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IN VIVO EVALUATION OF A PLANT-DERIVED RECOMBINANT VACCINE FOR DENGUE VIRUS

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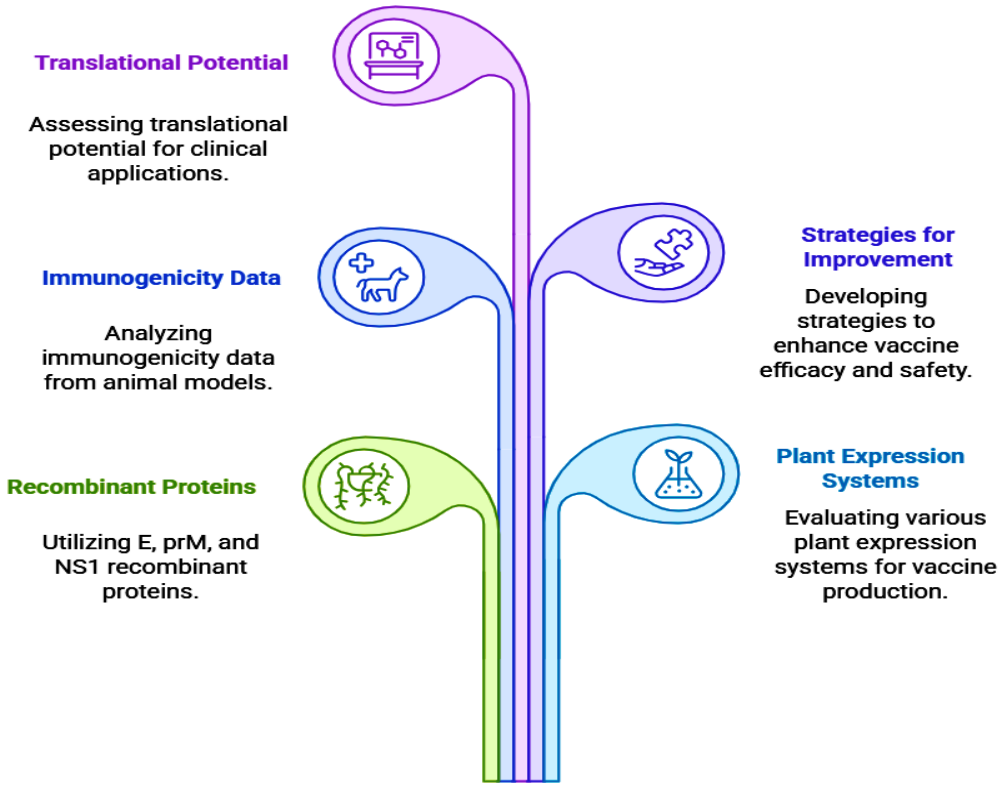
Abstract

Dengue virus infection remains a major public health concern in tropical and subtropical regions, with no universally safe and effective vaccine available. Traditional vaccine platforms face challenges including cost, cold-chain requirements, and the risk of antibody-dependent enhancement (ADE). Plant-based recombinant vaccines have emerged as a promising alternative due to their safety, scalability, and low production costs. This review explores the development and in vivo evaluation of plant-derived dengue vaccine candidates, focusing on recombinant proteins such as the envelope (E), pre-membrane (prM), and non-structural proteins (NS1). We examine various plant expression systems, immunogenicity data from animal models, and strategies for improving vaccine efficacy and safety. Furthermore, the review highlights the translational potential of plant-based vaccines and outlines future directions for preclinical and clinical development. This approach offers a novel and sustainable pathway toward effective dengue prevention.

Keywords: Plant-based vaccines, Dengue virus, Recombinant protein, In vivo evaluation, Molecular pharming

Graphical abstract:



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1. INTRODUCTION

1.1 Global Burden of Dengue

Dengue virus (DENV) infection is one of the most rapidly spreading mosquito-borne viral diseases worldwide, affecting over 390 million people annually, with nearly 96 million presenting with clinical manifestations. Endemic in more than 100 countries, dengue imposes a significant public health and economic burden, particularly in tropical and subtropical regions of Southeast Asia, Latin America, and Africa. The disease is caused by four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), each capable of causing illness. Infection with one serotype offers lifelong immunity to that serotype but only temporary cross-protection against the others [1]. Sequential infection with a different serotype increases the risk of severe forms of the disease such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), both of which are associated with high morbidity and mortality (Table 1).

Table 1: Dengue Virus Serotypes and Associated Clinical Manifestations

Serotype	DENV-1	DENV-2	DENV-3	DENV-4
Circulation	Global	Global	Global	Less prevalent
Associated Complications	Mild dengue	Severe DHF/DSS	Moderate to severe	Mild dengue
Cross-reactivity	Yes	Yes	Yes	Yes
Antibody-dependent Enhancement (ADE) Risk	High in secondary infections with heterologous serotype			

1.2 Need for an Effective, Safe, and Affordable Dengue Vaccine

The absence of specific antiviral treatment for dengue underscores the critical need for a preventive vaccine. A successful vaccine must induce a long-lasting, balanced immune response against all four DENV serotypes to prevent severe disease, including DHF and DSS. In addition to immunogenicity, an ideal dengue vaccine must be cost-effective, easy to manufacture, stable at ambient temperatures, and suitable for large-scale deployment, especially in resource-limited settings where dengue is most prevalent [2].

