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HARNESSING THE POWER OF PLANT-BASED VACCINES: A NEW FRONTIER IN BIOTECHNOLOGY

Nishi Mishra[1] and Prashant Gigaulia[2]

[1]Research Associate, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur

[2]Guest Faculty, Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur

**Corresponding author email: prashantgigaulia1408@gmail.com*

ABSTRACT

Bio-priming is an environmentally friendly method of seed treatment that combines the application of helpful microbes and hydration to improve seed germination, plant growth, and stress tolerance in crops. The process improves the vigour of the seedling, inhibits soil-borne diseases, and enhances nutrient intake without the need for chemical input. Bio-priming achieves these using microbial agents like Trichoderma, Bacillus and Pseudomonas to advance sustainable agriculture, particularly among small-scale farmers. Although there are challenges such as strain specificity and low awareness, its environmental and cost benefits make it as a future promising approach.

KEYWORDS: Biopharmaceutical production, Edible vaccines, Genetic transformation, Plant-based vaccines, Sustainable immunization

INTRODUCTION

Vaccination has revolutionised health by providing effective immunity, but vaccine scarcity for fatal diseases prompts efforts for safer, simpler vaccines. Traditional methods, relying on microbial fermentation or egg-based systems, face high costs, cold chain dependence, and labour intensity. The influenza vaccine, produced in eggs, is the most common example, involving virus injection and incubation over days, requiring many eggs and lengthy regulatory approval (Nachbagauer & Palese, 2020). Fermentation facilities' costs hinder cell-based vaccine development. Both methods are limited by time and expense, especially for temperature-sensitive vaccines needing cold storage. Recently, researchers have explored using biological systems like bacteria, insects, or plants to produce vaccines, due to concerns about immune reactions and early disease stages. Biotechnology has introduced plant-based vaccines as a promising, economical, scalable, and safer alternative, leveraging plants' ability to produce immunogenic proteins.

PLANT-BASED VACCINES

Plant-based vaccines are produced from genetically modified plants that produce pathogen proteins, prompting immune responses in humans or animals. These proteins are synthesised using stable or temporary transformation technologies, acting as biofactories (Shanmugaraj et al., 2020). Antigens can be purified or directly consumed through edible tissues. The first attempt to produce vaccines in plants was by Hiatt and colleagues in 1989. The concept of using transgenic plants for subunit vaccines was introduced by Dr. Arntzen and colleagues, demonstrating that it can surpass traditional vaccine limits. They produced the first subunit vaccine in tobacco by expressing the *Streptococcus mutans* surface protein antigen. Plant-based vaccines are easy to handle, don't need complex storage, are cost-effective, and scalable, and making them a cheaper production alternative. Edible vaccines from plants offer a needleless, convenient delivery method. Common plant bioreactors include tobacco, potato, tomato, corn, and rice.

Many transgenic plants have produced four vaccine types: bacterial, viral, parasite, and immune contraceptive. There are several plant-based vaccines that have been produced, with some of them currently at the clinical trial phase. Among them, the most common types of vaccines are against viruses and bacteria that cause fatal illness in humans and animals and usually *Nicotiana* plants are utilized as the bioreactor. However, to date, only two products have been licensed, namely plant-made *scFV mAb* used in the production of a recombinant HBV vaccine in Cuba and the Newcastle disease virus (NDV) vaccine for poultry, approved by the US Department of Agriculture (USDA). Plant-based vaccines are classified as genetically modified and should have a license from the US Food and Drug Administration (FDA). There is no plant-based vaccine that has been approved. Given this exciting yet challenging research, the first part of this article focuses on the conventional and refined expression technologies for improved plant-based vaccine production, while the latter part discusses challenges encountered during and after the production process. Edible vaccines are a form of plant-based vaccine where the transgenic plant's tissue (such as fruit or tubers) is consumed directly, offering needle-free immunization. Crops like banana, tomato, and potato have been explored for this purpose, particularly for mucosal immunity (Mason et al., 1992).

