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Plant-based edible vaccines: Can cholera be the case study in Africa?☆

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ABSTRACT

Vaccines are employed as a sanitary approach that is implemented to lessen the hurdles caused by infectious diseases on the safety of public health. A vaccine is biologically made from inactive components of microbes, to enhance immunity and as a defense mechanism adverse to parasitic, bacterial and viral illnesses. Nonetheless, the mode of production that involves purification is quite costly, more so, to low and middle-income countries, especially in Africa. Conventional oral cholera vaccines, though commercially available, face logistical challenges to be transported and distributed to target populations such as Africa. Edible vaccines derived from plants, on the other hand, offer cost-effective and bio-friendly production cost, they are easily administered to all age groups and can be grown near-user-site. This article thoroughly assesses the capability of plant-based edible vaccines as an option for immunization against cholera with exclusive concentration on the African continent.

1. Introduction

Cholera is still a persistent threat to global health, particularly in Africa, where the outbreaks weigh down the systems of healthcare and claim a lot of lives, yearly.[1] This calls for the crucial necessity for effective, innovative and most importantly, accessible preventive measures to control the infectious disease.[2] In response, plant-based edible vaccines have emerged as a promising vaccination strategy, offering a potential game-changer solution in the fight against cholera.[3] Although cholera control has improved, traditional vaccines remain expensive, difficult to access, and hard to scale.[4] As such, a new method is needed to address these pitfalls. Plant-derived edible vaccines provide a groundbreaking alternative, leveraging cutting-edge biotechnological tools to deliver life-saving protection against cholera. Imagine a future where cholera is a distant memory, and communities in Africa thrive without the fear of outbreaks.[5] This vision is within reach, if we plan to incorporate edible cholera vaccines (ECVs) made from plants and/or fruits into the already existing strategies like Water, Sanitation and Hygiene (WASH) infrastructure.[6] By harnessing the power of green factory vaccine development,[7] we can create a healthier Africa that is resilient to infectious diseases caused by bacteria, viruses or parasites. According to the World Health Organization (WHO), cholera affects up to five million people annually, alongside notable mortality cases. Consequently, the Global Task Force for Cholera Control (GTFCC) was formed in 2014 to spearhead the fight against cholera and eliminate its recurrence thereof by the year 2030.[8] In Africa, where healthcare facilities are regularly strained, the requirement for fail-safe vaccination schemes is urgently needed. Plant-based edible vaccines offer a beacon of hope, poised to revolutionize cholera control and prevention in Africa and beyond.

2. Cholera pathogenesis

Cholera is an acute diarrheal disease caused by the gram-negative bacterium *Vibrio cholerae*. The primary pathogenic mechanism involves the colonization of the small intestine and the production of cholera toxin (CT), a potent enterotoxin.[9] Upon ingestion, *V. cholerae* survives the acidic environment of the stomach and reaches the

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intestinal lumen, where it attaches to the epithelial cells using pili and other adhesion factors.[10] Once attached, the bacteria produce cholera toxin, which activates adenylate cyclase via the G protein signalling pathway. This results in increased cyclic adenosine monophosphate (cAMP) levels, leading to the secretion of chloride ions and water into the intestinal lumen while inhibiting sodium absorption.[11] The consequent loss of fluids and electrolytes manifests as severe watery diarrhoea, commonly referred to as "rice-water stools".[12] Without prompt rehydration therapy, rapid dehydration and electrolyte imbalances can lead to death within hours.

3. Cholera transmission

Cholera is primarily transmitted through the ingestion of contaminated water or food. Poor sanitation and inadequate access to clean drinking water create an ideal environment for *V. cholerae* to thrive and spread.[13] Faecal-oral transmission is the most common route, particularly in regions experiencing overcrowding, displacement, or natural disasters that disrupt water and sanitation systems.[14] Common sources of contamination include foods, particularly shellfish, irrigated with contaminated water; poor hygiene practices during food preparation; untreated or poorly treated water sources; and raw or undercooked seafood.[15] In endemic areas such as Africa, asymptomatic carriers also contribute to the persistence and propagation of the bacteria, further complicating control efforts.

