Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

ARE TRANSGENIC PLANT VACCINES, NEW IMMUNE BOOSTERS OF THE MILLENNIUM?

LUCY MOHAPATRA
Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, India
SAMBIT KUMAR PARIDA
Institute of Pharmaceutical Science and Research, Unnao, Uttar Pradesh, India
SASHIKANT SINGH
Varanasi College of Pharmacy, Varanasi, Uttar Pradesh, India
N T PRAMATHESH MISHRA
Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, India
*Corresponding authors: lmohapatra@lko.amity.edu; dr.lucymohapatra@gmail.com

ABSTRACT

Transgenic plant vaccines or simply edible vaccines consist of genetically modified products obtained by incorporating the genes of interest into some plants encoding specific antigen causing the disease. Compared to previously existing traditional vaccines the edible vaccines offer several advantages as safe, cost-effective, easily administrable, storable etc. Oral administration of these vaccines stimulates both mucosal (IgA mediated) and systemic (IgG mediated) immunity and therefore provides an improved mean to fight against infectious diseases. In addition to that the plants aid in preserving the antigenic possessions of such vaccines even after subsequent ruin in intestine. Some plants that have been used as edible vaccines recently in some part of the world include corn, bananas, potatoes, tomatoes etc. Despite of such advantages over the traditional vaccines, there exist tremendous challenges in the successful production and broad implementation of these third-generation vaccines. However, it has been considered that, there are numerous risks throughout the manufacture and distribution phases of this acquaintance, with possible impression on the atmosphere and on human health. Incessant efforts are continuing in several parts of the world to fabricate effectual plant vaccines for many anthropological and animal associated diseases. This paper analyses the current conservative approaches as well as the progress exertions by scientists to recover the creation of plant-based vaccines. This article further highlights on the jeopardies allied with the invention and manufacturing of these vaccines and how they can be controlled through suitable governing actions at all phases of manufacturing and delivery.

Key words Transgenic, Vaccines, Immunity, Antigenic, Immunoglobulins (Ig)

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

INTRODUCTION

Infectious diseases have become one of the greatest threats to the humanity in the present scenario. Every year in some corner of the world, new pathogens are being discovered, responsible for triggering some incurable life-threatening diseases. Vaccines have been used since long to prevent the manifestation of these transmittable ailments. Through the progress of vaccine technology and some global immunization programs, we could be able to eradicate completely some of the most dangerous, disabling and life-threatening infectious diseases such as smallpox from the world [1, 2]. Initially developed bacterial vaccines get transformed into more efficient subunit vaccine in due course nonetheless these products could not be easily accessible especially to the poor communities in the developing countries across the world because of their high production costs. Some statistics report that, 20% of infants remain un-immunized across the globe every year which lead to roughly two million needless demises every year [3]. The reason behind such grief events may be due to severe delineation in the herd immunity arising due to the restrictions on vaccines production, distribution and delivery across the world [4]. Besides being expensive, old-style vaccines usually comprise of deactivated or weakened pathogens and thus are not exclusively safe and sound. For example, type II vaccine-derived polio was first detected in Nigeria during 2006, became endemic in Africa and such cases are still found in many parts of the world today [5]. Hence, there is an urge to quest for some effortlessly administrable, storable, harmless and bio responsive natural vaccines to improve the current vaccination trends [6, 7]. Therefore, it was envisaged that plants could prove to offer a promising platform for an efficient production system for the third-generation vaccines i.e. the edible vaccines8.These vaccines have several rewards over outdated ones, such as low cost, ease of transportation and preservation, higher yield value due to large biomass of cultivation [8]. Apart from this, due the presence of plant cell wall they serve as a sustained-release outcome for the administration of the antigen to ensure maximum antibody titer in the blood [9,10].

