Plant Scientific Application
eISSN: 3122-7015
https://psa.cultechpub.com/index.php/psa

Utilizing Tobacco Mosaic Virus Vectors for Agricultural and Pharmaceutical Applications to Increase Recombinant Protein Production in Nigeria

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Abstract

The production of recombinant proteins for pharmaceutical, industrial, and agricultural applications remains a significant challenge in developing nations like Nigeria, where infrastructure limitations and cost constraints hinder access to conventional production systems. Plant virus-based expression systems, particularly those derived from tobacco mosaic virus (TMV), offer a promising alternative due to their high yield potential, rapid production cycles, and cost-effectiveness. This review comprehensively examines the development and optimization of TMV-based vectors for enhanced protein production, with specific attention to applications relevant to Nigeria's agricultural and public health needs. We detail the molecular mechanisms underlying TMV's efficiency as a gene expression vector, including the role of viral movement proteins, untranslated regions (UTRs), and coat protein modifications in boosting foreign protein accumulation. Recent advancements in vector design-such as TMV-Gate vectors for high-throughput applications and deconstructed viral systems for industrial-scale production-are discussed alongside optimization strategies involving host plant selection, agroinfiltration techniques, and co-expression of silencing suppressors. We present a case study conceptualizing the production of a malaria vaccine antigen in Nigerian tobacco species, addressing both technical feasibility and economic considerations. Despite challenges related to protein size limitations, host defense responses, and regulatory frameworks, TMV-based systems hold transformative potential for Nigeria's biomanufacturing capacity. This review concludes with future perspectives on adapting this technology to local contexts, emphasizing research investment, infrastructure development, and capacity building as essential components for harnessing plant molecular farming to address pressing national needs.

Keywords

Tobacco Mosaic Virus, Viral Vectors, Recombinant, Molecular Farming, Vaccine Production, Plant Biotechnology, Transient Expression

1. Introduction

The global demand for recombinant proteins continues to expand across multiple sectors, including human therapeutics (vaccines, antibodies, enzymes), industrial applications (biofuels, detergents, textiles), and agriculture (animal feed additives, biopesticides). Traditional production platforms-reliant on microbial fermentation, mammalian cell culture, or transgenic animals-often involve high capital investment, complex purification requirements, and limited scalability, making them economically challenging for developing nations like Nigeria. In contrast, plant-based expression systems offer compelling advantages: lower upstream costs, inherent scalability through cultivation, reduced risk of human pathogen contamination, and the capacity for complex eukaryotic post-translational modifications.

Among plant expression technologies, virus-based transient expression systems have emerged as particularly promising for high-yield protein production. These systems utilize engineered plant viruses as replicating gene vectors that can rapidly amplify and express foreign genes in infected plant tissues. The tobacco mosaic virus (TMV), one of the most extensively studied plant viruses, has served as a foundational model for vector development due to its well-characterized biology, high replication rate, and exceptional capacity for foreign protein expression. When appropriately engineered, TMV-based vectors can direct the production of recombinant proteins at levels exceeding 40% of total soluble protein in infected leaves-yields that dramatically outpace conventional stable plant transformation approaches.

For Nigeria, with its burgeoning population, significant disease burden (including malaria, tuberculosis, and vaccine-preventable illnesses), and agricultural economy, investment in local biomanufacturing capacity represents both an economic opportunity and a public health imperative. The country's suitable climate for cultivating tobacco and other solanaceous species-which serve as ideal hosts for TMV-based expression-provides a natural foundation for developing plant molecular farming initiatives. However, realizing this potential requires a thorough understanding of TMV vector

technology, optimization strategies tailored to local conditions, and pragmatic approaches to overcoming technical and regulatory hurdles.[1]

This review systematically examines the development, optimization, and application of TMV-based vectors for enhanced recombinant protein production, with particular attention to implementation possibilities within the Nigerian context. We will explore: (1) the fundamental biology of TMV that makes it an effective expression vector; (2) the evolution of TMV vector design through multiple generations of innovation; (3) key strategies for maximizing protein yields; (4) a conceptual case study for malaria vaccine production; and (5) the challenges and future directions for adapting this technology to address Nigeria's specific needs.