4. Epidemiology of cholera in Africa

Africa accounts for a substantial proportion of the global cholera burden. The WHO estimates that Sub-Saharan Africa (SSA) experiences approximately 60 % of all cholera cases reported globally.[16] Recurrent outbreaks are driven by a combination of socio-economic and environmental factors, like:

Limited Access to Clean Water: Over 300 million people in SSA lack safe drinking water, increasing vulnerability to waterborne diseases like cholera.[17]
Inadequate Sanitation: Open defecation[18] and poor sewage systems facilitate the spread of *V. cholerae*.[19]
Climate Factors: Seasonal rains and floods often exacerbate outbreaks by contaminating water supplies.[20]
Conflict and Displacement: Disrupted healthcare systems and overcrowded refugee camps in countries like South Sudan, Somalia, and the Democratic Republic of Congo create hotspots for cholera transmission.[21]

5. Regional impact

- **Sub-Saharan Africa**: The region is the epicentre of cholera in Africa, with Nigeria, the Democratic Republic of Congo,[22] Malawi, and Mozambique[23] reporting the highest number of cases in recent years. Recurrent outbreaks are linked to poor sanitation and limited healthcare access.[24]
- **Eastern Mediterranean Region (EMR)**: Countries such as Lebanon[25] Syria,[26] and Yemen[27] frequently report cholera outbreaks, exacerbated by conflict and population displacement.[28]
- **Caribbean Region**: Although less affected than Africa, cholera remains a concern, particularly in Haiti,[29] which experienced a devastating outbreak following the 2010 earthquake.

6. Magnitude of cholera outbreaks in Africa

Since 1 January 2022 up to 31 July 2024, a cumulative total of 7,023 deaths from 399,508 cholera cases were reported (Fig. 1), depicting a case fatality rate of 1.8.[30] The Democratic Republic of Congo, Ethiopia, Malawi, Mozambique, and Zimbabwe accounted for 72.2 % (288,570 cases) and 62.3 % (4,375 deaths) (Supplementary Material).[31]

7. Present status of conventional oral cholera vaccines in Africa

Conventional Oral Cholera Vaccines (OCVs) have been instrumental in controlling cholera outbreaks, not only in Africa but in other parts of the globe as well. They are prequalified and approved by the WHO and are orally administered in a double dose regimen, within a fourteen (14) day interval. OCVs provide 85 %-90 % protection against cholera for a period of 4 to 6 months. There are up to three distinct commercially available OCVs on the international market, namely Dukoral (crude, inactivated vaccine),[32] Vaxchora (live, attenuated vaccine)[33] and Shanchol (killed, whole-cell vaccine). WHO recommendations for situations in which OCVs are to be administered include during a response to a cholera outbreak, in cholera hotspots where there is ease of transmission from person to person, in vulnerable populations such as refugee

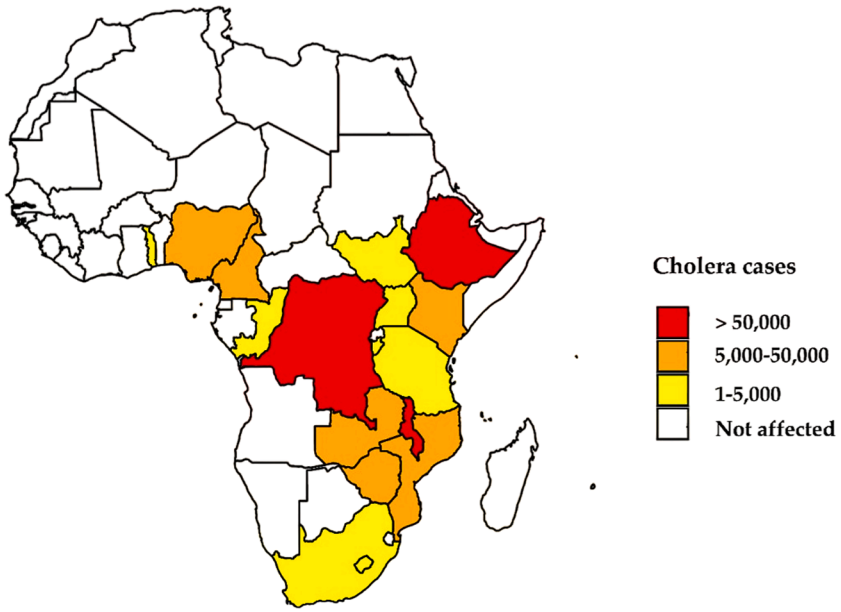

Fig. 1. Cholera cases in Africa from 1 January 2022 to 31 July 2024.