STRATEGIES FOR PRODUCTION OF PLANT-BASED VACCINES

STABLE TRANSFORMATION

A stable transformation system can be achieved through the plant genome (nuclear or plastid integration), usually via *Agrobacterium tumefaciens* or biolistics (gene gun). It is called stable or permanent due to the

permanent changes occurring in the recipient cells' genetics as the target transgene is integrated into the genome of host plant cells. This results in transgenic plants that can continually express the desired antigen across generations (Sala et al., 2003). Biolistic and genetically modified *Agrobacterium* strains can lead to the formation of stable transfection. However, as *Agrobacterium tumefaciens* does not naturally infect many plant species, it limits the application of the Agrobacterium strain for stable transformation of the desired gene. Generally, stably transgenic plant cells produce a lower amount of subunit antigen, in the range of 0.01 to 0.30% of total soluble plant protein.

TRANSIENT EXPRESSION

Transient expression allows short-term production of antigens by delivering the gene of interest into mature plant tissues, often using agroinfiltration or plant viral vectors. This method yields faster results and higher protein concentrations (Capell et al., 2020). The transgene is not incorporated into the genome of the plant cells. In this plant expression system, the regeneration of the whole plant is not required, and the frequency of its occurrence is higher. These characteristics overcome the pitfalls related to the stable integration. Two most commonly used methods that would achieve transient expression of a desired protein in plants are the Agrobacterium-mediated transformation of genetically modified plant virus and particle bombardment.

GENE DELIVERY METHOD FOR THE PRODUCTION OF PLANT-BASED VACCINES

As mentioned earlier, there are several methods that can be used to produce plant-based vaccines. Basically, these methods are divided into two categories, which are direct and indirect gene delivery.

DIRECT GENE DELIVERY METHODS

Direct gene delivery refers to the direct introduction of DNA or RNA into plant cells. One of the most widely used direct methods is the biolistic method, also known as the gene gun or microprojectile bombardment. This vector-independent approach is especially useful when *Agrobacterium*-mediated transformation is not feasible. It enables stable integration of the transgene and is applicable to a wide range of plant species and cell types (Ramachandran & Walden, 2008).

The biolistic method facilitates both nuclear and chloroplast transformation, though most recent plant-based vaccine developments favor chloroplast transformation. Chloroplast-derived vaccines have shown success in targeting bacterial diseases such as cholera, Lyme disease, anthrax, tetanus, and plague, as well as viral diseases including rotavirus and canine parvovirus (Verma et al., 2008). Chloroplast transformation provides advantages such as stable inheritance of transgenes across generations (Verma &

Daniell, 2007), high antigen yield, and reduced biomass requirements, facilitating simpler downstream processing such as freeze-drying or capsule formation (Daniell et al., 2001).

Several plant-derived vaccines have been tested in animal models. For example, mice immunized with chloroplast-derived anthrax vaccine survived exposure to anthrax toxin (Watson et al., 2004). In another case, a rabies vaccine was developed in *Zea mays* by transforming embryogenic callus with a construct driven by the cauliflower mosaic virus (CaMV) promoter using biolistics. Transgenic plants were grown, and their leaves expressing rabies virus glycoprotein were fed to mice, which showed protection against rabies virus challenge (Loza-Rubio et al., 2008).

INDIRECT GENE DELIVERY METHODS

Indirect gene delivery uses biological systems like *Agrobacterium* or plant viruses to integrate foreign genes into plant genomes.

AGROBACTERIUM-MEDIATED GENE TRANSFER

Agrobacterium tumefaciens and *A. rhizogenes* are Gram-negative soil bacteria commonly used as vectors. *A. tumefaciens* is preferred for stable nuclear transformation, as it naturally transfers its T-DNA into plant cell nuclei (Gelvin, 2003). In vaccine development, the tumor-inducing (Ti) genes in the plasmid are removed, and the target gene is inserted to form a recombinant disarmed Ti-plasmid. The bacterium's virulence genes facilitate the transfer and integration of this transgene into the plant genome (Tzfira & Citovsky, 2006). Although early *Agrobacterium*-mediated transformations used leaf soaking methods, these resulted in limited gene transfer due to the "position effect", where only edge-layer cells of explants were transformed (Arakawa et al., 1997). To enhance efficiency, agroinfiltration has been developed. This involves injecting a suspension of *A. tumefaciens* into plant tissue to allow transient transgene expression (Kapila et al., 1997).

