Potential of Plant Expression System for the Production of Vaccines and Biologics Against Bundibugyo Ebolavirus

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Abstract

The recent outbreak of Bundibugyo Ebolavirus (BDBV) in Africa received global attention due to its ability to cause severe hemorrhagic disease with high mortality rates. This virus belongs to the family *Filoviridae* and genus *Orthoebolavirus*. It is one of the Ebolavirus species known to infect humans. World Health Organization (WHO) has declared the current outbreak in Democratic Republic of Congo as Public health emergency of international concern on May 2026. Although there is significant progress in vaccine and therapeutic development against *Zaire Ebolavirus*, there are no approved vaccines or therapeutics currently available for BDBV. The vaccine development efforts using different platform technologies are currently in progress. Recently, plant expression systems have emerged as a promising platform for the rapid, scalable, and cost-effective production of recombinant vaccines and biopharmaceuticals. Furthermore, the production of Ebola virus antigens and the monoclonal antibody cocktail ZMapp in *Nicotiana benthamiana* provides proof-of-concept that the plant expression system can be utilized for the development of filovirus countermeasures. Here we examine the potential of plant expression system for the development of vaccines and biologics against BDBV.

Keywords: Bundibugyo Ebolavirus; molecular farming; outbreak preparedness; plant expression systems; recombinant vaccines.

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1. Context

Bundibugyo Ebolavirus (BDBV) is a zoonotic virus that belongs to the family *Filoviridae* and genus *Orthoebolavirus*. It is one of Ebolavirus species known to infect humans. The virus was first identified during a hemorrhagic fever outbreak in the Bundibugyo District of Uganda in 2007 [1]. Several suspected cases and fatalities were reported during this outbreak. The second outbreak was reported in the Democratic Republic of Congo (DRC) in 2012 resulting in 36 cases and 13 deaths. The World Health Organization (WHO) has declared the recent ongoing outbreak in DRC as public health emergency of international concern which highlights the urgent need for effective preparedness and response strategies to control the virus [2]. Currently massive international efforts are in progress to develop diagnostic reagents, vaccines, and antiviral drugs to slow down the disease spread and save lives.

Similar to other Ebolaviruses, BDBV is maintained through zoonotic cycles. The fruit bats are the suspected natural reservoirs for Ebola virus [3]. The virus is thought to be transmitted to humans through close contact with the infected animals and it subsequently transmitted from person to person through direct contact with infected individuals or contaminated materials. The incubation period ranges from 2 to 21 days and the case fatality rate reported during the 2007 and 2012 outbreaks were 30% and 50% respectively. In the recent outbreak, a total of 896 confirmed cases, including 232 deaths, have been reported from the DRC and Uganda has reported 19 confirmed cases including two deaths (As of June 18, 2026) [2, 4]. The outbreak in DRC continues to spread rapid with sustained transmission and increasing numbers of cases were reported.

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The rapid spread of BDBV suggests the urgent need for effective preventive and therapeutic interventions. Vaccines are crucial to control this outbreak and preventing future outbreaks. However, the lack of licensed vaccines specifically against BDBV highlights an important gap in current Ebola preparedness efforts. Although significant advances have been achieved in the development of vaccines and therapeutics against *Zaire Ebolavirus*, additional research, investment is required to develop effective countermeasures targeting other pathogenic Ebolavirus species, including BDBV [5]. Here we provide an overview on the potential of plant expression systems for the rapid production of vaccines and related biologics against BDBV and highlight how plant molecular farming could contribute to future outbreak preparedness and response efforts against Ebola virus.

2. Results

2.1. Need for Effective BDBV Vaccines

Vaccines are essential interventions for protecting humans and animals by reducing infection rates, severity, and mortality caused by infectious diseases. Vaccination programs are one of the effective public health measures contributed to the eradication of some diseases and the control of many infectious diseases [6, 7]. A significant progress has been achieved in the development of vaccines against *Zaire Ebolavirus* including the approval of vaccines such as *Ervebo* and *Zabdeno/Mvabea*. Ervebo (rVSV-ZEBOV) is a single-dose, live recombinant vesicular stomatitis virus vaccine expressing the *Zaire Ebolavirus* glycoprotein. Zabdeno is a non-replicating adenovirus type 26 vaccine expressing the glycoprotein, followed eight weeks later by Mvabea, a modified vaccinia Ankara vaccine expressing Ebola glycoprotein and additional filovirus antigens. [8]. WHO urges the development of Bundibugyo virus-specific

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vaccines and identified International AIDS Vaccine Initiative (IAVI) rVSV and ChAdOx1-Bundibugyo vaccine by the University of Oxford and the Serum Institute of India as potential candidates for clinical evaluation [9]. Currently there are no specific antiviral treatments or approved vaccines exist for BDBV. Hence, there is an urgent need to develop safe and efficacious vaccines against the BDBV. Although significant efforts are in progress to develop BDBV vaccine prototypes, efforts to establish cost-effective vaccine production platforms capable of supporting rapid deployment during outbreak situations should be considered.