camps where individuals are highly susceptible to contracting infectious diseases such as cholera, and during mass vaccination campaigns in cholera endemic regions.[34]

Notwithstanding, Africa still faces challenges in the acquisition and even distribution of OCVs, some of the issues faced are as follows: limited cold chain capacity, traditional vaccines require to be stored between 2℃–8℃, and this commodity is almost rare in rural settings. Logistical challenges are equally faced in Africa as there are substandard road networks, hindering vaccine delivery to hard-to-reach areas.[35] The cost of OCVs is relatively expensive, about $3-$5 per dose, making them inaccessible to many due to a lack of sufficient funds to procure them. Furthermore, the global demand for vaccines exceeds the supply thereof, leading to delayed deliveries and stockouts.[36] Not only that, but the number of medical personnel in most African countries does not tally to the number of people to be immunized, for that reason, conventional vaccines are not widely administered to the target population on the African continent.[37] Another limitation in the administration of OCVs is the hesitancy of the public to receive them, because of mistrust as regards vaccines, due to public misinformation and sometimes cultural or religious beliefs. These deployment barriers can affect vaccine uptake by the people.[38]

8. Plant-based edible cholera vaccines

Edible vaccines derived from plants or plant products are a promising strategy[39] to control cholera,[40] the plant biotechnology has proven efficient in different parts of the globe.[41] However, the innovation has not yet been explored on the African continent, i.e., the use of local plant varieties and carrying out clinical trials within the African population. Hence considering cholera, which is an endemic infectious disease, that continues to weigh down the public health safety of most countries in the SSA region, could be a test case because edible vaccines derived from plants offer several advantages.[42] This biotechnology involves the genetic engineering of plants to produce vaccine antigens, which stimulate an immune response against specific diseases, including cholera.[41] While the concept of edible vaccines made from plants was first researched over three decades ago,[43] there have been few openly accessible studies that delve into this biotechnique in the recent past.[44] Some of the specific studies carried out on edible cholera vaccines in various plants[45] are as follows (Table 1): potatoes,[46] rice,[47–48] maize,[49–50] tobacco[51] lettuce,[52–53] tomato,[54–55] carrot,[56–57] *Nicotiana benthamiana*,[58–59] sunflower,[60] and peanut.[61]

Plant-based edible vaccines offer several benefits, such as the elimination of the transportation and distribution chain as the plant candidates that vehicle the antigenic proteins are grown near-user-site. The mode of production of ECVs is cost-effective[62] and scalable,[45] with increased accessibility, especially in limited-resource settings. Unlike OCVs, plant-based edible vaccines provide the potential for long-term immunity and can be incorporated with other vaccines.[63]

9. Points to consider before developing a green factory edible vaccine

- Antigen selection: The safety and stability of the antigen to be engineered into the plant candidate should be rigorously assessed.[64] Establish quality control measures, standardized extraction and purification processes to ensure consistent vaccine potency.[65]
- Vaccine candidate: The choice of the plant to vehicle the antigenic protein should be thoroughly evaluated. Produce vaccines in farmer's preferred varieties, leveraging their natural ability to produce proteins.[66]
- Oral delivery: The number of antigen doses that would suffice for full immunization should be correctly quantified, to avoid overuse, misuse or underuse of the plant-based edible vaccine.[67] Formulate target-specific gut-associated lymphoid tissues (GALT) to ensure uniformity in immune responses.[68]

Table 1
Studies on edible cholera vaccines in various plant candidates.