- Syringe infiltration is a simple technique using a needleless syringe to deliver the bacterial suspension into leaves. It requires minimal equipment and allows rapid analysis of transgene expression (Yang et al., 2000).
- Vacuum infiltration involves submerging leaves in infiltration buffer containing *Agrobacterium* and applying a vacuum to replace air with the bacterial suspension. While this method ensures better penetration, it requires more specialized equipment and is less flexible for delivering multiple transgenes (Wroblewski et al., 2005).

Agrobacterium has also been used to deliver deconstructed viral vectors. In one case, agroinfiltration of lettuce with geminiviral replicon vectors produced high levels of virus-like particles (VLPs) from

Norwalk virus capsid protein and monoclonal antibodies (mAbs) against Ebola and West Nile viruses (Lico et al., 2008).

GENETICALLY ENGINEERED PLANT VIRUS

In this approach, a suitable plant virus is genetically modified to create a chimeric gene encoding a viral coat protein, thereby serving as a vector for delivering genetic material into plant cells. This strategy leads to the transient expression of antigens in the host plant. The recombinant virus facilitates expression of the desired protein or peptide, and antigen accumulation can be further enhanced by modifying the viral capsid proteins (Rybicki, 2009). Plant virus-mediated expression systems offer several advantages: they enable high-level recombinant protein production within a short period after infection, allow presentation of multiple antigen copies on the viral particle surface, and support large-scale infection of plants, making the system amenable to industrial-scale vaccine production (Gleba et al., 2007).

Table 1: Biotechnological Hosts for Plant-Made Vaccine Production for Human and Animal Diseases

Disease	Pathogen/Antigen	Plant Used	Transformation Method	Reference
Diarrheal	Norwalk virus	*Nicotiana benthamiana*	*Agrobacterium tumefaciens*	Tacket et al., 2000
Tuberculosis	*Mycobacterium tuberculosis*	*Arabidopsis thaliana*	*Agrobacterium*	Lakshmi et al., 2013
Avian H5N1	HA protein of H5N1	*Nicotiana benthamiana*	*Agrobacterium tumefaciens*	D'Aoust et al., 2008
Dengue	Dengue virus type 2 E glycoprotein (EIII)	*Nicotiana tabacum* cv. MD609	Agroinfiltration	Yusibov et al., 2011
Rabies	Rabies virus	*N. benthamiana*, Tomato	*Agrobacterium tumefaciens*	Loza-Rubio et al., 2008
Hepatitis B	HBsAg (Hepatitis B surface antigen)	Tomato	Stable transformation	Thanavala et al., 2005
Foot and Mouth Disease	FMDV (Foot and Mouth Disease Virus)	*Stylosanthes guianensis* cv. Reyan II	PEG-mediated transformation	Zheng et al., 2007
Nerve Agent	Acetylcholinesterase	Tobacco	Agroinfiltration	Saxena et al., 2006
Diabetes	Insulin	Safflower	Agroinfiltration	Boothe et al., 2010
HIV	HIV virus antigens	Tobacco	Agroinfiltration	Rosenberg et al., 2013
Ebola	Ebola virus	*Nicotiana benthamiana*	Agroinfiltration	Shoji et al., 2012

However, post-infection processing requires purification of the target products from infected plant tissues prior to use in vaccination. Additionally, this method results in plant death following viral infection**,** necessitating repeated cycles of infection and harvest for continuous production (Rybicki, 2009).

