of the vaccine is a critical step that ensures the final product is free from contamination before use in humans and animals. Many viral diseases, such as Zika, COVID-19, Avian Influenza, Hepatitis B and C, Ebola, and Newcastle disease (NDV), are targeted using a plant-based vaccine. The NDV is the most deadly and contagious viral disease in birds and poultry. To control the disease and reduce economic losses to the poultry industry, vaccines are produced in a plant. The maize plant is targeted to form a vaccine for NDV.[3] The potential application of this strategy in managing non-communicable diseases, notably type 1 diabetes, has also been recognized by researchers. The plants are engineered to express autoantigens, such as Insulin or GAD65, in the case of diabetes, and the diabetic vaccine is consumed orally, preventing immune cells from attacking pancreatic cells. Glutamic acid decarboxylase 65 (GAD65), a 65-kDa enzyme, is expressed in the brain and pancreas. In the pancreas, GAD65 is localized in insulin-producing beta cells. In type 1 diabetes, autoimmune responses mistakenly target GAD65, leading to beta cell destruction and impaired insulin production.[4] It is also seen that a type of cancer called colorectal or colon cancer is caused by the overexpression of a protein called GA733-2. The researchers target this protein to make a vaccine for colon cancer, where it can boost the immune system to give a response against colon cancer. The tobacco plant is targeted to make the vaccine for this cancer.[5]

In the preliminary stage of the animal model, positive results have been observed. There are many plants used for the production of vaccines, but the most commonly used is Nicotiana benthamiana because it is considered a more efficient system for protein expression and is easy to transform. With the transient expression system, many other molecular tools are used to increase the stability and expression of an antigen, examples of viral vectors include the Cowpea mosaic virus (CPMV) and the MagICON expression system.[6] This article presents a detailed review of the development of plant-based vaccines using the transient expression system and discusses various viral and non-viral diseases treated with this approach. (Figure 1).

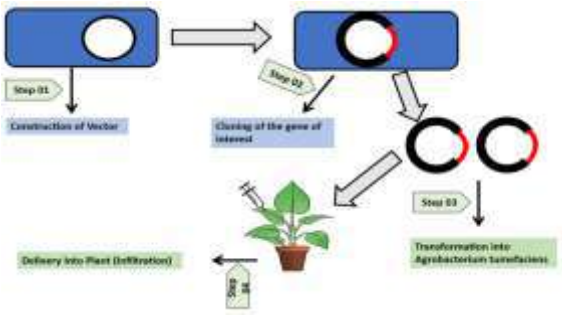

Fig.1: The basic procedure of the transient expression system in plants with steps. This schematic figure represents the main steps involved in the transient expression in a plant, including vector construction, cloning of the gene of interest, and transformation into Agrobacterium tumefaciens and the final delivery into plant leaves through agroinfiltration. This leads to the temporary expression of the recombinant protein is essential for the development of plant-based

Table 1: Showing Plant-based Vaccine vs Traditional Vaccine Platform Analysis

Feature	Plant-Based Vaccine Platform	Traditional Vaccine Platform	References
Safety and Scalability	Free from human/animal pathogens; reduced contamination risk. Easy scale-up with existing agricultural methods.	Risk of contamination with animal pathogens. More complex, limited scalability in some systems.	7
Production Speed	Rapid production using transient expression.	longer timelines for development.	8
Expression Systems	Uses plants like *Nicotiana benthamiana* with PVX vector or Agrobacterium tumefaciens.	Baculovirus, bacterial, or mammalian cell lines.	8
Post-Translational Modifications	Present but different from mammalian systems.	Closer to human PTMs in mammalian systems.	8
Cost	Low-cost production.	High production costs.	8
Platform Flexibility	Highly versatile, can express multiple antigens quickly.	Less flexible, often pathogen-specific.	8
Vaccine Format	Often uses virus-like particles (VLPs) with high safety.	Live-attenuated or inactivated viruses with the risk of reversion.	8

Methods

This narrative review aims to provide an updated analysis of plant-based vaccine production through transient expression systems, with a focus on recent advancements in the field. A systematic literature search was conducted for studies published between 2020 and 2025. The primary sources were academic databases including PubMed, Scopus, and Google Scholar. Only peer-reviewed articles that focused on plant-based vaccine production using transient expression systems were included in the review. Research articles were selected based on their relevance to plant species, expression systems, and vaccine yield.