Plant candidate	Description
Potato	Cholera toxin B subunit (CTB) was assembled with domain III of dengue virus and expressed in transgenic potatoes to develop plant-based edible vaccine
Rice	Rice grains carrying CTB antigens were grinded into powder and mixed with saline for consumption. The ECV proved safe in phase I of human trials
Maize	The y-zein promoter was used to express a synthetic gene encoding CTB in maize seeds and levels were determined enzyme-linked immunosorbent assay
Tobacco	Synthetic CTB gene was cloned in a plant expression vector under the control of ubiquitin promoter and transformed in tobacco plants by *in vitro* selection
Lettuce	20.5 % of inoculated lettuce explants showed positive results following a successful transformation and of the CTB gene using *Agrobacterium tumefaciens*
Tomato	Agrobiological properties were evaluated under *in vivo* conditions through biochemical characteristics of fruit quality, in transgenic tomatoes carrying CTB
Carrot	CTB was fused to an endoplasmic reticulum retention signal under the regulation of MLL promoter in carrot roots and the gene was detected by amplification
Nicotiana benthamiana	CTB rapid transient expression was conducted in *Nicotiana benthamiana* under *in vitro* cell culture for vaccine potential and to increase production yield
Sunflower	CTB-lumbrokinase was expressed in edible sunflower seeds as a transmucosal carrier to enhance thrombosis protection in mice and rats
Peanut	Peanuts expressing CTB were fed to offspring of female mice to epigenetically regulate Foxp3 promoter and induce IL-10 gene expression.

- Regulatory frameworks: There should be a contained process of securing a genuine license and a certified board to manage and control the vaccine accessibility, production, and distribution to avoid the potential escape of transgenic crops. Collaborate with regulatory agencies, and develop harmonized international standards.[69]
- Intellectual property: Patent innovation to protect proprietary technology and intellectual rights. Or collaborate with governments and public–private partners in the industry to share resources, knowledge and expertise to accelerate development.[70]
- Public awareness: Educate the community about genetically engineered bio-products before oral administration.[71] Ensure transparency and carry out thorough efficacy[72] and safety pre-clinical trials.[42] The local authorities can provide detailed information in native languages, braille and sign language to sensitize every person in society (Fig. 2).

10. Development of edible vaccines derived from plants

The vaccine is to be engineered with protective target specific formulations[73] to ensure that the antigen is released at the correct location in the gut.[74–75] The gene construct can include cardinal components such as a strong constitutive promoter cauliflower mosaic virus (CaMV) to drive expression in tissues of a plant,[76] a plant-specific protein storage[77–78] to stimulate the initiation process of translation. The gene of interest is critical as it is used to focus on the most preferred codons in the plant.[79] In the case of cholera, it is important to include the non-toxic[80–81] cholera toxin B subunit[82–83] in the expression cassette because

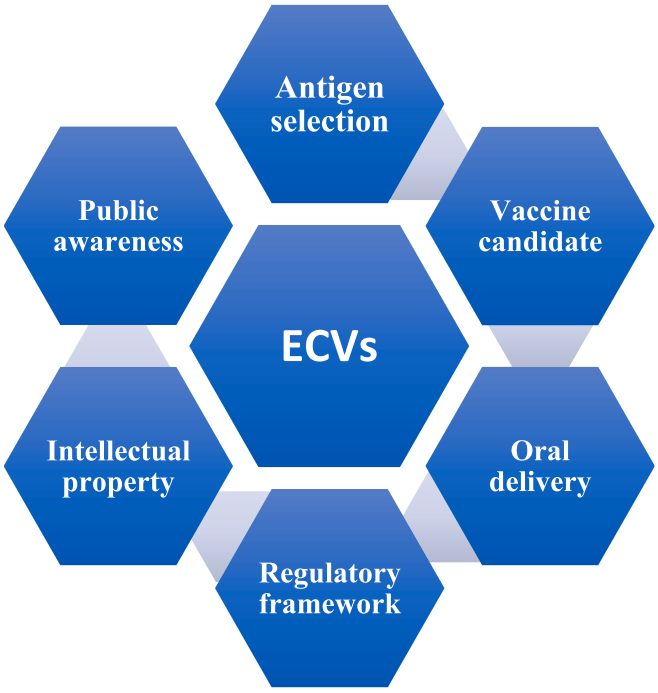

Fig. 2. Points to consider before developing plant-based edible cholera vaccines.