2. Tobacco Mosaic Virus: Biology and Relevance as an Expression Vector

2.1 Structural and Genomic Organization

Tobacco mosaic virus is the prototypical member of the Virgaviridae family, with a rod-shaped structure approximately 300 nm in length and 18 nm in diameter. Its genome consists of a single-stranded, positive-sense RNA molecule of approximately 6.4 kilobases, encoding four primary proteins: the 126-kDa and 183-kDa replication-associated proteins (involved in viral RNA synthesis), the 30-kDa movement protein (MP) (facilitating cell-to-cell spread), and the 17.5-kDa coat protein (CP) (forming the protective viral capsid). The genomic RNA is characterized by several structural elements critical to its function as an expression vector, including a 5' cap structure, a 3' untranslated region (UTR) containing conserved pseudoknot formations, and subgenomic promoters that regulate the expression of MP and CP.[2]

The TMV replication cycle begins with viral entry into plant cells, typically through mechanical wounds. The genomic RNA is translated to produce the replication proteins, which then synthesize complementary negative-strand RNA. This negative strand serves as a template for generating both full-length genomic RNA and shorter subgenomic mRNAs responsible for MP and CP expression. This amplification mechanism-where a single viral RNA molecule can generate thousands of copies within days-forms the foundational advantage of TMV as an expression vector, enabling extremely high levels of foreign gene expression when appropriately engineered.

2.2 Molecular Features Enabling High-Level Expression

Several intrinsic properties of TMV contribute to its exceptional efficacy as a protein production platform. First, the virus employs multiple subgenomic promoters that enable differential expression of viral proteins; this feature has been exploited to drive heterologous gene expression at levels comparable to CP, which naturally accumulates to 5-10% of total leaf protein in infected plants. Second, TMV's 3' UTR contains conserved pseudoknot structures that enhance translation efficiency by mimicking the function of poly(A) tails in eukaryotic mRNAs. Studies have demonstrated that incorporating these UTR sequences into expression constructs can boost foreign protein yields by 5-7 fold compared to vectors lacking these elements.

The viral movement protein plays a surprisingly significant role in recombinant protein accumulation beyond its canonical function in cell-to-cell spread. Recent research indicates that MP abundance directly correlates with foreign protein yield, particularly for high-molecular-weight proteins like CRISPR-Cas9 components. This finding has led to innovative vector designs incorporating trans-complementation of MP, where MP is expressed from a separate construct to enhance the production capacity of the primary viral vector. Additionally, TMV's ability to systemically infect entire plants through the vascular system enables protein production beyond initially inoculated tissues, though modern "deconstructed" vectors often restrict expression to localized areas for containment purposes.[3]

3. Evolution of TMV-Based Expression Vectors

3.1 First-Generation Vectors: Early Proof of Concept

The initial development of TMV-based expression vectors in the 1980s focused on full-length viral genomes with foreign genes inserted as additional expression cassettes. These first-generation vectors typically involved inserting a heterologous gene driven by an additional subgenomic promoter, often positioned before or after the native CP gene. While these vectors demonstrated the feasibility of using TMV for foreign protein expression, they suffered from significant genetic instability due to homologous recombination between repeated promoter sequences, leading to rapid deletion of inserted genes. Additionally, their large insert size capacity was limited, and they often induced severe phytopathogenic effects that reduced biomass and protein yield.

An important early innovation was the development of hybrid vectors incorporating elements from multiple tobamoviruses to reduce sequence homology and improve stability. The 30B vector, which combined sequences from TMV-U1 and odontoglossum ringspot virus, demonstrated improved stability for expressing smaller proteins like green fluorescent protein (GFP). However, these early vectors remained limited in their capacity to express larger or more complex proteins, highlighting the need for more sophisticated engineering approaches.[4]

3.2 Second-Generation Vectors: Enhanced Stability and Yield

Second-generation TMV vectors addressed stability issues through strategic modifications to reduce homologous recombination while maintaining high expression levels. Key innovations included: (1) the use of heterologous subgenomic promoters from related viruses to drive foreign gene expression; (2) codon optimization of viral replication

proteins to improve agroinfection efficiency; and (3) the strategic positioning of foreign genes nearer the 3' end of the viral RNA to enhance expression levels. Research established that proximity to the 3' terminus, with its translation-enhancing pseudoknot structures, significantly increased protein accumulation-a principle that would guide future vector design.