1.3 Limitations of Current Vaccines

The first licensed dengue vaccine, CYD-TDV (commercially known as Dengvaxia), developed by Sanofi Pasteur, marked a milestone in dengue prevention but also revealed significant limitations. It demonstrated varying efficacy among serotypes and was found to increase the risk of severe dengue in individuals who had not been previously infected with the virus. This outcome, attributed to antibody-dependent enhancement (ADE), raised concerns about its safety and restricted its use to seropositive individuals aged 9-45 years. Other candidates such as TAK-003 and TV003 are in late-stage development but still face challenges in balancing immunogenicity and safety across all serotypes [3].

1.4 Emergence of Plant-Based Recombinant Vaccines as Next-Generation Candidates

In recent years, plant-based recombinant vaccines have emerged as an innovative and promising platform for dengue immunization. Plants such as *Nicotiana benthamiana*, *Oryza sativa*, and *Lactuca sativa* are increasingly being used as biofactories for the expression of viral proteins, offering several advantages over traditional systems. These include low production cost, absence of human pathogens, ease of scaling up, and the ability to express complex viral antigens in their native conformations. Additionally, plant-derived vaccines are amenable to oral administration, which could simplify mass immunization campaigns. Given the limitations of conventional vaccines and the pressing need for safe and effective alternatives, plant-based recombinant vaccines represent a next-generation solution with considerable potential in combating dengue [4].

2. OVERVIEW OF PLANT-BASED VACCINES

2.1 Concept of Molecular Pharming and Edible Vaccines

Molecular pharming refers to the use of genetically engineered plants to produce pharmaceutical substances, including vaccines, antibodies, and therapeutic proteins. This innovative approach leverages plants as biofactories for the expression of recombinant antigens that can elicit immune responses. One of the most intriguing applications of molecular pharming is the development of edible vaccines, where the antigen is expressed directly in consumable parts of the plant such as fruits, leaves, or seeds. Upon ingestion, these plant tissues can stimulate mucosal immunity, offering a needle-free, user-friendly, and scalable vaccination strategy, particularly beneficial for low-resource settings [5].

2.2 Advantages of Plant-Based Vaccines

Plant-based vaccines offer multiple advantages over traditional vaccine platforms. They are cost-effective, as they eliminate the need for expensive fermentation and purification processes typically associated with microbial or mammalian cell culture systems. Scalability is another major benefit plants can be cultivated on a large scale using existing agricultural infrastructure. Additionally, these vaccines are stable at ambient

temperatures, reducing dependence on cold-chain logistics, which is particularly crucial for vaccine delivery in rural or tropical areas [6]. Importantly, plant-derived vaccines are inherently safe, as they do not harbor human or animal pathogens, thereby reducing the risk of contamination and enhancing biosafety (Table 2).

Table 2: Advantages of Plant-Based Vaccine Platforms

Feature	Plant-Based Vaccines	Traditional Vaccines
Cost	Low	High
Cold Chain Requirement	Not essential	Essential
Risk of Human Pathogens	None	Present
Scalability	High (vertical farming, hydroponics)	Moderate
Production Time	Rapid (especially with transient expression)	Longer
Biosafety	High	Variable

2.3 Common Plant Platforms for Vaccine Production

Several plant species have been used successfully as platforms for recombinant protein expression. *Nicotiana benthamiana*, a close relative of tobacco, is one of the most widely used species due to its fast growth, ease of genetic manipulation, and high biomass yield. *Solanum lycopersicum* (tomato) and *Lactuca sativa* (lettuce) are also frequently used for edible vaccine development because of their palatability and acceptance for oral administration [7]. *Oryza sativa* (rice) has also been employed, especially for expressing antigens in seeds, offering extended shelf-life and stability (Table 3).

Table 3: Plant Species Commonly Used for Vaccine Production

Plant Species	Scientific Name	Expression System	Advantages
Tobacco	*Nicotiana benthamiana*	Transient via agroinfiltration	High yield, rapid expression
Tomato	*Solanum lycopersicum*	Stable or transient	Edible vaccine potential
Rice	*Oryza sativa*	Stable transformation	Long shelf life, oral delivery potential
Lettuce	*Lactuca sativa*	Transient expression	Edible, suitable for oral immunization

2.4 Techniques for Plant Transformation

To produce recombinant vaccines in plants, several genetic engineering techniques are employed. Agrobacterium-mediated transformation is the most common method for introducing foreign genes into the nuclear genome of dicotyledonous plants. This technique utilizes the natural gene transfer ability of *Agrobacterium tumefaciens*. Alternatively, biolistic or gene gun transformation is used to deliver DNA directly into plant cells, especially monocots like rice and maize. For rapid and high-level expression, transient expression systems using plant viral vectors such as magnICON, geminivirus-based systems, or TMV-based vectors are gaining popularity. These systems bypass the time-consuming process of generating stable transgenic lines and can yield significant quantities of recombinant proteins within days [8].