it could induce potent humoral immunity to neutralize the toxin in the gut.[84] Endoplasmic reticulum retention signal SEKDEL is another pivotal component of the gene construct,[85] it helps in the maturation of newly synthesized secretory proteins and releasing them at the appropriate location in the gut. The choice of the suitable terminator is pertinent in the development of a stable plant-based edible vaccine, as it is responsible for the net control of protein expression[86] from the vaccine candidate.

When selecting the plant candidate to use for the delivery of edible vaccine, ensure that it has a long shelf-life, i.e., it should be thermostable with a low spoilage rate (e.g., rice seeds), the plant tissue should be amenable to genetic transformation and regeneration (e.g., potato internodes and leaves) and the plant variety of choice should have high crop yield[87] to enhance mass vaccination coverage.[88] Edible vaccines are developed by incorporating a transgene into the genome of the plant[89–90] via diverse molecular biology techniques[91] such as *Agrobacterium tumefaciens* gene-mediated transformation[92–93] (Fig. 3). Potato is the world's fourth staple food and is cultivated easily in most parts of the globe. It has been used and proven efficient as the candidate of choice in some diseases[94] as such, there is sufficient scientific data and optimized protocols on how to biologically engineer the potato plant tissues for delivery of antigenic proteins.[95]

11. Mode of action of edible vaccines derived from plants

When people consume transgenic plant material, they are exposed to the encoded antigens – the parts of a pathogen that trigger an immune response. Subsequently, the body's immune system recognizes these antigens and develops immunity against the pathogen.[96] As a result, plant-derived edible vaccines stimulate both the systemic immune system and the mucosal immune network.[97] Mucosal immunity is the first line of defense that offers protection to vulnerable entry points, such as urogenital, respiratory and gastrointestinal tracts, where most pathogens invade.[98] This system relies on antibodies such as Immunoglobulin A (IgA) to neutralize pathogens.[99–100] When administered, edible vaccines present antigens to mucosal surfaces like gut-associated lymphoid tissue (GALT). This exposure activates IgA production and stimulates localized immunity at pathogen entry points in the mucosal tissues.[101]

The systemic immune system on the other hand goes beyond the local response,[102] it triggers the production of IgG antibodies.[103] IgG are capable of neutralizing pathogens[104–105] in the entire organism and are vital for long-term immunity.[106] Mucosal immunity cross-primes the systemic immune system to activate T-cells and B-cells that circulate throughout the body, in turn inducing a system-wide immune response.[103] This occurs when the microfold (M) cells acknowledge the antigenic protein from the vaccine released within the Peyer's patches (PP) lymphoid tissues of the small intestines.[107] Extending this mechanism of defense, the M cells then pass on the antigens to macrophages,