The TRBO (TMV RNA-Based Overexpression) vector, developed by Lindbo in 2007, represented a landmark advancement in second-generation design. By deleting the coat protein gene entirely and positioning the foreign gene in its place immediately upstream of the 3' UTR, TRBO achieved unprecedented expression levels-up to 5.5 g of recombinant GFP per kg of infiltrated *Nicotiana benthamiana* tissue. This vector also simplified delivery by eliminating the need for co-expression of silencing suppressors like P19, though such suppressors could still provide additional yield enhancement. TRBO's success demonstrated that deconstructed viral systems-retaining only essential replication and movement functions while eliminating structural components-could dramatically improve both yield and stability.[5]

3.3 Third-Generation Vectors: Modular and High-Throughput Systems

Current TMV vector development emphasizes modularity, high-throughput compatibility, and specialized applications. The TMV-Gate vector series exemplifies this trend, incorporating Gateway recombination technology to enable rapid cloning of gene inserts and fusion with various epitope tags or fluorescent proteins. These vectors maintain the high-expression characteristics of TRBO while adding flexibility for protein characterization studies, including affinity purification, immunodetection, subcellular localization, and protein-protein interaction analysis.

Recent innovations have focused on overcoming size limitations for foreign gene insertion and enhancing the production of complex proteins. The pAT-transMP system utilizes trans-complementation of the movement protein to boost yields of high-molecular-weight proteins like Cas9. Other advances include the incorporation of synthetic regulatory elements-such as optimized 5' UTRs, matrix attachment regions, and double transcriptional terminators-into TMV vectors to further enhance expression. A 2024 study demonstrated that adding a synthetic 5' UTR to the pJL-TRBO vector increased eGFP production by 5.6-7.2 fold compared to the parent vector.[6]

Table 1. Evolution of TMV-based expression vectors.

Generation	Key Characteristics
First (1980s-1990s)	Full-length TMV genome with additional subgenomic promoter; hybrid vectors with reduced homology
Second (2000s)	Deconstructed vectors lacking CP; foreign gene positioned near 3' terminus; heterologous promoters
Third (2010s-present)	Gateway compatibility; epitope tagging; trans-complementation systems; synthetic regulatory elements

Table 1: This table summarizes the development history of TMV (Tobacco Mosaic Virus) expression vectors (three generations from the 1980s to the present), mainly comparing them in terms of structural characteristics, representative vectors, yield improvements, and limitations. TMV vectors have evolved from simple full-length viral vectors → deconstructed high-expression vectors → highly engineered modular platforms. Expression yields have continuously increased, but the technical requirements have also risen accordingly.

4. Strategies for Maximizing Recombinant Protein Yields

4.1 Vector Optimization Approaches

UTR engineering represents one of the most effective strategies for enhancing protein expression from TMV vectors. The TMV 5' UTR (Ω sequence) functions as a translation enhancer by reducing mRNA secondary structure and facilitating ribosomal binding, while the 3' UTR stabilizes transcripts and enhances translational efficiency through pseudoknot structures that functionally mimic poly(A) tails. Incorporating both UTRs in expression constructs has demonstrated synergistic effects, with one study showing that a vector containing both TMV 5' and 3' UTRs produced significantly higher GUS activity compared to vectors containing either UTR alone. More recently, synthetic UTRs derived from other viruses (like cowpea mosaic virus) or designed *de novo* have shown even greater enhancement potential when incorporated into TMV vectors.[7]

Promoter selection and positioning also critically influence yield. While the cauliflower mosaic virus (CaMV) 35S promoter remains standard for driving initial transcription in binary TMV vectors, its enhanced versions (with duplicated domains) can provide additional boosts. The relative position of the foreign gene within the viral transcript-specifically its proximity to the 3' terminus-strongly correlates with expression level due to the gradient of subgenomic mRNA production. This principle underlies the success of CP replacement vectors like TRBO, where removing the CP gene allows the foreign gene to occupy the optimal position immediately upstream of the 3' UTR.