3. DENGUE VIRUS STRUCTURE AND VACCINE TARGETS

3.1 Structure and Function of Dengue Virus

Dengue virus (DENV) belongs to the *Flaviviridae* family and exists as four distinct but closely related serotypes: DENV-1 to DENV-4. It is an enveloped, positive-sense single-stranded RNA virus, approximately 11 kb in length. The viral genome encodes a single polyprotein that is post-translationally cleaved into three structural proteins - Capsid (C), Pre-membrane (prM), and Envelope (E) and seven non-structural (NS)proteins - NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [9].

The Envelope (E) proteinplays a key role in viral attachment, entry into host cells, and membrane fusion. It is the primary target for neutralizing antibodies and has been extensively used in vaccine design. The Pre-membrane (prM) proteinis involved in viral assembly and protects the E protein from premature fusion during viral maturation. However, improper maturation of prM can lead to the generation of partially immature virions, which may contribute to non-neutralizing antibody production and enhanced disease [10].

Among the non-structural proteins, NS1 is a secreted glycoprotein that plays roles in immune evasion and viral replication. Importantly, it induces strong humoral and cellular immune responses, making it a promising vaccine target. NS3 functions as a serine protease and helicase, while NS5 has RNA-dependent RNA polymerase activity and contributes to viral replication. These proteins also serve as targets for T-cell responses and are being explored for inclusion in next-generation vaccine constructs (Table 4).

Table 4: Key Antigenic Targets of Dengue Virus for Vaccine Design

Protein	Function	Vaccine Relevance
Envelope (E)	Virus binding and fusion	Major target for neutralizing antibodies
Pre-membrane (prM)	Virion maturation	Helps in VLP formation
NS1	Immune evasion, biomarker	Protective immunity; diagnostic use
NS3	Protease/helicase activity	Cell-mediated immunity
NS5	RNA polymerase	Limited vaccine use due to internal nature

3.2 Immunogenic Epitopes for Vaccine Development

Effective vaccine development relies on identifying conserved, immunodominant epitopes that can elicit a protective immune response across all four dengue serotypes. The E protein, particularly Domain III (EDIII), contains type-specific and cross-reactive epitopes capable of inducing strong neutralizing antibodies. In contrast, the prM protein contains epitopes that predominantly lead to cross-reactive, non-neutralizing antibodies, which can enhance infection through the process of ADE [11].

The NS1 proteinhas emerged as a potent immunogen capable of stimulating both B-cell and T-cell responses. Epitopes within NS1 can induce protective immunity without the risk of ADE, making it an attractive component in subunit and recombinant vaccine platforms. Additionally, $CD8^+$ T-cell epitopes within NS3 and NS5 are conserved across serotypes and may provide cross-protective cellular immunity, offering an advantage for T-cellbased vaccine strategies [12].

3.3 Antibody-Dependent Enhancement (ADE) and Its Impact on Vaccine Design

One of the most significant challenges in dengue vaccine development is antibody-dependent enhancement (ADE). ADE occurs when non-neutralizing or sub-neutralizing antibodies, generated during a primary infection, facilitate viral entry into Fc receptor-bearing cells during subsequent infections with a different serotype. This phenomenon leads to increased viral replication and a higher risk of severe disease manifestations like DHF and DSS [13].

The risk of ADE has critical implications for vaccine design. Vaccines must elicit a balanced and robust neutralizing antibody response against all four serotypes simultaneously. Partial immunity or serotype-biased responses can predispose individuals to ADE upon natural infection. For this reason, vaccine platforms, including plant-derived systems, must be carefully designed to incorporate protective epitopes while avoiding components (e.g., certain prM epitopes) that could promote ADE. Research into epitope-focused immunogen design, multivalent formulations, and T-cell based strategies is essential to mitigate ADE risk and enhance vaccine safety [14].

4. PLANT-DERIVED DENGUE VACCINE CANDIDATES

4.1 Recombinant E and prM Protein Expression in Transgenic Plants

The Envelope (E) and Pre-membrane (prM) proteins are primary antigens used in dengue vaccine development due to their critical roles in virus attachment, entry, and immune recognition. In plant-based vaccine platforms, these proteins have been successfully expressed in transgenic systems to mimic the native conformation of the virus and induce protective immunity [15].

Several studies have demonstrated the expression of E/prM protein complexes in plant species such as *Nicotiana benthamiana* and *Lactuca sativa* using Agrobacterium-mediated transformation or transient expression vectors. These recombinant proteins, when properly folded and glycosylated, can stimulate the production of neutralizing antibodies in vivo. However, careful antigen design is required to avoid eliciting non-neutralizing antibodies that may contribute to antibody-dependent enhancement (ADE) [16].

4.2 NS1 Protein as a Diagnostic and Vaccine Candidate

Non-structural protein 1 (NS1) has dual utility in dengue research as a diagnostic biomarker and a vaccine antigen. It is secreted in high concentrations during infection and is a known target for immune responses that do not contribute to ADE. This makes NS1 an especially attractive component for inclusion in subunit vaccines.

In plant systems, NS1 has been expressed with success using transient expression methods in *Nicotiana benthamiana*. Studies show that plant-derived NS1 can elicit robust humoral and cellular immune responses in animal models, including the production of high-affinity IgG and activation of T-cells. The antigen also holds promise for use in diagnostic test kits, offering a scalable and low-cost source for NS1 protein production [17].