The inclusion criteria were as follows:

1. Studies published between 2020 and 2025 focusing on plant-based vaccine production through transient expression systems.
2. Studies that provide detailed information about the plant species used, the expression systems employed, and the corresponding yield.
3. Peer-reviewed research from databases such as Google Scholar, Web of Science, Science Direct, Scopus, and Research Gate.

Data were extracted from the selected studies and analyzed using thematic analysis to identify common trends in plant-based vaccine production, expression system effectiveness, and scalability. This review emphasizes recent developments and identifies emerging gaps in the field, providing directions for future research.

Types of Plant-based Vaccines and Delivery Strategy

Subunit vaccines

Subunit vaccines produced by plants' transient systems utilise the system to rapidly generate immunogenic proteins from a pathogen or multiple pathogens. To produce subunit vaccines, the gene encoding the target antigen is transiently expressed in plant cells, primarily Nicotiana benthamiana, using vectors such as Agrobacterium tumefaciens or viral vectors. The subunit vaccines allow high-yield, scalable production of recombinant proteins; however, they do not elicit robust or long-lasting immune responses. They require appropriate adjuvants or repetitive doses. Subunit vaccines have been successful in providing immunity against hepatitis B and rabies and are being considered for Zika virus, Ebola, and many other viral diseases.[9] (Table 2).

Virus-like particles (VLPs)

VLPs that are produced by a plant's transient system are non-infectious and self-assembling particles. They are composed of viral structural proteins that imitate the morphology of viruses, but they lack

genetic material; therefore, non-infectious. As stated before, the transient expression system in N. benthamiana is preferred to ensure rapid, high-yield VLP production.[6] Agrobacterium tumefaciens is used as a viral vector. These VLPs stimulate a strong humoral and cellular immune response by enhancing the uptake of antigen-presenting cells, as VLPs present repetitive surface epitopes. Unlike subunit vaccines, VLPs often do not require additional adjuvants. These plant-derived VLP vaccines are proving to be appropriate candidates for viral diseases such as Influenza and SARS-CoV-2.[3] (Table 2).

Edible vaccines

Edible vaccines are produced from genetically engineered plants that express antigens from pathogens in their edible tissues. As they are consumed, the antigen becomes orally administered, resulting in the stimulation of mucosal and systemic immune responses. The host plants are commonly potato, banana, lettuce, and tomato due to their palatability and their ability to be consumed raw.[10] These antigens are expressed by integrating the genes into the plant genome so that long-term expression across generations is ensured. Edible vaccines offer low production cost, ease of administration, and the elimination of cold chain logistics. (Table 2). However, consistent antigen dosage, avoiding degradation in the GIT, are still some challenges.[11] (Figure 2).

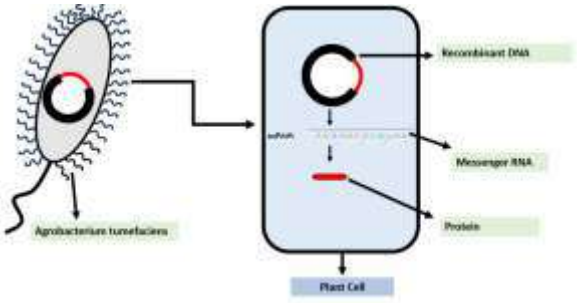

Fig.2: Agrobacterium-mediated gene transfer procedure

Delivery Strategies

The delivery methods of vaccines include.