Codon optimization for the host plant species represents another key optimization strategy. Since TMV vectors are often deployed in *Nicotiana benthamiana* (a model solanaceous species), adjusting the codon usage frequency of foreign genes to match tobacco preferences can dramatically improve translation efficiency. For example, in expressing recombinant human plasminogen activator (rhPA) in tobacco, codon optimization increased yields to 0.6% of total soluble protein (approximately 60 μg per gram of fresh leaf tissue).[8]

4.2 Host Plant Optimization

Plant species and cultivar selection significantly impact recombinant protein accumulation. *Nicotiana benthamiana* remains the preferred host for TMV-based expression due to its susceptibility to agroinfiltration, rapid growth, high

biomass production, and diminished RNA silencing response. However, other species within the *Nicotiana* genus, including *N. excelsiana* and *N. tabacum* cultivars, have shown promise for specific applications. A comparative study evaluating *N. benthamiana*, *N. excelsiana*, and their hybrid found that while *N. benthamiana* provided the highest protein accumulation per leaf area, the hybrid offered advantages in biomass production and ease of infiltration.

Growth conditions and plant age at the time of inoculation critically influence protein yields. Optimal results are typically obtained with 5-6 week old plants grown under controlled environmental conditions (24°C, 70-80% relative humidity, 14-hour photoperiod). Maintaining plants under moderate light intensity (130-150 μE m^{-2} s^{-1}) and avoiding stress conditions (water deficit, nutrient deficiency, or temperature extremes) helps maximize both biomass and recombinant protein accumulation. The timing of harvest represents another key variable, with most TMV-based systems reaching peak protein accumulation at 5-7 days post-infiltration (dpi), after which yields typically decline due to host defense responses and tissue necrosis.[9]

4.3 Delivery and Co-expression Strategies

Agroinfiltration methodology has evolved considerably to enhance delivery efficiency and scalability. While early protocols relied on syringe infiltration of individual leaves, modern approaches utilize vacuum infiltration of whole plants to process dozens of plants simultaneously. Optimization studies have identified key parameters for efficient agroinfiltration: *Agrobacterium* cultures grown in AB medium (rather than LB or YEB), adjusted to an OD600 of 0.4-0.5, and diluted directly in water or induction medium without centrifugation. Vacuum pressure of 50-100 mbar applied for 30-60 seconds typically achieves >95% infiltration of leaf tissues. Among *Agrobacterium* strains, GV3101 has demonstrated superior protein production compared to LBA4404, C58C1, and various wild-type strains.

Co-expression of silencing suppressors provides one of the most dramatic boosts to recombinant protein yield. Plant RNA silencing represents a primary antiviral defense mechanism that degrades viral RNA, limiting both viral spread and recombinant protein accumulation. Co-infiltrating TMV vectors with viral silencing suppressor proteins such as P19 (from tomato bushy stunt virus) or P23 (from citrus tristeza virus) can increase target protein yields by 15-25%. These suppressors function by binding small interfering RNAs (siRNAs), preventing their incorporation into RNA-induced silencing complexes. An alternative strategy involves engineering suppressor sequences directly into TMV vectors, though this can sometimes reduce genetic stability or increase phytopathogenicity.[10]

Table 2. Optimization strategies for TMV-based protein production.