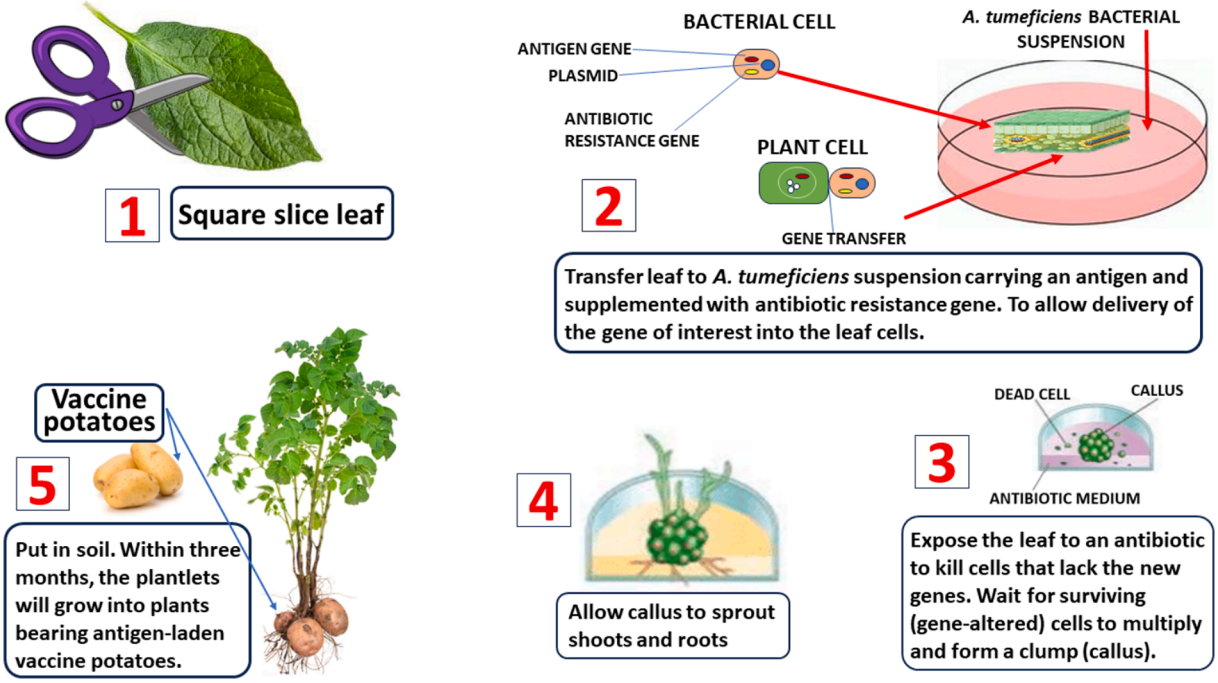

Fig. 3. Potato-based edible vaccine transformed using *A. tumefaciens* gene-mediated method.

which generate antiserum responses.[108] Thus, ingesting vaccines made from plants can train the immune system's memory helper cells to recognize the pathogen as a threat and produce cytotoxic T cells[109] to attack infected cells when a disease agent like *V. cholerae* arrives.[108] The Memory T cells swiftly stimulate antibody secretion,[110] and the antibodies promptly neutralize the invader (Fig. 4).

Plant-based edible vaccines simplify vaccination by offering a convenient and non-invasive mode of delivery.[97] They promise to be a potent tool against infectious diseases such as cholera, especially in resource-limited regions like Africa.[111] Edible vaccines made from plants eliminate the need for needles,[112] cold chain storage and trained medical personnel which are inadequate facilities in most areas of Africa.[113]

12. Problems associated with edible vaccines derived from plants and possible solutions

While plant-based edible vaccines promise to be a better immunization approach[114] to control cholera in Africa,[115] they are not without limitations. Some of the challenges faced in the production of green factory vaccines to be administered orally are that the plant-based edible vaccine may pose a risk of hypersensitivity reactions, particularly allergic reactions[116] due to the presence of plant-derived allergens, such as lectins. This can be mitigated by screening potential allergens and knocking them out/down[117] or developing hypoallergenic plant varieties.[118] Another challenge is the difference in the glycosylation patterns between plants and humans, which may impact the efficacy of the vaccine.[119] To address this problem, scientists may engineer plant pathways to produce human-like glycans[120] or conduct comparative studies on plant- and human-derived vaccines with minimized glycosylation differences.[121] Similarly, certain plants such as potatoes are not eaten raw, and overcooking the transgenic tubers might weaken the antigenic protein present in them.[63] However, Lauterslager *et al.,* 2001 reported that recombinant CT-B in potato tuber is stable upon cooking and that it preserved more than 40 % of its biological activity.[122] Even though there must be a coating tuber matrix to provide some degree of protection to the antigens against rapid digestion by gastric enzymes. Additionally, the tubers can be mildly cooked by steaming or air-frying to avoid degrading the vaccine with elevated temperature levels.[123] Alternatively, leaves can be used in place of tubers or alongside the tubers.[124–125] The two transgenic tissues of the potato plant (leaves and tubers) indicated an increased accumulation of synthetic heat-labile enterotoxin B subunit (LT-B) gene in 1998 when Mason and others expressed the Norwalk virus capsid protein in them.[76]