Initially, plant virus expression systems primarily utilized engineered RNA viruses such as tobacco mosaic virus (TMV)**,** potato virus X (PVX)**,** alfalfa mosaic virus (AIMV)**,** cucumber mosaic virus (CMV), and cowpea mosaic virus (CPMV) as expression vectors. These plant viruses do not replicate in mammalian cells, making them safe and effective vehicles for expressing vaccine antigens for both human and veterinary applications. Upon administration either via injection or orally the plant-derived antigens are processed by antigen-presenting cells (APCs)**,** which present them to T-lymphocytes**,** thereby initiating humoral and/or cellular immune responses**.** Furthermore, edible vaccines have the potential to stimulate mucosal immunity, offering an additional layer of protection (Streatfield, 2005).

TRANSLATIONAL MILESTONES IN PLANT BASED VACCINE RESEARCH

Several plant-based vaccine candidates have advanced to preclinical and clinical stages. Notably, the expression of hepatitis B surface antigen (HBsAg) in potatoes and lettuce has successfully elicited immune responses in animal models (Thanavala et al., 2005). Similarly, tomatoes engineered to express the Norwalk virus capsid protein induced mucosal immunity in human trials (Tacket et al., 2000). More recently, Ward et al. (2020) reported results from a Phase 3 clinical trial (NCT03321968) of a quadrivalent, recombinant, virus-like particle (VLP) influenza vaccine produced in *Nicotiana benthamiana* via transient expression. This VLP-based platform incorporates haemagglutinin (HA) protein trimers, combined into a quadrivalent vaccine formulation (QVLP). Although multiple vaccines have been developed and distributed against COVID-19, several companies continue to explore plant-based production platforms, with some candidates progressing into clinical evaluation (Refs. 109, 110). Among them, Medicago (Quebec, Canada) announced a plant-derived QVLP COVID-19 vaccine, approved by the FDA, which is administered either alone or with adjuvants such as AS03 or CpG1018, provided by GSK (Brentford, Middlesex, UK).

CHALLENGES AND LIMITATIONS

The ultimate goal of plant-based vaccines is to produce stable transgenic vaccines which are safe for consumption while reducing the production cost. Besides all the underlying issues that may affect the efficacy of plant-based vaccines, the regulatory guideline is regulated by the U.S. Department of Agriculture (USDA) and especially the growth of transgenic plants, production and purification of plant-based vaccines, and all phases of clinical trial until the marketable stage shall be strictly implemented.

Therefore, the manufacturers shall ensure their responsibility to follow the Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) so that the upstream to downstream production of plant-based vaccines is strictly controlled for quality management.

The first issue is the selection of an antigen and the right plant expression host because it helps to determine the safety of the vaccine produced, also be used to produce a thermostable vaccine. The consistency of dosage is another obstacle for researchers have to face, as dosage produced may vary within the plants of the same species, from fruit to fruit and from generation to generation due to the size and ripeness of the fruits and vaccine manufacturing, according to good manufacturing practice (GMP) procedures.

On top of that that, it is also quite difficult to evaluate the required dosage for every patient. Levels of innate and adaptive immune responses generated in different individuals may vary based on the types of antigens being exposed in the body. Between two patients with different body weight as well as their age, the dosage of the plant-based vaccine required will be different. If this issue is not monitored carefully, an immunological tolerance will be induced when the patient is overdosed, while a reduction in antibody production will occur when the patient is underdosed. Besides that, gene silencing might be induced due to the accumulation of mRNA in the transgenic plant cells, as the growth of the plants is stopped and the fruit formation is reduced while the antigen content is increased. In such cases, consumption of plant-based vaccines may induce allergic reactions and a few side effects such as toxicity on the central nervous system, cytokine-induced sickness, and autoimmune diseases.

Today, companies like Medicago and Kentucky Bio-Processing have overcome these limitations. Globally, the World Health Organization (WHO), U.S. Food and Drug Administration (FDA), and European Medicines Agency (EMA) are updating frameworks to accommodate plant-derived biopharmaceuticals. Medicago's COVID-19 vaccine was among the first plant-based vaccines to gain regulatory approval in Canada, signalling a turning point in global acceptance. These obstacles can be removed with several practices, like; Rapid design and deployment of vaccines using transient systems. Decentralized production in developing countries using local crops, cost-effective vaccines for livestock and poultry diseases and drought and heat-tolerant plant hosts for vaccine production.