2.2. Plants as Vaccine Production Platform

The advancements in plant molecular biology and genetic engineering have expanded the pharmaceutical applications of plants. Plants can be engineered to act as bioreactors for recombinant protein production, and their pathways can be manipulated to increase the beneficial compounds. Plant expression systems have been used for the production of biopharmaceuticals for more than three decades, the concept termed as molecular farming [10]. The plant biotechnology could offer potential solutions in the development of vaccines, antibodies, diagnostic reagents and antiviral peptides in the fight against emerging and reemerging infections [11-14]. Furthermore, several reports have highlighted the potential of plant expression systems for the production of vaccines against a wide range of human and animal pathogens [15-18]. The advantages of plant expression systems for the production of vaccines for Ebola, SARS-CoV-2 and many others have been discussed in earlier reports and safety and feasibility of the plant-derived products have been demonstrated in numerous preclinical and clinical studies [19-25]. The advantages and limitations of available expression systems were provided in table 1.

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111 Table 1. Comparison of available expression systems for biopharmaceutical protein production
112 (Adapted from Shanmugaraj et al.,[26])

Expression System	Advantages	Limitations
Bacteria	• Easy to manipulate • Low cost • High expression • Ease of scale up • Short turnaround time • Established regulatory procedures and approval	• Improper folding • Lack of post-translational modifications which may affect the protein function • Endotoxin accumulation
Mammalian Cells	• Proper folding and authentic post-translational modifications • Existing regulatory approval	• High production cost • Expensive media and culture condition requirements
Yeast	• Rapid growth and scalable • Easy to manipulate • Simple and inexpensive media requirements and culture conditions • Post-translational modifications of recombinant proteins	• Difficulty in cell disruption due to the thick and hard cell walls • Hyperglycosylation of proteins • Limited glycosylation capacity
Insect cells	• High expression levels • Ability to produce complex proteins including secreted, membrane, and intracellular proteins • Proper folding and post-translational modifications	• High cost and time consuming • Expensive media and culture condition requirements
Plant	• Rapid and affordable • Optimized growth conditions • Free from pathogen and bacterial toxin contaminants • Economical • Post-translational modification somewhat similar like mammalian system	• Regulatory compliance • Limited glycosylation capacity

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The recombinant proteins can be produced in plants either by stable or transient expression systems. The stable expression can be achieved through transformation of foreign genes into the nuclear genome (transgenic plants) or in the plastid (chloroplast) genome (transplastomic plants). In particular, plastid genome engineering in edible plants shown promising results and has the potential for the development of oral vaccines. Notably, antigens produced in lettuce chloroplasts confer protection against several diseases [27-29]. The recombinant proteins can also be produced using plant cell suspension cultures [30-33]. Among these approaches, transient expression in *Nicotiana benthamiana* has emerged as one of the most widely used systems because of its rapid protein production and high yields. Vaccine antigens against several viral pathogens have been produced in these three platforms.

Plant-derived vaccines can significantly reduce the manufacturing costs and the energy requirements compared to other production platforms [34]. The post-translational modifications, folding and assembly of plant-made proteins are often comparable to those in mammalian system [35]. However, certain plant-specific features particularly in the protein glycosylation patterns must be considered [36, 37]. However, the recent advances in glycoengineering have enabled the production of recombinant proteins with humanized glycan structures, further enhancing the suitability of plant systems for pharmaceutical manufacturing. Though plant expression systems were emerged as a potentially scalable and cost-efficient biofactories, industrial-scale validation remains limited and efforts are needed for commercialization.