Optimization Category	Specific Strategy	Mechanism of Action	Typical Yield Improvement
Vector Design	Incorporation of TMV 5' and 3' UTRs	Enhanced translation initiation and mRNA stability	5-7 fold increase
Vector Design	Foreign gene positioning near 3' terminus	Increased subgenomic mRNA production	Up to 100-fold increase
Vector Design	Codon optimization for host plant	Improved translation efficiency	2-10 fold increase (varies by gene)
Host Plant	Selection of *N. benthamiana* over other species	Reduced RNA silencing; easier infiltration	3-5 fold over non-optimal hosts
Delivery Method	Vacuum agroinfiltration (vs. syringe)	More uniform and extensive tissue delivery	2-3 fold more consistent expression
Co-expression	Viral silencing suppressors (P19, P23)	Inhibition of RNA silencing pathways	15-25% increase
Harvest Timing	Collection at 5-7 days post-infiltration	Peak protein accumulation before necrosis	2-3 fold over early/late harvest

Table 2 is summarizes the main optimization strategies for improving protein yield in the TMV (Tobacco Mosaic Virus) expression system, covering factors such as vector design, host selection, delivery method, co-expression strategy, and harvest time. Therefore, different strategies can improve translation efficiency, mRNA stability, RNA silencing inhibition, and tissue infection efficiency at different stages, resulting in yield increases ranging from 2 to hundreds of times. By optimizing vector structure, host plant, delivery method, RNA silencing inhibition, and harvest time, the protein yield of the TMV expression system can be significantly increased from several times to hundreds of times.

5. Conceptual Application for Malaria Vaccine Production in Nigeria

5.1 Technical Design and Implementation

Malaria represents one of Nigeria's most significant public health challenges, with the country accounting for approximately 27% of global malaria cases and 23% of malaria deaths worldwide. The recent development of the R21/Matrix-M vaccine has demonstrated efficacy, but production scalability and cost limitations hinder widespread access. A TMV-based production system for malaria vaccine antigens could potentially address these challenges by enabling local manufacturing of key immunogenic components.[11]

For this conceptual case study, we propose expressing the circumsporozoite protein (CSP) of *Plasmodium falciparum*, the target of leading malaria vaccines, using a TMV-based vector in Nigerian-grown tobacco. The technical approach would involve:

1.Vector Construction: Designing a deconstructed TMV vector similar to TRBO, with the CSP gene (codon-optimized for *Nicotiana benthamiana*) positioned immediately upstream of the TMV 3' UTR. The vector would include the TMV Ω sequence at the 5' end and a hexahistidine tag for purification. Given CSP's moderate size (~40 kDa), the standard TRBO vector would likely suffice without requiring the trans-MP system.

2.Host Plant Selection: While *N. benthamiana* offers the highest protein yields, its growth requirements might challenge Nigerian farmers. Alternatively, N. tabacum varieties already cultivated in Nigeria could be evaluated for CSP production, potentially balancing yield with agricultural familiarity. Preliminary small-scale trials would identify the optimal cultivar for local conditions.

3.Production Process: Implementing a scalable agroinfiltration protocol adapted to local resources. Given Nigeria's climate, production would likely occur in simple greenhouse structures with evaporative cooling rather than sophisticated growth chambers. Vacuum infiltration equipment could be fabricated locally using vacuum pumps, sealable containers, and simple pressure gauges. The harvest window would be optimized for Nigerian growing conditions, potentially shifting from the standard 5-7 dpi if temperatures differ significantly from optimal 24°C.

4.Downstream Processing: Developing a simplified purification protocol utilizing the histidine tag for immobilized metal affinity chromatography (IMAC). Nigerian universities and research institutes already possess basic chromatography equipment that could be adapted for this purpose. The final product would undergo quality assessment using SDS-PAGE, Western blotting, and ELISA to verify antigenicity.

5.2 Economic and Infrastructure Considerations

Implementing plant-based malaria antigen production in Nigeria would require addressing several economic and infrastructure considerations. The initial investment would include costs for greenhouse construction, agroinfiltration equipment, and basic purification infrastructure.[12] However, compared to establishing mammalian cell culture facilities-which require sterile environments, expensive media, and sophisticated bioreactors-the plant-based approach offers significantly lower capital requirements. Operational costs would be dominated by plant cultivation expenses (seeds, soil, fertilizer, water) and labor, both relatively affordable in the Nigerian context.