4.3 Fusion Proteins and Virus-Like Particles (VLPs) from Plants

To enhance immunogenicity, plant-derived vaccines often utilize fusion protein strategies or virus-like particles (VLPs). Fusion proteins typically combine immunodominant dengue epitopes with carrier proteins like cholera toxin B subunit (CTB), ricin B chain, or heat-labile enterotoxin subunits, which act as mucosal adjuvants. These constructs have been expressed in edible plant species such as tomato, lettuce, and rice, and

have demonstrated significant mucosal and systemic immune responses in animal models upon oral delivery [18].

VLPs are another highly promising approach. These are self-assembling, non-infectious nanoparticles that structurally resemble the native virus but lack genetic material. Dengue VLPs produced in plants especially in *Nicotiana benthamiana* have been shown to display authentic quaternary epitopes necessary for neutralizing antibody responses. Plant-derived VLPs are advantageous because they can be produced rapidly, are inherently safe, and induce strong immunogenicity without the risk of reversion to virulence [19].

4.4 Case Studies of Promising Candidates

Several experimental plant-based dengue vaccine candidates have demonstrated success in preclinical settings. One of the most notable examples is the expression of dengue VLPs in*Nicotiana benthamiana* via a magnICON transient expression system. This platform yielded high levels of tetravalent VLPs that mimicked the morphology of native dengue virions. Immunization of mice with these VLPs, particularly when adjuvanted, elicited serotype-specific neutralizing antibodies and T-cell responses with cross-serotype coverage [20].

Another promising candidate involved the expression of recombinant EDIII (Envelope Domain III) fused with CTB in transgenic lettuce. Oral administration of this fusion protein to mice led to significant mucosal IgA and systemic IgG production, demonstrating the potential of edible vaccines. Similarly, rice-expressed NS1 protein has been evaluated for both immunization and diagnostic applications with encouraging outcomes (Figure 1).

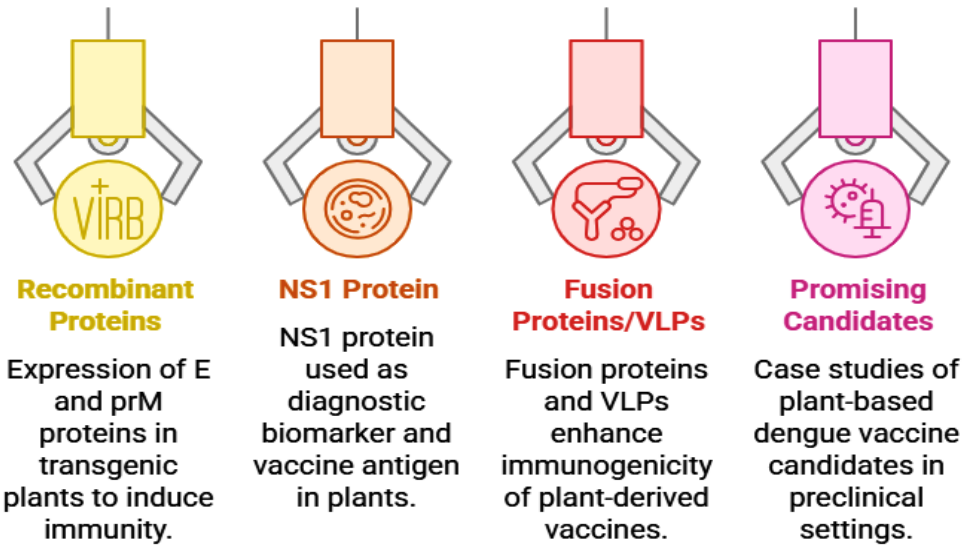

Figure 1: Plant derived Dengue vaccine

5. IN VIVO PRECLINICAL EVALUATION OF PLANT-BASED DENGUE VACCINES

5.1 Animal Models Used

In vivo preclinical evaluation of plant-derived dengue vaccine candidates primarily involves the use of murine models, particularly BALB/c mice and AG129 mice (which are deficient in type I and II interferon receptors and hence susceptible to dengue infection). BALB/c mice are widely employed for immunogenicity and safety studies due to their well-characterized immune responses. In contrast, AG129 mice are preferred for dengue virus challengestudies, enabling the assessment of protective efficacy. In advanced stages of preclinical development, non-human primates (NHPs), such as rhesus macaques, may also be utilized due to their closer physiological resemblance to humans, although ethical and cost considerations limit their routine use [21].

5.2 Immunization Strategies

Different immunization routes have been explored to evaluate the effectiveness of plant-derived dengue antigens. Oral immunization is particularly appealing when using edible plants like lettuce or rice, providing mucosal immunity while avoiding needles. However, oral vaccines may require higher doses or bio-encapsulation to overcome degradation in the gastrointestinal tract. Intranasal administration offers another needle-free alternative, especially effective for mucosal immune responses. Parenteral routes (e.g., subcutaneous or intramuscular injection) are the most commonly used in preclinical studies, ensuring consistent antigen delivery and systemic immunity [22].