2.3. Commercial Progress and Translational Potential

The commercial feasibility of this platform has been demonstrated by several molecular farming companies specializing in the development of plant-derived products including

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Medicago Inc. (Canada), Protalix BioTherapeutics (Israel), ORF Genetics (Iceland), and Baiya Phytopharm (Thailand) showcasing the translational potential of plant-based production technologies. A major milestone in the field was the successful development, regulatory approval, and commercialization of Elelyso® (taliglucerase alfa), the first plant-produced recombinant therapeutic protein approved for human use. This product was developed by Protalix BioTherapeutics for enzyme replacement therapy for the treatment of patients with Gaucher disease. Elelyso is produced by genetically engineered carrot cell suspension cultures and approved by U.S. Food and Drug Administration in 2012. Another recombinant enzyme Elfabrio® (pegunigalsidase alfa) produced in plant cell cultures by Protalix BioTherapeutics received regulatory approval for the treatment of Fabry disease [38-40]. Their regulatory approval and commercial manufacturing demonstrated the feasibility of plant expression systems for producing clinical-grade therapeutic proteins.

The Coronavirus Disease (COVID-19) pandemic further highlighted the potential of plant expression systems for rapid vaccine development. Medicago developed a plant-derived virus like particle (VLP) vaccine against COVID-19 (Covifenz®). This vaccine was produced in *N. benthamiana* through transient expression. This plant made vaccine demonstrated robust efficacy in clinical trials and received regulatory approval in Canada in 2022 [41]. This demonstrates the ability of plant expression systems to rapidly produce complex vaccine candidates in response to emerging infectious disease outbreaks. In parallel, several groups utilized plant expression systems to produce SARS-CoV-2 antigens, antibodies, diagnostic reagents, and VLP-based vaccine candidates, further demonstrating the versatility and adaptability of this platform during public health emergencies. These achievements demonstrate that plant expression systems have evolved from an experimental technology into a commercially viable manufacturing platform

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capable of supporting large-scale production of complex biologics. The development of these products provides a strong foundation for the future development of plant-derived vaccines and therapeutics against emerging pathogens, including BDBV.

2.4. Plant-Based Production of Ebola Therapeutics

Transient expression in plants is rapid compared to conventional platforms as there is no requirement for the generation of stable cell lines making it particularly attractive for responding to emerging infectious disease outbreaks. Plant transient expression has the potential to produce recombinant biopharmaceuticals often within the timeframe of two to three weeks of getting the target gene sequence for protein characterization and preclinical research. Therefore, large scale clinical grade materials can be produced in minimal investment [42]. Figure 1 illustrates the workflow from antigen selection to recombinant protein production in plants. A summary of vaccine candidates and monoclonal antibodies (mAbs) against Ebola virus that have been produced using plant-based expression systems is provided in Table 2.

Table 2. Selected list of plant-produced vaccine candidates and monoclonal antibodies against Ebolavirus.

Vaccine Candidates					
Protein	**Plant Expression Host**	**Transformation Method**	**Expression Platform**	**Expression Level (Yield)**	**Reference**
Zerola	*N. tabacum* cv. Petit Havana SR1	*A. tumefaciens*-mediated transformation	Stable nuclear expression	1-2 µg/g of FLW	[43]

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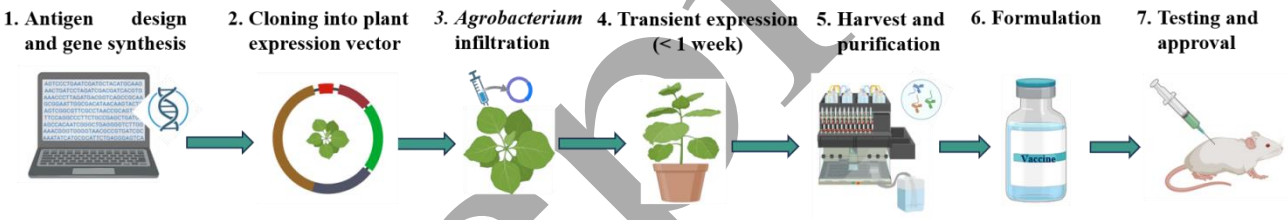
LTB-EBOV GP1-E1 and GP1-E2	*N. tabacum* cv. Petit Havana SR1	*A. tumefaciens*-mediated transformation	Stable nuclear expression	14.7 µg/g of FLW	[45]
VP40	*N. tabacum* cv. Petit Havana SR1	*A. tumefaciens*-mediated transformation	Stable nuclear expression	0.6 to 2.6 µg/g of FLW	[46]
Heavy chain-GP1 fusion protein	*N. benthamiana*	Agroinfiltration	Transient expression	~50 µg/ g of FLW	[47]
Antibodies					
mAb 13F6-Full and mAb 13F6-large single-chain	*N. tabacum*	*A. tumefaciens*-mediated transformation	Stable nuclear expression	24 mg and 7 mg per kg leaves	[48]
h13F6	*N. tabacum* cv Wisconsin 38	*A. tumefaciens*-mediated transformation	Stable nuclear expression	3-17 ng per mg of TSP	[44]
6D8 mAb	*N. benthamiana*	Agroinfiltration	Transient expression	87.2 ± 25.2 µg/g of FLW	[49]
ZMAb (m1H3, m2G4, and m4G7)	*N. benthamiana*	Agroinfiltration	Transient expression	NA	[50]
ZMapp (c13C6, c2G4 and c4G7)	*N. benthamiana*	Agroinfiltration	Transient expression	NA	[50]
MB-003 (h-13F6, c13C6, and c6D8 mAbs)	ΔXT/FT *N. benthamiana*	Agroinfiltration	Transient expression	NA	[51, 52]