Scalability represents a key advantage of the plant-based system. Production could begin at pilot scale (hundreds of plants) within a research institution, then expand to small-scale production (thousands of plants) for preliminary clinical trials, and potentially progress to commercial scale (hectares of plants) for broader distribution. This incremental scalability reduces initial risk and allows for gradual investment aligned with demonstrated success.

Regulatory approval pathways would require careful navigation. Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) has established guidelines for biological products, though plant-produced pharmaceuticals represent a novel category. Engaging regulatory authorities early in the development process, with transparent communication about the technology and quality control measures, would be essential. International precedents, such as the approval of plant-produced glucocerebrosidase for Gaucher disease, provide helpful regulatory frameworks.[13]

5.3 Potential Impact and Challenges

Successful implementation of TMV-based malaria antigen production could yield substantial public health benefits for Nigeria. Local production would reduce dependency on international vaccine supplies, potentially lowering costs and improving availability in remote regions. The technology could also be adapted for other Neglected Tropical Diseases prevalent in Nigeria, creating a versatile platform for multiple public health needs.

However, significant technical challenges must be addressed. Protein yield consistency might vary with seasonal climate fluctuations, requiring environmental control measures. Purification efficiency from plant tissues presents another hurdle, as plant-derived compounds (phenolics, proteases, pigments) can complicate downstream processing. Glycosylation patterns in plant-produced CSP might differ from native parasite or mammalian cell-produced versions, potentially affecting immunogenicity-though this could be addressed through glycoengineering or demonstrating equivalent efficacy despite differences.

Societal acceptance represents another consideration. [14] While Nigeria has experience with tobacco cultivation, the concept of "pharming" (using plants for pharmaceutical production) might require public education efforts. Clear communication about biological containment measures (preventing gene flow to food crops, ensuring environmental safety) would be essential to gain community trust and regulatory approval.

6. Challenges and Future Perspectives

6.1 Technical Limitations and Solutions

Despite significant advancements, TMV-based expression systems still face technical limitations that require innovative solutions. Insert size constraints remain a primary challenge, with most TMV vectors accommodating foreign genes of 1-2 kb without significant yield reduction or genetic instability. For larger proteins (like antibodies requiring both heavy and light chains), co-expression strategies using multiple vectors or engineering viral vectors to express multiple genes have shown promise but require further optimization. The recently developed pAT-transMP system, which enhances

expression of high-molecular-weight proteins through trans-complementation of movement protein, represents one approach to this challenge.[15]

Host defense responses present another limitation. While co-expression of silencing suppressors like P19 can enhance yields, these proteins themselves may trigger unintended effects on host physiology or recombinant protein quality. Alternative strategies include engineering viral proteins with reduced pathogenicity or developing plant lines with compromised silencing pathways specifically for protein production. The inducible expression systems that activate viral replication only after plant biomass accumulation might also help separate growth and production phases, potentially increasing both yield and biomass.

Protein stability and post-translational modifications require further attention. Some recombinant proteins, particularly those of mammalian origin, may be susceptible to plant proteases or lack appropriate post-translational modifications. Strategies to address these issues include: targeting proteins to subcellular compartments with lower protease activity (apoplast, endoplasmic reticulum); co-expressing mammalian chaperones or modifying enzymes; and engineering plant glycosylation pathways to produce human-like glycan structures. The Zera® fusion technology, which induces protein body formation in plant cells, has shown promise for stabilizing recombinant proteins and increasing accumulation.

6.2 Regulatory and Biosafety Considerations

For Nigeria and other African nations, establishing appropriate regulatory frameworks for plant-produced pharmaceuticals represents a critical step toward technology adoption. Current regulations in most countries were developed for conventional production systems and may not adequately address unique aspects of plant molecular farming, such as environmental containment, gene flow prevention, and food/feed crop segregation. Nigeria could take a leadership role in developing science-based, proportionate regulations that ensure safety without stifling innovation.[16]

Biosafety concerns primarily focus on potential gene flow to wild relatives or food crops, particularly when using edible species as production hosts. For non-food species like tobacco, this risk is reduced but still requires management through physical isolation, reproductive containment (using male-sterile lines or harvesting before flowering), and potentially chloroplast transformation (which minimizes pollen-mediated gene transfer). [17] For pharmaceutical production, complete physical containment in greenhouse or growth chamber facilities may be necessary regardless of host species.