FUTURE PROSPECTS

Despite the challenges in production and application, the future of plant-based vaccines remains promising. Current research focuses on enhancing antigen yield in transgenic plants to elicit stronger immune responses. One strategy involves codon optimization of bacterial or viral genes to match plant

nuclear gene expression patterns (Rybicki, 2009). Selecting the appropriate subcellular compartment for protein targeting is also critical to achieve optimal yield and stability. Another promising approach is the fusion of antigen genes with immunomodulatory proteins such as mucosal adjuvants, which enhance the immune response. Examples include bacterial enterotoxins like the cholera toxin B subunit (CTB)**,** plant-derived secondary metabolites, and mammalian or bacterial immunomodulators (Yuki & Kiyono, 2003). This technique has shown efficacy in preventing diarrheal diseases. For instance, fusing CTB with enterotoxin proteins from rotavirus and *E. coli* produced a tricomponent subunit vaccine in transgenic potato that conferred protection against all three pathogens. Moreover, ongoing studies aim to address dose variability in edible plant vaccines. Rigano et al. (2003) demonstrated that food-processing methods such as batch-processing and freeze-drying can preserve the native antigenic structure and immunogenicity of materials derived from transgenic plants like tomato, potato, and *Arabidopsis*.

CONCLUSION

Plant-based vaccines are emerging as a promising alternative to conventional immunization strategies, offering low-cost, scalable, and safe production platforms. Depending on the gene delivery method, both stable and transient gene expression can be achieved. Among these, chloroplast transformation via **biolistic (particle bombardment) methods** has shown high potential for efficient antigen production. Continued refinement of gene delivery techniques remains essential for optimizing vaccine yield and efficacy. Despite their advantages, plant-based vaccines raise certain bioethical concerns**.** These include the potential transfer of allergens from transgenic plants to humans or animals, and the theoretical risk of reactivation of bacterial or viral vectors used in vaccine constructs. However, with stringent biosafety protocols and regulatory oversight, these risks can be minimized. Overall, the benefits of plant-based vaccines including rapid scalability, lower production costs, and suitability for global disease control are expected to outweigh the challenges. As scientific, regulatory, and public awareness advances, these vaccines hold strong promise in contributing to universal healthcare and pandemic preparedness. Realizing this potential will require coordinated efforts in research, policy-making, and public education**.**

REFERENCES

Arakawa, T., Chong, D. K. X., and Langridge, W. H. R. (1997). Expression of cholera toxin B subunit in transgenic potato plants. *FEBS Letters*, *411*(2-3), 185–190. https://doi.org/10.1016/S0014-5793(97) 00709-3

Boothe, J., Nykiforuk, C., Shen, Y., Zaplachinski, S., Szarka, S., Kuhlman, P., ... and Moloney, M. M. (2010). Seed-based expression systems for plant molecular farming. *Plant Biotechnology Journal*, *8*(5), 588–606. https://doi.org/10.1111/j.1467-7652.2009.00499.x

Capell, T., Twyman, R. M., Armario-Najera, V., Ma, J. K. C., Schillberg, S., and Christou, P. (2020). Potential applications of plant biotechnology against SARS-CoV-2. *Trends in Plant Science, 25*(7), 635–643. https://doi.org/10.1016/j.tplants.2020.04.009

D'Aoust, M. A., Lavoie, P. O., Couture, M. M., Trépanier, S., Guay, J. M., Dargis, M., ... and Landry, N. (2008). Influenza virus-like particles produced by transient expression in *Nicotiana benthamiana* induce a protective immune response against a lethal viral challenge in mice. *Plant Biotechnology Journal, 6*(9), 930–940. https://doi.org/10.1111/j.1467-7652.2008.00391.x