5.3 Dosage, Adjuvants, and Immune Response Kinetics

The optimal dose and adjuvant selectionsignificantly influence the immunogenicity of plant-derived dengue vaccines. Adjuvants like cholera toxin B subunit (CTB) and heat-labile enterotoxin subunit B (LTB) are frequently used in oral and mucosal vaccines to enhance antigen uptake and immune activation. Aluminum hydroxide (alum) is commonly used in injectable formulations due to its safety and proven efficacy. The immune response typically develops over several weeks, with booster doses administered at 2-4 week intervals to enhance antibody titers and memory response [23].

5.4 Humoral Immune Response

The most direct measure of vaccine-induced protection is the generation of neutralizing antibodies. In vivo studies have shown that plant-expressed dengue antigens, such as E protein, prM-E complexes, and NS1, elicit high titers of IgG antibodiesin immunized animals. The Plaque Reduction Neutralization Test (PRNT50) remains the gold standard for evaluating the functionality of these antibodies. PRNT assays measure the ability of serum from vaccinated animals to reduce viral plaque formation by 50%, providing a quantitative estimate of neutralizing capacity against each DENV serotype [24].

5.5 Cell-Mediated Immunity

In addition to humoral responses, effective dengue vaccines must activate cell-mediated immunity to support viral clearance. Plant-derived vaccines have been shown to induce the production of key cytokines such as interferon-gamma (IFN-γ), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α) in splenocytes or lymphocytes isolated from immunized animals. These cytokines indicate a balanced Th1/Th2 response. Some studies have also reported cytotoxic T-lymphocyte (CTL) activation, suggesting the presence of antigen-specific $CD8^+$ T-cell responsesespecially when antigens like NS3 or NS5 are included in the vaccine construct [25].

5.6 Protective Efficacy

The ultimate goal of any preclinical study is to determine the protective efficacyof the vaccine. In challenge experiments, AG129 mice vaccinated with plant-derived dengue antigens were exposed to a lethal dose of dengue virus. Several formulations demonstrated significant protection against mortality, reduced viremia, and attenuated clinical symptoms such as weight loss and lethargy. Survival rates, coupled with virological and histopathological assessments, confirm the ability of plant-based dengue vaccines to confer protective immunity [26]. These findings support further development and optimization toward clinical translation (Table 5).

Table 5: Summary of In Vivo Preclinical Studies on Plant-Derived Dengue Vaccines

Vaccine Type	Plant Host	Animal Model	Immune Response	Protective Efficacy
E protein VLPs	*N. benthamiana*	BALB/c mice	High IgG, PRNT50	80–100% survival
NS1 subunit	*O. sativa*	AG129 mice	IFN-γ, IL-4 ↑	Partial protection
prM-E fusion	*L. sativa*	BALB/c	Neutralizing Abs	Reduced viremia
Oral VLPs	*S. lycopersicum*	Mice	Mucosal IgA ↑	Not evaluated

6. SAFETY AND TOXICITY ASSESSMENT

6.1 Acute and Chronic Toxicity in Animal Models

The evaluation of toxicological profiles is a fundamental component of preclinical vaccine assessment. Plant-derived dengue vaccine candidates have undergone both acute and chronictoxicity studies in animal models most commonly in mice and occasionally in rabbits or rats. Acute toxicity is assessed by administering a high single dose of the vaccine and observing animals over a short duration (typically 14 days) for signs of morbidity, mortality, behavioral abnormalities, or organ dysfunction. Chronic toxicity studies involve repeated dosing over several weeks to monitor the effects of sustained antigen exposure. In most reported studies, plant-derived vaccine formulations including those containing recombinant E, prM, or NS1 proteins did not induce any significant toxicity, suggesting a favorable safety profile [27].

6.2 Allergenicity and Hypersensitivity Studies

One concern with plant-based vaccines is the potential for allergenicity, particularly when plant-derived proteins or residual plant metabolites are present in the final formulation. To assess hypersensitivity reactions, in vivo models such as guinea pigs or mice are used to monitor for local or systemic allergic responses following repeated immunization. These include measurements of histamine release, serum IgE levels, and observation of symptoms such as itching, rashes, respiratory distress, or anaphylaxis. Thus far, most plant-based dengue vaccine candidates have shown low allergenic potential, especially when purification steps are employed to remove plant-specific allergens. However, caution is warranted when using edible plants, and further clinical validation is required [28].

6.3 Histopathology of Liver, Spleen, and Lymphoid Tissues

Histopathological examination provides insight into potential organ-specific toxicity or immune-related tissue damage following vaccine administration. Commonly analyzed organs include the liver, spleen, and

lymphoid tissues such as lymph nodes and Peyer's patches. Tissue samples from vaccinated animals are stained and examined microscopically for signs of inflammation, necrosis, fibrosis, or lymphoid hyperplasia. Studies involving plant-derived dengue antigens have not reported any pathological lesions or adverse tissue reactions. The absence of hepatotoxicity and normal architecture of lymphoid tissues reinforce the safety of these vaccines [29]. Additionally, serum biochemical markers like ALT, AST, and creatinine are often used to confirm normal hepatic and renal function (Figure 2).