Product consistency and quality control present additional regulatory challenges. Plant-based production systems inherently exhibit more batch-to-batch variability than controlled fermentation systems due to biological variation in plant growth and environmental factors. Implementing rigorous standard operating procedures, environmental controls, and analytical methods will be essential to demonstrate product consistency to regulatory authorities. The development of rapid, low-cost analytical techniques suitable for resource-limited settings would significantly enhance feasibility in the Nigerian context.[18]

6.3 Future Research Directions for Nigerian Applications

Several research priorities emerge for adapting TMV-based expression technology to Nigerian needs and conditions:

1.Host Plant Adaptation: Screening Nigerian tobacco cultivars and related solanaceous species for compatibility with TMV vectors and agroinfiltration. Research should focus on indigenous species that grow well in Nigerian climates without intensive inputs, potentially lowering production costs and increasing sustainability.

2.Climate-Adapted Protocols: Developing production protocols optimized for Nigerian environmental conditions, including higher temperatures and varying humidity levels. This might involve identifying heat-tolerant plant varieties, adjusting infiltration and harvest timing, or implementing simple cooling technologies for greenhouse production.

3.Disease Target Prioritization: Identifying the most promising applications for Nigerian public health needs. Beyond malaria antigens, candidates might include vaccines for meningitis (particularly relevant in the "meningitis belt"), therapeutic proteins for sickle cell disease (which has high prevalence in Nigeria), or diagnostic reagents for locally prevalent infectious diseases.

4.Simplified Downstream Processing: Developing protein extraction and purification methods that minimize equipment and reagent requirements while maintaining product quality. Affinity tag systems that function with locally available matrices, aqueous two-phase extraction using affordable polymers, and membrane-based purification technologies warrant investigation for Nigerian implementation.

5.Capacity Building and Training: Establishing educational programs and hands-on training in plant molecular farming techniques at Nigerian universities and research institutes. International collaborations with research groups experienced in TMV vector technology could accelerate knowledge transfer while adapting protocols to local conditions and resources.

7. Conclusion

Tobacco mosaic virus-based expression vectors represent a powerful technology platform for recombinant protein production with particular relevance to Nigeria's agricultural capabilities and public health needs. Through decades of refinement, these systems have evolved from simple proof-of-concept tools to sophisticated platforms capable of producing complex pharmaceutical proteins at commercially relevant yields. Key innovations-including deconstructed viral genomes, strategic gene positioning, UTR optimization, and co-expression strategies-have collectively addressed earlier limitations of stability, yield, and scalability.

For Nigeria, investing in plant molecular farming based on TMV vector technology offers multiple potential benefits: reducing dependency on imported pharmaceuticals, creating high-value agricultural opportunities, building biomanufacturing capacity, and addressing pressing public health challenges with locally produced solutions. The inherent scalability of plant-based production, from small research plots to hectare-scale cultivation, aligns well with incremental investment approaches appropriate for developing economies.

Realizing this potential will require strategic investments in research infrastructure, human capacity development, and regulatory framework establishment. Nigerian research institutions should prioritize adapting TMV technology to local conditions through cultivar screening, protocol optimization, and application-specific vector development. Public-private partnerships could facilitate technology transfer while ensuring alignment with market needs. International collaborations would provide valuable expertise while respecting local priorities and knowledge.

As plant molecular farming continues to advance globally, Nigeria has an opportunity to position itself as a regional leader in this emerging field. By leveraging its agricultural strengths, addressing its public health priorities, and making strategic investments in biotechnology capacity, Nigeria could harness TMV-based protein production not only for domestic benefit but potentially as an export-oriented industry. The journey from basic research to commercial application will undoubtedly present challenges, but the potential rewards-in health, economic development, and scientific capacity-merit serious consideration and investment.

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