Till today numerous plant vaccines have been formed and many of these have been tested clinically for their safety and efficacy at different phases of clinical trials. Most of these vaccines are usually produced in *Nicotiana* plants. To date, scFV mAb utilized in the making of a recombinant HBV vaccine in Cuba and Newcastle disease virus (NDV) vaccine for fowls are the only two licensed plant vaccines permitted by the US department of agriculture (USDA) [11]. As they are categorized beneath the genetically modified crop class, they are not accepted widely by common people [12]. This may be because, of public fallacy as to how these vaccines will be delivered in a real-world sense. However, with additional expansion of the technology, investigators and controllers emphasized that to govern the level of revelation, boundaries on transfer would be required. Hence, the pattern of edible vaccines administration involving ingestion of biologically engineered fruit/vegetables/cereals will be prescribed by a health care operative. This pattern was inescapably enforced to additionally advance to encounter typical rations for pharmaceuticals, to remove dose inconsistency. In such case, the final artefact possibly will not be detectable as a plant material, moderately a

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

packed pill/capsule. This existing pattern specifies that transgenic plant made vaccines are not just any food ingredients and therefore they will have to meet guidelines which are still developing inside national regulatory authorities like United States Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA). Presently in US and many other parts of the world, the manufacture and supply of transgenic plants is being regulated by USDA [13]. This regulatory organization is largely anxious about genetic restraint and plummeting the hazard of genetic transmission.

In sight of this exhilarating yet demanding forte, the first part of this paper discourse on the technologies for the production and up-to-date developments in diverse transgenic plant vaccines while the latter part highlights the challenges encountered in the large-scale utility and acceptability of these vaccines.

How to prepare edible vaccine?

The steps involved in the making of plant vaccines begin with the incorporation of transgene into the plant cells. Generally, transgenes are known as exogenous sequences of DNA present in the genome of any species which may include genes from the same species or novel genes from completely different species transferred naturally through genetically engineering technology [13,14]. Hence, for preparing an edible vaccine the genes encoding specific antigens are obtained from the microbes responsible to cause the disease and then inserted into the desired plant structure of selected plant species. Usually these genes can be handled by the plants in two different ways - **stable transformation** or **transient transformation** systems. Generating stably transformed cells begins the integration of foreign gene into the plant cells genome. The symbol of stable transformed cells is that the extraneous gene become an integral part of the plant genome and finally gets replicated. Hence, the newly formed plant cells will also express the new gene. Stable transformations can be accomplished through nuclear/plastid integration [15]. Biolistic and or genetically modified *Agrobacterium* strain, could help in forming a stable transformation [16]. Whereas, transiently transformed cells prompt the extraneous gene without assimilating it into their genome and hence, the novel gene is never replicated by the plant cells. As a result, these cells express the gene for a finite passé say for few days and then the foreign gene is vanished through cell division or by some other influences. *Agrobacterium*-mediated renovations of genetically adapted plant virus and particle bombardment are the dual utmost utilized approaches that would accomplish transient transformation [15].

Some of the important methods of gene transfer in plant cells are represented in **Fig. 1** and briefly discussed over here:

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

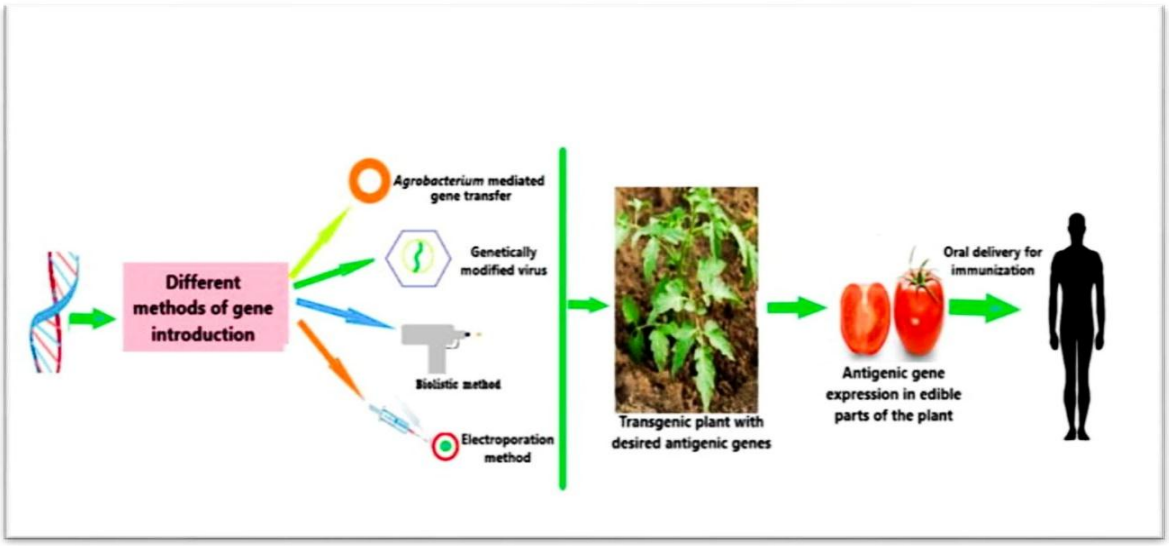

Fig. 1 Methods of gene transfer in plant cells.

1. Biolistic method/Gene gun method: It is also known as micro-projectile bombardment technique. It is a vector-independent method. This method is based on the gene gun that fires metal (gold, tungsten) coated DNA particles of interest at the plant cells and later they can grow in the new plant, which are then cloned to produce the desired product in huge amount [17].

2. Gene transfer via *Agrobacterium* strain: In this method, the desired gene is unified into the plasmid of a suitable species of *Agrobacterium* and then grown along with the desired plant cells that get transformed afterwards. This is a very commonly followed method as *Agrobacterium* is a common plant pathogen which has the ability of infecting several dicotyledonous plant species including tobacco, potato, tomato, cornet [18,19]. The method of *Agrobacterium* mediated gene transfer and edible vaccine production has been represented in **Fig. 2**.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

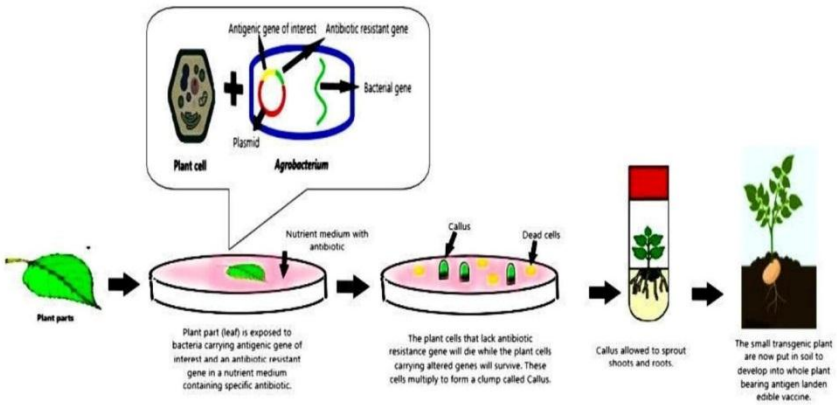

Fig. 2 Method of *Agrobacterium* mediated gene transfer and edible vaccine production

3. Electroporation method: In this method both plant cells and desired genetic materials are mixed and then exposed to a high voltage electrical field. The electricity increases the cellular permeability of the plant cells, because of which the genetic material enters the cell. Sometimes this approach requires mild enzymatic treatment as well [18,19].

How do they work on the body?