Oral administration

Oral vaccines utilise plant-derived antigens to stimulate systemic (IgG) and mucosal (IgA) immunity.[12] They reduce the requirement for preservation through refrigeration and the need for personnel. They are also needle-free.[13] (Table 2).

Injectable administration

Injectable vaccines often use adjuvants and can be administered parenterally to induce a robust, long-lasting immune response.[14] This method allows precision of dosing and is suitable for vaccines that require a rapid immune response.[6] It includes COVID-19 vaccines.[2] (Table 2).

Intranasal administration

The intranasal administration of vaccine requires plant-derived extracellular vesicles to deliver mRNA to mucosal surfaces.[15] It increases mucosal immunity and is less invasive than injections.[11] It is promising; however, it is still under research and hasn't been made widely available for use. (Table 2).

Advantages of Plant-based Vaccine

Plant-based vaccines are considered cost-effective because they don't require expensive bioreactors, unlike bacterial or mammalian systems. It required 3-7days to produce the vaccine, which is very crucial for the rapid production during pandemics. Plant and animal pathogens are different, so there is no chance that plants are hosts for human pathogens. It reduces the chance of contamination by viruses, prions, and other contaminants. A simple agricultural practice is needed to produce a vaccine from plant leaves. As a result, it is easy to scale up vaccines. Using a single syringe to inject a mixture of different genetic constructs into the plant's leaves, the plant produces multiple vaccines in a single leaf. Complex proteins are produced mainly by plants and require proper folding, glycosylation, and assembly; these processes are very important for vaccine functionality. For instance, Virus like particles or VLPs.[16]

Challenges

A natural phenomenon through which plants attack foreign DNA is known as post-transcriptional gene silencing. It is the main hurdle in vaccine production because it reduces the amount of vaccine protein. Researchers are resolving this issue by applying silencing suppressor proteins such as p19 and CMV 2b. On the other hand, some plants show low protein yield and need upgradation to improve expression levels. The researchers are solving the technical problems by improving protein production and making plant-based vaccines more effective.[6]

Ethical Concerns with Edible Vaccines

Edible vaccines are promising for immunisation by

Table 2: Overview of Plant-based vaccines included in the literature review

Target pathologies	Host plant	Antigen	Immunogenic response	Delivery method	Vaccine type	Reference
Newcastle disease (NDV)	Potato	Hemagglutinin-neuraminidase protein (HN) and fusion protein (F)	Oral immunisation induced mucosal IgA and serum IgG responses. The intraperitoneal route only induced a systemic IgG response	Oral and injected	Subunit	3
Covid 19	*N. benthamiana*	SARS-CoV-2 spike glycoprotein	Unadjuvanted coVLP vaccine induced anti-spike IgG and neutralising antibodies Th1-biased cellular responses characterised by IFN-γ were also recorded. Adjuvanted coVLP enhanced immunogenicity by inducing a more balanced Th1/Th2 response, along with higher neutralising antibody titers. CoVLP with ASO3 induced higher immunogenicity	Injection	VLP	2
Hepatitis E	*N. benthamiana*	HEV ORF2 (110-610 aa) capsid protein	Higher production of HEV-specific IgG was noticed in mice by immunisation through HEV 110-610 aa	Intra-muscular injection	VLP	6
Bovine respiratory disease	*N. benthamiana*	Leukotoxin A (LktA) and PipE + CBT as adjuvant	The immunogenic response was analysed by ELISA, measuring IgG (mucous) and IgA (BAL and faeces). High production was observed	Oral	Subunit	1
Type 1 diabetes (T1D)	Tobacco plant	Human proinsulin (hpINS) and glutamic acid decarboxylase (GAD)	The vaccine wasn't significantly effective in preventing T1D	Oral administration	Antigen-specific vaccine	4
Colorectal cancer	Tobacco plant	Recombinant GA733-2 (rGA733-2) and rGA733 fused to Fc domain (rGA733-Fc)	The plant-derived RGA733-FC induced 68% apoptosis of MC38 and 54% in HCT116	Not mentioned	-	5
Hepatitis C	*N. benthamiana*	HVR1 fused to CTB	High anti-CTB and anti-HCV (HVR1) sera IgG responses were recorded, enhanced by a booster dose, indicating immunogenic memory	Intranasal	Subunit	3
Rabies	*N. benthamiana*	Rabies virus glycoprotein (RVGP) + human IgG Fc +KDEL sequence. (RVGP-FcK)	The RVGP-FcK enhanced immunogenicity against Rabies virus due to the Fc domain by promoting better binding to antigen-presenting cells	Not mentioned	Subunit	9