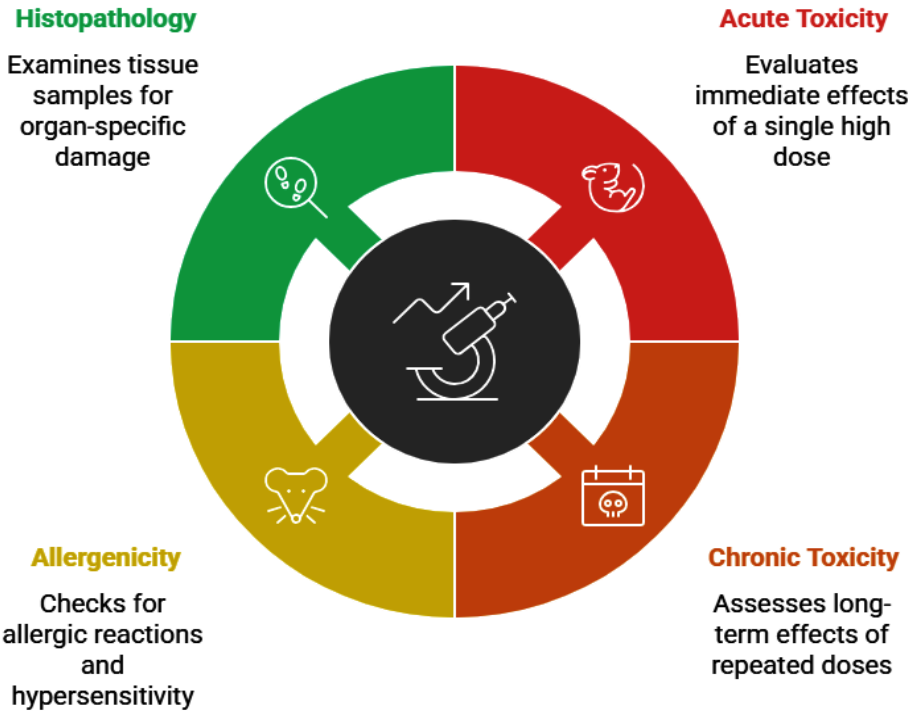

Figure 2: Safety assessment of Plant Derived Vaccines

7. Comparison with Other Vaccine Platforms

7.1 Live Attenuated Vaccines

Live attenuated dengue vaccines, such as CYD-TDV (Dengvaxia) and TV003/TV005, have been among the most clinically advanced candidates. These vaccines use weakened but replication-competent versions of the virus to stimulate a broad immune response. Dengvaxia, developed by Sanofi Pasteur, was the first to be licensed, but it showed variableefficacy among serotypes and posed safety risks for seronegative individuals due to antibody-dependent enhancement (ADE). TV003, developed by the NIH, has demonstrated promising results in early trials, with a more balanced tetravalent response. However, live attenuated vaccines require cold-chain storage, involve complexmanufacturing, and carry risks of reversion to virulence, particularly in immunocompromised individuals [30].

7.2 DNA and RNA Vaccines

Nucleic acid-based vaccines, including DNA and mRNA platforms, offer flexibility and rapid design potential. They work by delivering genetic instructions to host cells to produce dengue antigens, typically E or prM proteins. Several DNA vaccines have shown immunogenicity in preclinical models, but limited efficacy in humans has slowed progress. mRNA vaccines, which gained popularity during the COVID-19 pandemic, are now being investigated for dengue. They offer precise antigen expression, no risk of genomic integration, and fast manufacturing. However, cold chain dependency, short-lived expression, and the need for lipid nanoparticle (LNP) delivery systems present significant logistical and technical challenges [31].

7.3 Recombinant Protein Vaccines from Microbial or Insect Cell Cultures

Recombinant subunit vaccines produced in bacterial (e.g., E. coli) or insect cell (e.g., baculovirus-Sf9) systems are widely used for generating dengue antigens such as E protein, EDIII, or NS1. These platforms allow high-yield production and have well-established purification protocols. However, they may suffer from incorrect folding or lack of post-translational modifications critical for proper antigenicity. Insect cells can provide glycosylation, but they often require costly bioreactor infrastructure and stringent biosafety controls. Moreover, some formulations require potent adjuvants to overcome poor immunogenicity 32].

7.4 Pros and Cons of Plant-Based Vaccine Systems

Plant-derived vaccine platforms offer a unique blend of affordability, biosafety, and scalability. Unlike microbial or mammalian systems, plants are free from humanpathogens, reducing the risk of contamination. They are also cost-effective due to lower production and infrastructure requirements and have the potential for room-temperaturestability, which is critical for mass immunization in tropical regions [33].

Additionally, edible vaccines derived from plants like *Lactuca sativa* or *Oryza sativa* could enable needle-free oral immunization, improving vaccine acceptance and coverage. However, challenges persist, including low yield in some plant systems, batch-to-batchvariability, and regulatory hurdles due to the use of genetically modified organisms (GMOs). Also, the purification of plant-expressed proteins to meet clinical-grade quality standards remains a technological barrier (Table 6).