13. Why is it critical to implement plant-based edible vaccines in Africa?

Infectious diseases account for over one hundred public health emergencies in Africa, year in, and year out. According to the Africa Centre for Disease Control and Prevention, almost 20 % of these infectious diseases are classified as vaccine-preventable diseases of high priority that require immediate mitigation actions.[126] Mass production of medical countermeasures is among other investments that would ensure that the people of Africa have health security. ECVs offer potential to counteract the public health threats posed by cholera, on the African continent.[127]

The Corona Virus Disease of 2019 (COVID-19) pandemic exposed Africa's long-lasting struggles of acquiring basic life-saving supplies, such as vaccines. Although several COVID-19 vaccines[128] candidates were developed timely worldwide, they were not equally distributed to other parts of the world even at the peak of the pandemic, mainly due to barriers of intellectual property[129] and financial constraints. Consequently, many high-income nations could promptly attain the WHO's 70 % vaccination target of their population, whereas barely 32 % of African countries[130] were able to meet the primary vaccination target and this was merely three years post the vaccine availability, i.e., December 2023.[131] This situation highlights the critical need of region-specific solutions like plant-based edible vaccines developed from local cultivars, to enhance sustainable in-house production of vaccines as well as implement apt policies to protect human life in Africa and beyond.[62]