Mucosal immunity is measured to be the chief and most important line of defense mechanism of our body. This is because almost all pathogens march into the body by the mucosal surfaces of any of the suitable tracts such as urogenital, respiratory or gastrointestinal route. Some literatures suggest that mucosal immunization is more effective in enhancing the immunity of an individual against diseases than any other routes. Oral route is the most competent alleyway of mucosal immunization as vaccines administered through oral route can fabricate both mucosal immunity and systemic immunity [20]. Once the plant-based vaccines are consumed orally and masticated properly, they reach the intestine and release the antigen after the intestinal enzyme action. Generally, they break near the payer's patches and the antigens present in them meet some specialized cell called M cells. Payer's patches in the intestine are an augmented foundation of IgA immunoglobulin fabricating plasma cells and hence serve as the mucosal immune effector sites. These M cells pass the antigen to the macrophages that further display them to the local lymphocytes (helper T cells) producing memory T cells and simultaneously stimulate B lymphocytes to produce antibodies (IgG, IgE, IgA) to destroy the ingested antigen immediately. However, memory cells produced during this process counterbalance the attack by real infectious pathogen rapidly and effectively thus preventing the infection [21]. The mechanism by virtue of which edible plant vaccines can arouse both mucosal and systemic immunity has been depicted in **Fig.**

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

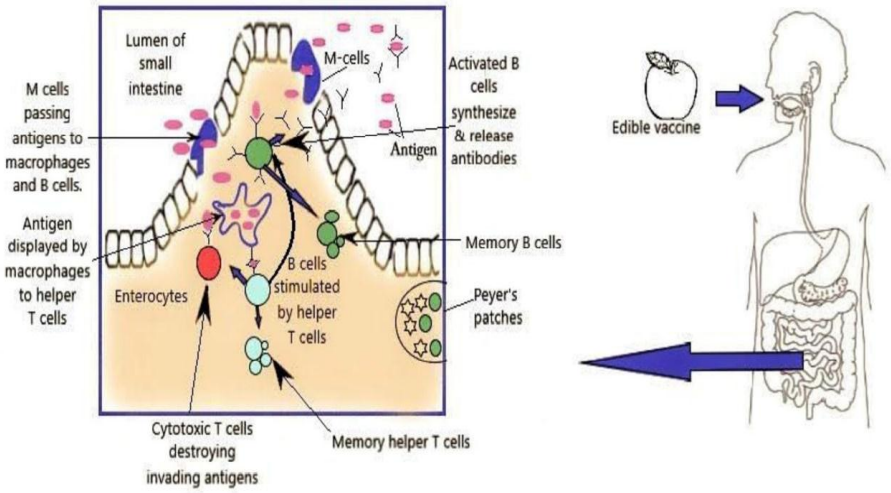

Fig. 3 Mechanism of action of edible plant vaccines stimulating both mucosal and systemic immunity.

3. Reasons why we can use plant vaccines?

As stated earlier in this article, plant vaccines offer several advantages over the conventional vaccines and thus can prove their wider utility. Some these advantages are being discussed here below:

1. These vaccines come up with better safety profile as they do not contain any live attenuated or killed microbes as that of the conventional vaccines. Hence, they do not present the risk of virulence regain by these microbes that may be even being more dangerous [22].
2. Moreover, they are cost effective as equated to the conventional subunit vaccines since of their less making cost. Traditional vaccines do require several sophisticated equipment (fermenters or bioreactors) and techniques for their production and development. But plants can be grown in the fields or sometimes may be in a greenhouse. If the vaccine is formed in either of the fit for human consumption parts of the plant (grain, fruit, leaves etc.,) then they might not even necessitate purification [22].
3. Another advantage of edible vaccines is the simple and easy mean of their administration (oral administration). Thus, they abolish the necessity of skilled medical recruits every time for immunization. Moreover, the risk of contamination is also abridged as they do not need specific sites and manufacturing area to be sterilized [23].
4. Edible vaccines can provide an efficient mode of action for immunization as unlike traditional vaccines as they bring forth both mucosal and systemic immunity. In addition

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

to that, they do not require adjuvants as that of many conventional vaccines to stimulate immune response [24].

5. These vaccines also propose better stowage prospects as seeds of transgenic plants can be easily dried and stored for years [25].

6. Apart from that the, availability of such vaccines can also be enhanced as their production processes can be scaled up rapidly by breeding [26].