enabling oral administration of plant-derived vaccines; however, they still raise ethical concerns. These concerns must be addressed to ensure safety and build public trust. There is a general scepticism towards genetically modified organisms (GMOs); therefore, a resistance has been seen in communities towards edible vaccines. The concerns include the safety of consuming GMOs and the environmental impact.[11] Rigorous and extensive preclinical and clinical trials are necessary to ensure the safety and efficacy of the vaccines. Monitoring should continue even after licensure to maintain the public's trust. These are some concerns regarding edible vaccines that must be considered. They can cause challenges in acceptance and implementation. Addressing these issues can serve as a prompt and efficient step towards the advancement of mankind in the field of technology and towards achieving public health goals.[17]

Real World Applications and Industry Insight

Plant-based vaccine entered the real world with COVIFENZ®, a COVID-19 virus-like particle (VLP) vaccine produced by Medicago using N. benthamiana. VLPs are noninfectious multiprotein assemblies that resemble native viruses but lack the viral genome that causes diseases, making them safer candidates for vaccine development. They have been approved in Canada, which is a notable milestone for plant-based vaccines using a transient system.[3,18] In addition to the COVID-19 vaccine, plant-derived Influenza VLP vaccines have successfully completed Phase I and Phase II clinical trials, which builds confidence in plant-derived biologics. These developments point to an ever-faster timeline from production to regulatory approval; for instance, COVIFENZ® moved from development to market in about 24 months amid the pandemic. Medicago is considered a pioneer in plant-based VLP vaccines. Although LMICs are not always explicitly discussed in the literature, several sources emphasise that the cost, speed of scale-up, and potential for oral delivery of plant-based vaccines align with health priorities in low- and middle-income countries.[6,19]

Edible vaccine for Poultry - Newcastle disease Virus (NVD)

Transgenic potatoes have also been investigated as a delivery system for the production of edible vaccines against NDV, a major poultry disease. This plant-based system is a safe, cost-effective, and effective alternative to conventional injectable vaccines, particularly in agriculture. It may also be useful for boosting mucosal immunity.[3]

Transient Expression for Ebola and Malaria

Transient expression systems have been employed to manufacture clinical-grade biologics, including the ZMapp antibody cocktail against Ebola and a transmission-blocking vaccine candidate against malaria, underscoring the platform's ability to address poverty-related and neglected diseases.[7]

ZMapp and Ebola vaccine through the magnICON System

Additional research illustrates ZMapp manufacturing in the magnICON system, which further confirms the utility of plant expression systems for the fight against epidemic diseases such as Ebola.[20]

Case Studies

HEV-Based Chimeric VLPs

Chimeric virus-like particles based on hepatitis E virus (HEV) capsid were engineered in Nicotiana benthamiana to express green fluorescent protein (GFP) to track expression and confirm surface localization, and four copies of the influenza M2e peptide to potentiate mucosal immune responses. M2e is crucial for viral propagation and is involved in the uncoating process of the influenza virus. Geometry was optimised for antigen presentation by using flexible glycine-rich linkers. Expression analysis revealed that high levels of recombinant protein could be obtained from HEV core at 20% of total soluble protein (TSP), HEV/GFP at 10%, and HEV/4M2e at 6-8% TSP. HEV/GFP refers to a recombinant form of the Hepatitis E virus (HEV) that incorporates the green fluorescent protein (GFP) as a reporter gene, which allows for real-time tracking of viral infection in cell cultures and animal models.[21,22] After purification, yields of HEV/4M2e ranged from 60 to 80μg/g of leaf biomass, a parameter that supports commercial-scale applicability. Structural characterisation by transmission electron microscopy (TEM) and atomic force microscopy (AFM) established that IVLPs were intact, spherical particles approximately 21-24 nm in diameter, displaying various antigens on their surface. (Table 3). In preclinical trials on mice, the vaccines elicited strong systemic IgG responses against both HEV and