Daniell, H., Lee, S. B., Panchal, T., and Wiebe, P. O. (2001). Expression of the native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts. *Journal of Molecular Biology, 311*(5), 1001–1009. https://doi.org/10.1006/jmbi.2001.4916

Gelvin, S. B. (2003). *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying" tool. *Microbiology and Molecular Biology Reviews, 67*(1), 16–37. https://doi.org/10.1128/MMBR.67.1.16-37.2003

Gleba, Y., Klimyuk, V., and Marillonnet, S. (2007). Viral vectors for the expression of proteins in plants. *Current Opinion in Biotechnology, 18*(2), 134–141. https://doi.org/10.1016/j.copbio.2007.02.004

Kapila, J., De Rycke, R., Van Montagu, M., and Angenon, G. (1997). An *Agrobacterium*-mediated transient gene expression system for intact leaves. *Plant Science, 122*(1), 101–108. https://doi.org/10.1016/S0168-9452(96)04541-4

Lakshmi, P. S., Verma, D., Yang, X., Lloyd, B., Daniell, H. (2013). Low cost tuberculosis vaccine antigens in capsules: Expression in chloroplasts, bio-encapsulation, stability and functional evaluation in vitro. *PLOS ONE, 8*(1), e54708. https://doi.org/10.1371/journal.pone.0054708

Lico, C., Santi, L., Twyman, R. M., Pezzotti, M., and Avesani, L. (2008). The use of plants for the production of therapeutic human peptides. *Plant Cell Reports, 27*(6), 1031–1042. https://doi.org/10.1007/s00299-008-0530-0

Loza-Rubio, E., Rojas, E., Gómez, L., Olivera, M. T., Gómez Lim, M., and Tapia, C. (2008). Development of a *capsid* recombinant vaccine against rabies using *Zea mays* as expression system. *Vaccine, 26*(37), 5070–5074. https://doi.org/10.1016/j.vaccine.2008.07.010

Mason, H. S., Lam, D. M. K., and Arntzen, C. J. (1992). Expression of hepatitis B surface antigen in transgenic plants. *Proceedings of the National Academy of Sciences, 89*(24), 11745–11749. https://doi.org/10.1073/pnas.89.24.11745

Nachbagauer, R., and Palese, P. (2020). Is a universal influenza virus vaccine possible? *Annual Review of Medicine, 71*, 315–327. https://doi.org/10.1146/annurev-med-041818-011541

Ramachandran, S., and Walden, R. (2008). Gene transfer and expression in plants. In Kole C., Michler C., Abbott A., Hall T. (Eds.), *Transgenic Crop Plants* (pp. 35–66). Springer. https://doi.org/10.1007/978-3-540-68959-8_2

Rigano, M. M., Alvarez, M. L., Pinkhasov, J., Jin, Y., Sala, F., Arntzen, C. J., and Walmsley, A. M. (2003). Production of a fusion protein consisting of a tuberculosis antigen and a mucosal adjuvant in edible plant tissues for use as a primary oral TB vaccine. *Vaccine*, *22*(5–6), 689–697. https://doi.org/10.1016/S0264-410X(03)00658-X

Rosenberg, Y., Sack, M., Montefiori, D., Forthal, D., Mao, L., Hernandez-Abanto, S., ... and Schillberg, S. (2013). Pharmacokinetics and immunogenicity of broadly neutralizing HIV monoclonal antibodies produced in transgenic tobacco plants. *The Plant Biotechnology Journal, 11*(7), 819–829. https://doi.org/10.1111/pbi.12074

Rybicki, E. P. (2009). Plant-produced vaccines: promise and reality. *Drug Discovery Today, 14*(1–2), 16–24. https://doi.org/10.1016/j.drudis.2008.10.002

Sala, F., Rigano, M. M., Barbante, A., Basso, B., Walmsley, A. M., Castiglione, S., and Benvenuto, E. (2003). Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. *Vaccine, 21*(7–8), 803–808. https://doi.org/10.1016/S0264-410X(02)00649-7