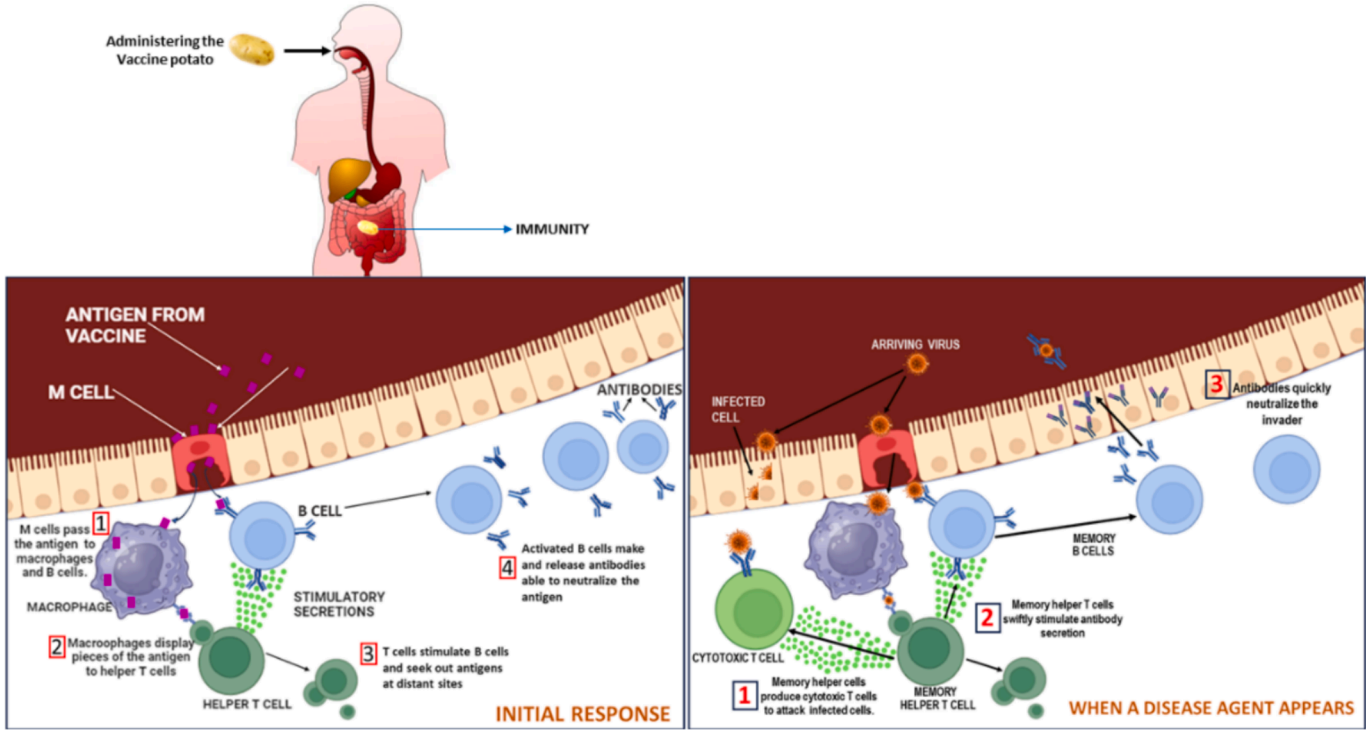

Fig. 4. Mode of action of a transgenic potato-based edible vaccine.

14. Is Africa geared for local manufacture of plant-based edible vaccines?

The presence of public health challenges in Africa cannot be over emphasized.[132] Manufacturing vaccines locally remains a relatively new technology in most low and middle-income countries.[133] Implying that scientists may not have the necessary skills or even biological tools to expressively involve governments to delve into the local production sector.[134] Vaccine production is a complex landscape that requires well-coordinated involvement of willing stakeholders to spearhead a working environment, if this agenda is to be implemented. In addition, plant-based edible vaccines may encounter hurdles in sustaining long-term production[44] as the technique is time-bound and it relies on financial aid from host governments and development partners. However, the level of engagement from African researchers in the production of vaccines proves the continent's preparedness to practically explore this technology.[135] For instance, WHO classified Nigeria in the Maturity Level 3 (ML3) for importation of vaccines alongside pharmaceutical regulations. Egypt was declared ML3 for local production as well as importation of vaccines.[136] This indicates that now is the time for other African countries to formally investigate conversations of local production of vaccines for sustainability and self-reliance.[135]

15. Confidently expecting – Local manufacture of vaccines

It is time to acknowledge the vital role of plant-based edible vaccines in shaping a healthier, more resilient future through collaborative and inclusive strategies, specifically in combating cholera outbreaks.[137] To amplify the impact of plant-based edible vaccines, governance platforms should foster mutual trust among vaccine developers, stakeholders, and governments.[136] This synergy will enable governments to share manufacturing priorities, gather public input, and support local manufacturers in partnering with vaccine developers to inform policies and regulations that drive local production.[119] Effective governance platforms will also facilitate ongoing capacity-building for plant-based edible vaccine developers, empowering them to advocate for sustainable, cholera-focused vaccine production. This, in turn, will contribute to a more resilient and self-reliant healthcare infrastructure in Africa, ultimately protecting communities from the devastating impact of cholera.

16. Conclusion

The development of plant-based edible cholera vaccines presents a compelling solution to Africa's cholera burden. They do not only offer health benefits but also economic advantages. By harnessing local agricultural infrastructure, these vaccines can stimulate economic growth, create jobs, and reduce healthcare expenditures. Similarly, plant-based edible vaccines can surmount traditional logistical challenges, i.e., improve accessibility, and provide cost-effective protection. As research progresses, plant-based edible cholera vaccines promise to transform cholera prevention and control in Africa. Future endeavors will focus on scaling up production, knocking-down allergens from transgenes, conducting expansive pre-clinical and clinical trials, and integrating these vaccines into existing healthcare frameworks. Efforts to prioritize community-centered approaches, would foster trust and awareness about plant-based edible vaccines and create standardized quality control platforms to address the dosage issues. This people-focused strategy will be crucial in achieving widespread adoption and ultimately, a cholera-free Africa.

Availability of data and materials

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

Consent for publication

Not applicable.

CRediT authorship contribution statement

Beenzu Siamalube: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Emmanuel Ehinmitan:** Writing – review & editing, Software, Data curation. **Lupupa Kachenga:** Data curation, Writing – review & editing, Validation. **Steven Runo:** Supervision, Resources, Methodology. **Maina Ngotho:** Supervision, Investigation, Formal analysis. **Justus Onguso:** Supervision, Project administration, Conceptualization.

Ethics approval and consent to participate

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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