Table 3: Structural and Immunological Characteristics of Plant-Derived VLPs [8,22]

VLP Construct	Size (nm)	Surface Antigen	Yield (μg/g)	Immunogenicity	Delivery Mode
HEV Core VLP	22	None	100–200	High IgG response	Intraperitoneal
HEV/GFP	24 ± 8	GFP	80	GFP-specific IgG	Intraperitoneal
HEV/4M2e	21 ± 6	M2e ×4	60–80	Serum IgG, mucosal IgA	Oral, i.p.

GFP antigens. Notably, the orally delivered HEV/4M2e vaccine induced mucosal IgA responses, indicating the potential for non-invasive vaccine delivery.[23]

Regulation and Biosafety of the Protocol of the Transient Expression System

Biosafety, containment, and gene flow concerns

Production of recombinant proteins using transgenic plants is now recognised as a cost-effective and efficient method for the development of pharmaceuticals. This method, however, comes with its threatening prospects. The risks may include accidental cross-pollination between model species and commercial food crops, and harmful exposure to domestic or non-target animals. Even though transient expression systems pose less environmental risk than stable expression systems, they are not completely risk-free. Regulatory concerns include the possibility that residues of recombinant DNA may remain in the soil in extracted biomass or plant tissues post-harvest and disrupt the ecosystem, instability of viral vectors, and inconsistency in levels and quality of desired proteins and the need for regulatory guidelines to ensure safety, quality, and efficacy of plant-produced biopharmaceuticals for human use. These biosafety concerns also shape public scepticism regarding plant biotechnology. Strictly speaking, host plants of transient expression systems are not genetically modified crops. Certain regulatory bodies, such as the USDA, may exempt them from GMO regulations, but the general public does not distinguish between stable GM crops and GE transient host plants. Therefore, this review presents an overview of the various approaches employed in plant molecular pharming.[24]

Regulatory Authorities Involved

Biosafety protocols vary slightly across jurisdictions, as leading countries in biotech have their own regulatory authorities. The globally acknowledged regulatory bodies include the FDA in the US, EMA in the EU, and HC (Health Canada). USDA-APHIS regulates the cultivation of GM plants, and FDA oversees the safety aspects if the product is pharmaceutical.[25]

Biosafety Steps

The typical steps that are important to take before cultivating a transient expression system include risk assessment, where potential pathogenicity or allergenicity is assessed, and biosafety level (BSL) classification. BSLs are established by the WHO, the CDC, and the NIH and adopted by national regulatory bodies of different countries. There are four biosafety levels (BL1-P to BL4-P) depending on the nature of the recombinant DNA, its potential to harm the environment, and its mobility and dispersal risk.[26] The Cartagena Protocol is an international set of rules that focuses on handling the safety of the cross-border movement of GMOs. The authorization requirements involve a notification process for GM plants grown in fields and a Permit process for transgenic plants producing pharmaceuticals.[27] The role of Good Manufacturing Practice (GMP) comes in after the extraction of the desired protein and successful preclinical evaluations. It is a set of procedures that ensures the safety of the biological product. Factors affecting the stability of GM products and their shelf-life concerns should be noted. Product characterization and quality assurance are a requirement for approval. The final commercialization comes after a detailed process, clinical trials, and applications. A Clinical Trial application (CTA) or Investigational New Drug (IND) application is to be approved by the FDA/EMA to initiate clinical trials in humans; only then is the product directed towards commercialization if it has promising results.[28] Development of any product that would require genetic engineering in plants goes

through this rigorous process, and becomes more meticulous in the case of pharmaceuticals. The development to commercialisation of a vaccine using transient expression is more efficient and can be achieved within weeks or a few months at most, whereas a traditional approach typically takes 10-15 years.[29]