Table 6: Comparative Evaluation of Vaccine Platforms for Dengue

Platform	Advantages	Limitations
Live Attenuated (e.g., Dengvaxia)	Strong immune response	ADE risk, not safe for all age groups
DNA/RNA Vaccines	Rapid design and production	Cold chain, delivery systems
Microbial Cell Expression	High yield	Endotoxin contamination risk
Insect Cell Expression	Proper folding of proteins	Expensive culture systems
Plant-Based Vaccines	Safe, scalable, no cold chain	Regulatory hurdles, slower public acceptance

8. Regulatory and Manufacturing Challenges

8.1 Regulatory Considerations for Plant-Based Biopharmaceuticals

The regulatory landscape for plant-derived biopharmaceuticals, including vaccines, is still evolving. Agencies such as the World Health Organization (WHO), U.S. Food and Drug Administration (FDA), and Central Drugs Standard Control Organization (CDSCO) in India recognize the potential of plant-based systems but require comprehensive safety, efficacy, and quality data before approval. The FDA's Center for Biologics Evaluation andResearch (CBER) has specific guidance on biologics derived from novel platforms, including plant-based systems [34]. Regulatory frameworks emphasize the need for rigorous preclinical and clinical trials, identity and purity verification, absence of contaminants, and consistency in production. Moreover, WHO has encouraged the use of plant-derived vaccines in the context of low-cost solutions for endemic diseases, but formal international harmonization of guidelines remains limited.

8.2 Good Manufacturing Practice (GMP) Compliance

For plant-based dengue vaccines to be approved for human use, the manufacturing process must be in full compliance with Good Manufacturing Practice (GMP) standards. This includes controlled environments, validated equipment, proper documentation, and traceability throughout the production chain. Ensuring batch-to-batch consistency in protein yield and antigen quality is particularly challenging in plant-based systems due to biological variability. Therefore, the implementation of standard operating procedures (SOPs), quality control (QC) assays, and analytical validation is critical to satisfy regulatory requirements [35].

8.3 Containment Strategies for Genetically Modified Plants

The use of genetically modified (GM) plants for pharmaceutical production raises biosafety and environmental concerns. To prevent unintentional spread of transgenes to the ecosystem or food chain, strict containment measuresmust be enforced. These may include greenhouse cultivation, physical isolation, or the use of male sterile or non-food plant species. Additionally, biocontainment technologieslike plastid transformation (which prevents gene flow through pollen) or inducible expression systems can further mitigate risks. Regulatory agencies require comprehensive environmental risk assessmentsand biosafety protocols before approving field trials or commercial cultivation of GM plants [36].

8.4 Scale-Up Potential: Vertical Farming, Hydroponics, and Bioreactors

Scalability is a major strength of plant-based vaccine platforms. Traditional open-field cultivation, while cost-effective, is often unsuitable for pharmaceutical-grade production due to risks of contamination and limited environmental control. Instead, controlled-environment agriculture (CEA) methods such as vertical farmingand hydroponicsoffer sterile, high-yield production systems ideal for vaccine manufacturing. These methods allow precise regulation of temperature, humidity, light, and nutrient conditions, ensuring consistent output. Additionally, plant cell suspension cultures in bioreactors are emerging as a scalable, GMP-compliant alternative for producing recombinant proteins in a closed, contained system [37]. Bioreactors can support the production of secreted proteins in a liquid medium, facilitating easier downstream processing and purification (Figure 3).

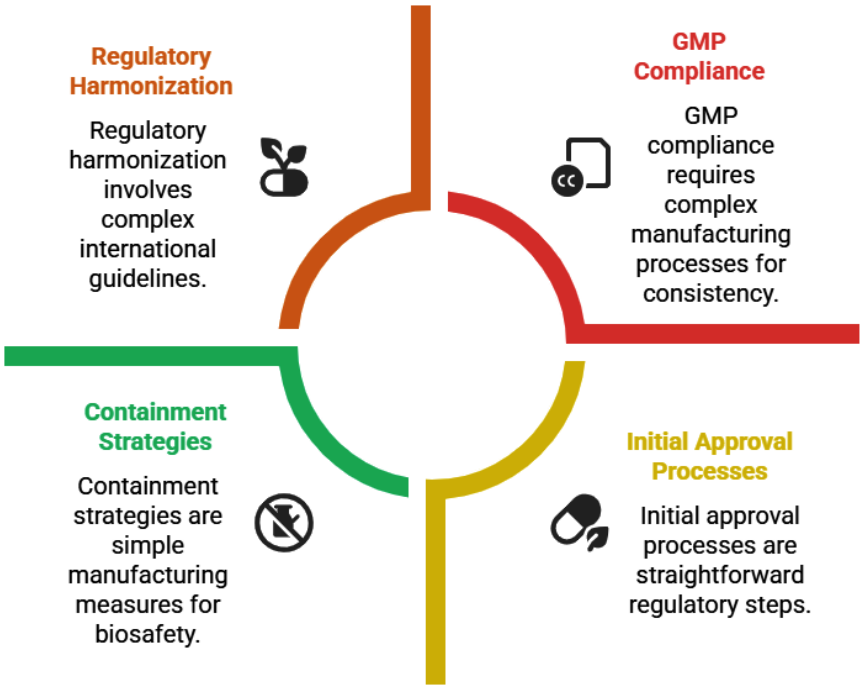

Figure 3: Challenges and strategies in plant based biopharmaceutical production

9. Future Perspectives and Translational Potential

9.1 Advancements in Plant Synthetic Biology and CRISPR for Vaccine Enhancement

Recent advances in synthetic biology and genome editing technologies such as CRISPR/Cas9 have opened new frontiers in optimizing plant systems for vaccine production. Synthetic biology enables the design of customized promoters, enhancers, and gene circuits to enhance transgene expression, control antigen localization, and improve protein yield and functionality. CRISPR can be used to knock out endogenous genes that interfere with recombinant protein production or to precisely insert vaccine genes into genomic "safe harbors." These tools can also help generate transgenic plants with improved stability, increased expression levels, and faster production timelines, enhancing the viability of plant-based vaccine platforms for commercial deployment [38].