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h-13F6ΔXF and h-13F6agly	ΔXTFT *N. benthamiana*	Agroinfiltration	Transient expression	NA	[53]
h13F6 mAb	*N. benthamiana*	Agroinfiltration	Transient expression	0.5 mg/g of FLW (10% TSP)	[54]
6D8 mAb	*N. benthamiana*	Agroinfiltration	Transient expression	0.5 mg/g of FLW	[55]

Note: FLW- Fresh Leaf Weight; TSP-Total Soluble Protein; NA- Not Available

Figure 1: Schematic Workflow for Rapid Production of Plant-Derived Vaccines and Biologics against BDBV.



The subunit and VLP based vaccines are quicker and safer alternatives to inactivated or attenuated vaccines as they do not contain infectious viral material and favorable safety profiles. The Ebola virus antigens have been produced in plants, demonstrating the feasibility of plant-based vaccine development against filoviruses [43, 45, 46]. In addition, Ebola immune-complex vaccines were produced in plants and shown to induce antigen-specific immune responses in animal studies [47]. Most of the plant-produced proteins exhibit potent immunogenic properties and induced antigen-specific immune responses in suitable animal studies.

VLP-based vaccine represents another promising strategy for Ebola virus disease. VLPs mimic the structural organization of native virions, but lack the genetic material. It induces

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strong immune response and also has excellent safety profile. VLPs can efficiently stimulate antigen-presenting cells and induce both humoral and cellular immune responses. The successful development of plant-derived VLP vaccines against several viral diseases further supports the applicability of this platform to filoviruses. Notably, Medicago successfully produced approximately 10 million doses of an H1N1 influenza VLP vaccine within one month as part of the DARPA Blue Angel program, which demonstrated the capacity of plant expression systems for rapid vaccine production [56]. Similarly, iBio has also developed plant-based VLP production technologies.

In addition to vaccine antigens and diagnostic reagents, plants have also been explored for the production of therapeutic antibodies for the passive immunotherapy. Several mAbs and mAb derivatives against viral pathogens have been expressed in different plant species [57]. Notably, ZMapp, a cocktail of three monoclonal antibodies (13C6, 2G4, 4G7) was produced in *N.benthamiana* through transient expression [50]. It was used under emergency compassionate protocols during the 2014–2016 West African Ebola outbreaks. The successful development of ZMapp provided a landmark proof-of-concept that plant expression systems can efficiently produce complex mAb cocktails against highly pathogenic filoviruses and demonstrated the translational potential of plant molecular farming for therapeutic biologics [19, 43, 46, 47]. This further supports the feasibility of utilizing similar approaches for BDBV vaccine development.

Other than vaccines and therapeutic antibodies, plant transient expression is an excellent platform to develop diagnostic proteins such as viral antigens or virus-specific antibodies, which can be used immediately as components of assays and kits [58, 59]. The development pipeline for vaccines and therapeutics is longer due to the need for *in vitro, in vivo* and clinical studies. In addition, proper GMP conditions need to be followed which further increase the development