Approval hurdle for plant-based biologics

Common challenges that might hinder the approval process for cultivating host plants include fear-based media narratives that fuel public mistrust. Regulatory approval bodies are sensitive to public sentiment; moreover, public opposition can halt incoming development funds. GE plants aimed for pharmaceutical products undergo stricter regulatory scrutiny, so they are less likely to be granted non-regulatory status for commercialisation. Another major problem is that, globally, biosafety guidelines are not unified, leaving more room for accidents. Regulatory bodies, except a few, do not have separate guidelines for the cultivation of transient expression host plants, which unnecessarily delays the approval process, as they are then scrutinised under the protocol for stable systems, which requires more investigation and time since it has greater potential.[30]

Most edible vaccines are not effective against diseases because stomach acid is strong enough to degrade them before they reach the immune system. For this, a cover is needed around the vaccine to protect it from degradation. Many plants have high yields but are non-edible; for instance, N. benthamiana requires an additional purification process, which increases production costs. Another main limitation of this process is its instability, which means some proteins need a cold environment for vaccine storage, which can be challenging in rural or underdeveloped areas. The process of lyophilisation is used for long-term storage in a powder form, but it reduces its effectiveness in some cases.[11]

To advance the development of plant-based technology, it is important to harmonize global regulatory frameworks, apply Good Manufacturing Practices (GMP) specific to plant-derived systems, and use reliable assays to ensure antigen consistency. The focus must be on high-yield production platforms, such as transient expression in N. benthamiana, while developing stable transgenic plant models for long-term vaccine production is also essential.

Low-cost purification methods such as membrane filtration, magnetic bead separation, and purification tags require further refinement. To improve edible vaccine strategies that use crops like tomato and maize, improvements in bioencapsulation to protect antigens and stimulate mucosal immunity will be needed. Optimising oral and nasal delivery systems with potent adjuvants and carriers can be vital to strengthen IgA responses. Multi-epitope constructs and synthetic gene fusions should be prioritised that target different stages of disease progression. Using plant-based virus-like particles (VLPs) and recombinant fusion proteins can significantly increase immunogenicity. Artificial intelligence and omics technologies will help in novel antigen discovery and efficient codon optimisation if integrated correctly. The development of thermostable or lyophilised vaccine formulations will reduce dependence on cold-chain logistics. Furthermore, plants must be harnessed to produce veterinary vaccines to weaken zoonotic disease transmission. Ultimately, public engagement, early-stage clinical trials, and vigorous global collaboration will be crucial to the successful adoption and implementation of plant-based vaccines.

Despite these advantages, many limitations and challenges remain. Variability in expression levels, the limited success of mostly edible vaccines against diseases, the complexity of downstream processing, and regulatory standardization continue to hinder large-scale commercial adoption.

Conclusion

A plant-based vaccine is an alternative to the conventional vaccine manufacturing platform. This method enables rapid, flexible, and scalable production of recombinant vaccine antigens without requiring stable genetic modification of the host plant. It has several advantages over the traditional method, including reduced production time, lower infrastructure costs, and improved biosafety. These features make a plant-based transient system prominent for rapid vaccine development during an infectious disease outbreakIn conclusion, transient expression systems in plants offer a promising, sustainable platform for next-generation vaccine production.