9.2 Multiplexed Vaccines Against Dengue and Other Flaviviruses

Given the structural similarities among flaviviruses, the development of multiplexed or multivalent vaccines that target dengue, Zika, and yellow fever simultaneously is a promising direction. Plant systems offer a flexible platform for producing chimeric antigens, fusion proteins, or virus-like particles (VLPs) that incorporate epitopes from multiple viruses. This strategy could be especially valuable in regions where co-circulation of flaviviruses increases the risk of misdiagnosis and coinfections. Multiplexed vaccines not only improve public health outcomes but also reduce the cost and complexity of immunization programs [39].

9.3 Public Acceptance and Ethical Considerations of Plant-Based Edible Vaccines

While plant-based edible vaccines offer advantages like needle-free delivery, low cost, and ease of administration, public perception and regulatory acceptance remain challenges. Concerns about the use of genetically modified organisms (GMOs), potential cross-contamination with food crops, and unclear labeling practices can affect public trust. To ensure ethical deployment, there is a need for transparent risk communication, community engagement, and clear labeling of edible vaccine products. Education campaigns and regulatory oversight will play critical roles in increasing public confidence in these novel vaccine platforms [40].

9.4 Potential for mRNA Expression in Plant Systems (Plant-Derived RNA Vaccines)

Inspired by the success of mRNA vaccines in the fight against COVID-19, researchers are now exploring the expression of RNA molecules in plants for direct use as vaccines. This involves synthesizing stabilized mRNA transcripts in plant cells that encode viral antigens and can be purified for formulation into lipid nanoparticles. While this field is still in its infancy, plant-derived RNA vaccines offer an innovative alternative to synthetic mRNA production, potentially reducing costs and environmental impact. Further

research is needed to optimize RNA stability, translation efficiency, and large-scale purification protocols in plant-based systems [41].

9.5 Integration with AI and Bioinformatics for Epitope Prediction and Design

The integration of artificial intelligence (AI) and bioinformatics is revolutionizing vaccine design, including for plant-based platforms. Machine learning algorithms can predict B-celland T-cell epitopes, identify immune-dominant regions, and model antigen structures to guide recombinant construct development. These tools can streamline the process of selecting antigen sequences with the highest likelihood of inducing protective immunity while avoiding those that may trigger ADE or allergic reactions [42]. Coupled with synthetic biology, AI-assisted design can accelerate the creation of next-generation, precision vaccines that are tailor-made for expression in plant systems (Table 7).

Table 7: Emerging Technologies and Future Directions in Plant Vaccine Development

Technology	Application	Expected Impact
CRISPR/Cas9	Gene editing for stable expression	Higher expression, improved safety
Synthetic Biology	Modular vaccine design	Multivalent vaccine constructs
AI/Bioinformatics	Epitope prediction, codon optimization	Personalized and rapid vaccine design
Edible Vaccines	Oral delivery through food plants	Needle-free immunization, global accessibility
mRNA in Plants	Transient expression of RNA	Future platform for plant RNA vaccines

10. CONCLUSION

The in vivo evaluation of plant-derived recombinant vaccines for dengue virus has demonstrated encouraging outcomes in terms of immunogenicity, safety, and protective efficacy. Animal model studies, particularly in murine systems, have consistently shown robust humoral and cell-mediated immune responses, with significant levels of neutralizing antibodies and cytokine production following immunization with plant-expressed dengue antigens such as E, prM, and NS1 proteins. Challenge studies have further confirmed the protective potential of these vaccine candidates, offering solid preclinical evidence supporting their advancement to the next stages of development [43].

Plant-based expression systems provide a promising and innovative platform for the production of dengue vaccines. Their advantages - cost-effectiveness, cold chainindependence, biosafety, and rapid scalability make them particularly suited for use in low- and middle-income countries where dengue burden is highest. In addition to dengue, the flexibility of plant molecular pharming can be extended to develop multivalent ormultiplexed vaccines against other flaviviruses, presenting a broader solution for arboviral disease management [44].

To realize the full potential of plant-derived dengue vaccines, there is an urgent need for collaborative, interdisciplinary research encompassing plant biotechnology, virology, immunology, regulatory science, and public health. Translational trials, including human safety and efficacy studies, along with harmonized regulatory frameworks, will be critical to bring these next-generation vaccines from the laboratory to global deployment [45].

Plant-derived vaccines stand at the cusp of transforming preventive healthcarenot only for dengue but for a range of infectious diseases threatening global health. With continued investment and innovation, they may soon play a pivotal role in pandemic preparedness and equitable vaccine access.

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