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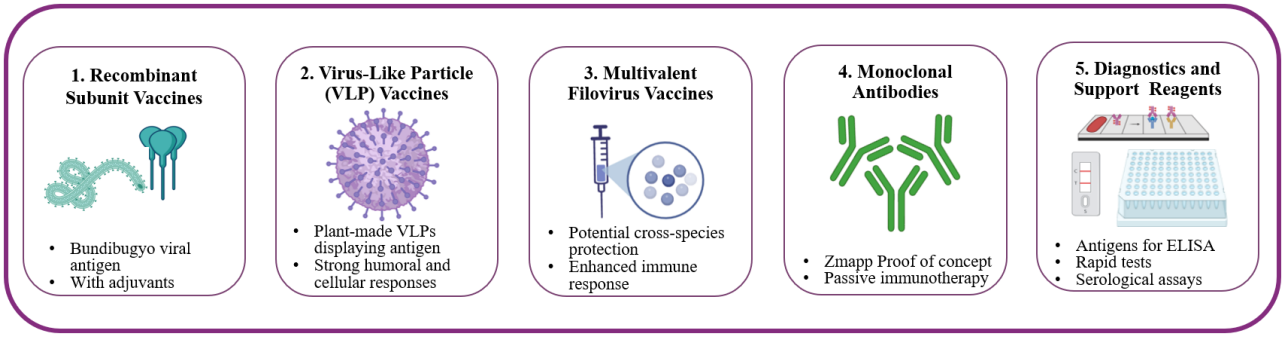
time and costs. However, the development pathway for diagnostic reagents is comparatively less complex and generally requires shorter timelines for validation and deployment.

2.5. Prospects for Plant-Derived Vaccines against BDBV

The successful production of Ebola virus antigens, virus-like particles, and therapeutic antibodies in plants provides a strong foundation for the development of BDBV-specific countermeasures. In the context of BDBV, plant expression systems could be utilized for the production of recombinant subunit vaccines based on the viral protein as well as VLP-based vaccines that mimic the native structure of the virus. These approaches have been widely investigated in plants for numerous viral pathogens, and plant-produced antigens have consistently demonstrated the ability to induce robust humoral and cellular immune responses.

Although significant progress has been achieved in the development of vaccines against *Zaire ebolavirus*, there is not much progress in BDBV vaccine development. The recurring outbreaks, potential for sustained human-to-human transmission and high mortality necessitates the urgent need to accelerate the development of effective and accessible countermeasures. In this context, plant expression systems offer a promising platform for the rapid development of next-generation vaccines, diagnostic reagents and monoclonal antibodies against this neglected Ebolavirus (Figure 2).

Figure 2: Potential applications of plant expression systems in the development of BDBV countermeasures. Plant expression systems can support the development of recombinant subunit



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vaccines, VLP-based vaccines, therapeutic monoclonal antibodies, multivalent filovirus vaccines, and diagnostic reagents against BDBV.

The successful production of Ebola virus antigens in plants demonstrates that the technological foundation required for BDBV vaccine development already exists. The viral protein which represents the principal target for protective immunity could be rapidly expressed in plants as a recombinant subunit vaccine antigen. Similarly, plant expression systems could facilitate the production of BDBV-specific VLPs that closely mimic the native viral structure while maintaining a favorable safety profile. Once the genetic sequence of a target antigen is available, the vaccine candidates can be produced in a short timeframe using transient expression [60]. This capability may be particularly valuable for emerging and reemerging filovirus strains requiring rapid countermeasure development. Further, plant-made vaccines have the potential to support large-scale vaccine production at affordable cost and improve accessibility especially in low and middle income countries where coverage is challenging and the outbreaks occur frequently [61, 62]. Currently, the research on plant-derived vaccines targeting BDBV remains limited, highlighting the need for systematic investigations in this research field. In addition, future studies should explore the feasibility of developing multivalent vaccines capable of providing protection against multiple pathogenic Ebolavirus species, including *Zaire Ebolavirus*, *Sudan Ebolavirus*, and *Bundibugyo Ebolavirus*. Overall, the advancements made in the plant-produced Ebola biologics provide solid evidence that the plant expression systems could play an important role in future BDBV vaccine development.

3. Conclusion

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In conclusion, the efforts toward the development of plant-made vaccines against BDBV could provide effective, scalable, and economically feasible vaccination strategies. Thus, increased research and collaborative efforts in this direction are essential to accelerate the fight against this deadly disease. Several vaccine candidates produced in plants have progressed to clinical trials that provide evidence of safety and efficacy of this platform. Looking ahead, an internationally harmonized regulatory framework for the production of recombinant vaccine proteins in plants should be developed which will be critical for facilitating product approval and global distribution.

Declarations

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Conflict of Interest

None. The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

Conceptualization, B.S.; literature review, B.S., A.N., P.S., T.B.; writing—original draft preparation, B.S., A.N. writing—review and editing, B.S., A.N., P.S., T.B.; supervision, B.S. All authors have read and agreed to the published version of the manuscript.

Availability of data and material

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The data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declaration of generative AI use

The authors used artificial intelligence tools for the purpose of correcting grammatical errors and improving language clarity in the manuscript.

Ethics approval and consent to participate

Not applicable.

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Informed Consent Statement

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