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REFERENCES

1. Chung YH, Church D, Koellhoffer EC, Osota E, Shukla S, Rybicki EP, et al. Integrating plant molecular farming and materials research for next-generation vaccines. Nature Reviews Materials. 2022; 7: 372-88. doi: 10.1038/s41578-021-00399-5
2. Ward BJ, Gobeil P, Séguin A, Atkins J, Boulay I, Charbonneau PY, et al. Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19. Nature medicine. 2021; 27: 1071-8. doi: 10.1038/s41591-021-01370-1
3. Su H, Van Eerde A, Rimstad E, Bock R, Branza-Nichita N, Yakovlev IA, et al. Plant-made vaccines against viral diseases in humans and farm animals. Frontiers in plant science. 2023; 14: 1170815. doi: 10.3389/fpls.2023.1170815
4. Bassin EJ, Piganelli JD, Little SR. Auto-antigen and Immunomodulatory Agent–Based Approaches for Antigen-Specific Tolerance in NOD Mice. Current Diabetes Reports. 2021; 21: 9. doi: 10.1007/s11892-021-01376-6
5. Park SH, Ji KY, Kim HM, Ma SH, Park SY, Do JH, et al. Optimization of the human colorectal carcinoma antigen GA733-2 production in tobacco plants. Plant biotechnology reports. 2021; 15: 55-67. doi: 10.1007/s11816-020-00657-y
6. Mardanova ES, Vasyagin EA, Ravin NV. Virus-like particles produced in plants: A promising platform for recombinant vaccine development. Plants. 2024; 13: 3564. doi: 10.3390/plants13243564
7. Boes A, Reimann A, Twyman RM, Fischer R, Schillberg S, Spiegel H. A plant-based transient expression system for the rapid production of malaria vaccine candidates. Methods in Molecular Biology. 2016; 1404: 597-619. doi: 10.1007/978-1-4939-3389-1_39
8. Ewa WG, Agata T, Milica P, Anna B, Dennis E, Nick V, et al. Public perception of plant gene technologies worldwide in the light of food security. GM crops & food. 2022; 13: 218-41. doi: 10.1080/21645698.2022.2111946
9. Lee Y, Park J, Kim Y, Hwang H, Jin C, Oh Y, et al. Transient plant expression of recombinant rabies virus glycoprotein: a strategy for vaccine production. 원예과학기술지. 2024; 42: 264-78. doi: 10.7235/HORT.20240023
10. Karakas I, Tonk FA. Plants that can be used as plant-based edible vaccines, current situation and recent developments. Virology & Immunology Journal. 2022; 6: 000302. doi: 10.23880/vij-16000302
11. Vo DK, Trinh KT. Molecular farming for immunization: current advances and future prospects in plant-produced vaccines. Vaccines. 2025; 13: 191. doi: 10.3390/vaccines 13020191
12. Kaldis A, Uddin MS, Guluarte JO, Martin C, Alexander TW, Menassa R. Development of a plant-based oral vaccine candidate against the bovine respiratory pathogen Mannheimia haemolytica. Frontiers in Plant Science. 2023; 14: 1251046. doi: 10.3389/fpls.2023.1251046
13. Kwong KW, Xin Y, Lai NC, Sung JC, Wu KC, Hamied YK, et al. Oral vaccines: a better future of immunization. Vaccines. 2023; 11: 1232. doi: 10.3390/vaccines11071232
14. Kumar M, Kumari N, Thakur N, Bhatia SK, Saratale GD, Ghodake G, et al. A Comprehensive overview on the production of vaccines in plant-based expression systems and the scope of plant biotechnology to combat against SARS-CoV-2 virus pandemics. Plants. 2021; 10: 1213. doi: 10.3390/plants10061213
15. Xu H, Cai L, Hufnagel S, Cui Z. Intranasal vaccine: Factors to consider in research and development. International journal of pharmaceutics. 2021; 609: 121180. doi: 10.1016/j.ijpharm.2021.121180
16. Chattopadhyay A, Jailani AA, Mandal B. Exigency of plant-based vaccine against COVID-19 emergence as pandemic preparedness. Vaccines. 2023; 11: 1347. doi: 10.3390/vaccines11081347
17. Yang YT, Reiss DR. Regulation and Testing of Vaccines. Vaccine Law and Policy. 2023: 15-23. doi: 10.1007/978-3-031-36989-6_2
18. Gupta R, Arora K, Roy SS, Joseph A, Rastogi R, Arora NM, et al. Platforms, advances, and technical challenges in virus-like particles-based vaccines. Frontiers in immunology. 2023; 14: 1123805. doi: 10.3389/fimmu.2023.1123805
19. Uvarova EA, Belavin PA, Deineko EV. Design and assembly of plant-based COVID-19 candidate vaccines: recent development and future prospects. Vavilov Journal of Genetics and Breeding. 2022; 26: 327. doi: 10.18699/VJGB-22-39
20. Zahmanova G, Aljabali AA, Takova K, Minkov G, Tambuwala MM, Minkov I, et al. Green biologics: harnessing the power of plants to produce pharmaceuticals. International journal of molecular sciences. 2023; 24: 17575. doi: 10.3390/ijms242417575
21. Nayak B, Quadri A, Yadav R, Kumar H, Pandey S, Saraya A, et al. Pseudo virus production for Hepatitis E virus using GFP reporter expressing Lentiviral genome backbone to study neutralizing immune response by transduction assay. Journal of Clinical and Experimental Hepatology. 2023; 13: S183-4.
22. Kumar U, Goyal P, Madni ZK, Kamble K, Gaur V, Rajala MS, et al. A structure and knowledge-based combinatorial approach to engineering universal scFv antibodies against influenza M2 protein. Journal of Biomedical Science. 2023; 30: 56. doi: 10.1186/s12929-023-00950-2
23. Mardanova ES, Vasyagin EA, Kotova KG, Zahmanova GG, Ravin NV. Plant-Produced Chimeric Hepatitis E Virus-like Particles as Carriers for Antigen Presentation. Viruses. 2024; 16: 1093. doi: 10.3390/v16071093
24. Caradus JR. Processes for regulating genetically modified and gene edited plants. GM Crops & Food. 2023; 14: 1-41. doi: 10.1080/21645698.2023.2252947
25. Knödler M, Reunious PW, Buyel JF. Risk assessment and bioburden evaluation of Agrobacterium tumefaciens-mediated transient protein expression in plants using the CaMV35S promoter. BMC biotechnology. 2023; 23: 14. doi: 10.1186/s12896-023-00782-w
26. Pereira R. The Cartagena protocol on biosafety and the regulation of transboundary movement of living modified