Shanmugaraj, B., Malla, A., and Ramalingam, S. (2020). Plant-based edibles for the prevention and management of COVID-19. *Frontiers in Plant Science, 11*, 598283. https://doi.org/10.3389/fpls.2020.598283

Shoji, Y., Farrance, C. E., Bautista, J., Bi, H., Musiychuk, K., Horsey, A., ... and Yusibov, V. (2012). A plant-based system for rapid production of influenza vaccine antigens. *Influenza and Other Respiratory Viruses, 6*(3), 204–210. https://doi.org/10.1111/j.1750-2659.2011.00285.x

Streatfield, S. J. (2005). Plant-based vaccines for animal health. *Revue Scientifique et Technique-Office International des Epizooties, 24*(1), 189–199. https://doi.org/10.20506/rst.24.1.1554

Tacket, C. O., Mason, H. S., Losonsky, G., Clements, J. D., Levine, M. M., and Arntzen, C. J. (2000). Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. *Nature Medicine, 6*(5), 555–559. https://doi.org/10.1038/75093

Thanavala, Y., Mahoney, M., Pal, S., Scott, A., Richards, A., and Arntzen, C. J. (2005). Immunogenicity in humans of an edible vaccine for hepatitis B. *Proceedings of the National Academy of Sciences, 102*(9), 3378–3382. https://doi.org/10.1073/pnas.0409899102

Tzfira, T., and Citovsky, V. (2006). Agrobacterium-mediated genetic transformation of plants: biology and biotechnology. *Current Opinion in Biotechnology, 17*(2), 147–154. https://doi.org/10.1016/j.copbio.2006.01.009

Verma, D., and Daniell, H. (2007). Chloroplast vector systems for biotechnology applications. *Plant Physiology, 145*(4), 1129–1143. https://doi.org/10.1104/pp.107.106989

Verma, D., Kanagaraj, A., Jin, S., Singh, N. D., Kolotilin, I., and Daniell, H. (2008). Chloroplast-derived vaccine antigens and biopharmaceuticals: protocols for expression, purification, and functional evaluation. *Methods in Molecular Biology, 483*, 163–192. https://doi.org/10.1007/978-1-59745-407-0_11

Ward, B. J., Makarkov, A., Séguin, A., Pillet, S., Trepanier, S., Dhaliwall, J., ... and Landry, N. (2020). Efficacy, immunogenicity, and safety of a plant-derived, quadrivalent, virus-like particle influenza vaccine in adults: a randomized, double-blind, placebo-controlled trial. *The Lancet, 396*(10261), 1491–1503. https://doi.org/10.1016/S0140-6736(20)32014-6

Watson, J., Koya, V., Leppla, S. H., and Daniell, H. (2004). Expression of Bacillus anthracis protective antigen in transgenic chloroplasts of tobacco, a non-food/feed crop. *Vaccine, 22*(31–32), 4374–4384. https://doi.org/10.1016/j.vaccine.2004.04.043

Wroblewski, T., Tomczak, A., and Michelmore, R. (2005). Optimization of *Agrobacterium*-mediated transient assays of gene expression in lettuce, tomato and Arabidopsis. *Plant Biotechnology Journal, 3*(2), 259–273. https://doi.org/10.1111/j.1467-7652.2005.00123.x

Yang, Y., Li, R., and Shen, J. (2000). Transient expression of foreign genes in *Nicotiana* plants using *Agrobacterium* infiltration. *Plant Molecular Biology Reporter, 18*(3), 207–218. https://doi.org/10.1007/BF02825033

Yuki, Y., and Kiyono, H. (2003). New generation of mucosal adjuvants for the induction of protective immunity. *Reviews in Medical Virology, 13*(5), 293–310. https://doi.org/10.1002/rmv.397

Zheng, Z., Qian, Y., and Lin, W. (2007). Expression of FMDV VP1 protein in stylosanthes using PEG-mediated transformation. *Transgenic Research, 16*(6), 751–762. https://doi.org/10.1007/s11248-007-9080-8

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