organisms. Transgenic insects: Techniques and applications. 2022: 533-51. doi: 10.1079/97818006 21176.0027
27. Buyel JF. Product safety aspects of plant molecular farming. Frontiers in Bioengineering and Biotechnology. 2023; 11: 1238917. doi: 10.3389/fbioe.2023.1238917
28. Zafar S, Akhtar A, Sayed E, Onaiwu E, Arshad MS, Ahmad Z. Vaccine formulation design: challenges and opportunities. RSC Pharmaceutics. 2025; 2: 490-516. doi: 10.1039/D5PM00017C
29. Hundleby PA, D'Aoust MA, Finkle C, Atkins J, Twyman RM. Regulation of molecular farming products. Methods in Molecular Biology. 2022; 2480: 313-33 doi: 10.1007/978-1-0716-2241-4_17
30. Akher SA, Wang KY, Hall K, Hunpatin OS, Shan M, Zhang Z, et al. Harnessing transient expression systems with plant viral vectors for the production of biopharmaceuticals in Nicotiana benthamiana. International Journal of Molecular Sciences. 2025; 26: 5510. doi: 10.3390/ijms26125510

Author Contributions

AR: Conception and design of the work
MA: Writing the original draft, proofreading, and approval for final submission
ZA: Manuscript writing for methodology design and investigation
WF: Validation of data, interpretation, and write-up of results
TK: Revising, editing, and supervising for intellectual content
HK: Data acquisition, curation, and statistical analysis