Plants transformed for edible vaccines and recent advances

Several plant species have been studied and used to produce desired gene products in many parts of the world till today. The plants are selected in regard of ease of transformation, extensive genomic sequence knowledge, ease of cultivation and no doubt high volume yield. Based on these foresaid factors the plant that has been extensively studied and utilized for such purpose is the Tobacco plant [26]. The very idea that, plants can be used to prompt vaccine antigen was first demonstrated by Curtiss and Cardineau in 1990, when they made tobacco plant to successfully express the *Streptococcus mutans* surface protein antigen A (SpaA) [27,28]. This revelation was confidentially shadowed by the countenance of hepatitis B surface antigen (HbsAg), the *E. coli* heat–labile enterotoxin responsible for diarrhoea, rabies virus glycoprotein and the Norwalk virus capsid protein in the same plant thereafter in several scientific studies [28]. Other plants that have been used for such purpose include alfalfa, potato, maize, arabidopsis, tomato, carrot, lettuce, cowpea, spinach and even unicellular algae such as *Chlamydomonas* [29]. Fruits (apple, banana, grapes, melon, kiwi) and vegetables (cauliflower, cucumber, soybean, squash sugar beet, sweet potato) have been also used for this purpose [30]. Despite of all such scientific efforts and outcomes only a few plants based human vaccines have reached clinical trials so far. A subunit vaccine of enterotoxigenic *E. coli* (ETEC) produced either in potato or maize has made its entry to the 1st phase of clinical trial [31, 32]. Norovirus capsid protein VP1 was also formed in potato tubers in the equivalent technique and almost 20% of vaccinated volunteers developed specific serum IgG titers in the phase 1 clinical trials [33, 34]. Similarly plant based vaccines developed against Hepatitis B virus (in lettuce, potato), Rabies virus (in spinach), H5N1, H1N1, H7N9 and Influenza virus (in *Nicotiana benthamiana*) and Cholera virus (in rice) have made their entry to the phase 1 clinical trials [35]. However, some studies revealed that both phases I and II clinical trial of the VLP (virus like particles) composed of HA (hemagglutinin) protein of H5N1 influenza virus (A/Indonesia/5/05) (H5-VLP) have been accomplished. It was found that the plant-based vaccine was able to induce HA inhibiting titer at all weathered doses. In addition to that, after 6 months of vaccination with such vaccine in a phase-II clinical trial the volunteer group showed cross-protective $CD4^+$ T-cell responses representing sturdy orientation of enduring cell-mediated immunity by plant-made H5-VLP vaccines [35, 36].

Not only this but also antigens of pathogens affecting animals have been also expressed in plants and many of these products have been evaluated in the respective animal species. The first USDA-approved plant-made vaccine was the “Newcastle disease vaccine for poultry” i.e. meant for veterinary use [35]. Similarly, S1 glycoprotein

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

gene responsible to cause chicken bronchitis has been introduced into potato [37]. Plant-based vaccines for protecting pigs from enterotoxigenic E. coli (ETEC) and foot and mouth disease virus (FMDV) have been well characterized and were produced in alfalfa [38]. Rabies virus causes a zoonotic disease which means that the disease is basically transmitted from animals to human beings. It is usually found that this very virus is first transmitted from wild animals such as bats, raccoons, and foxes to pet animals and then to humans. Hence, to prevent the prognosis of this dreadful disease in human vaccination of pet animals is mandatory. As a reliable solution to this problem, now rabies G protein has been expressed in several plant species, including tobacco, tomato, spinach, carrot, maize and oral immunization has tremendously reduced the mortality rate from virus challenge of several animal species [39]. No doubt, the cost of immunization for the regular farm animals tends to fringe profit for the farmers from selling products such as meat, milk, eggs etc. Therefore, plant-based vaccines are going to prove themselves as appositive feature for the animal use. This is because; they can be manufactured at low cost and effortlessly administered to the animals.

Plausible hazards of transgenic plant vaccines: why these vaccines are not used widely?

Now the query is that despite of such intensive research and excellent outcomes based on edible vaccines, what are the facts that restrict their extensive use across the globe? The common challenges faced by plant-based-vaccines development include technical aspects, regulatory aspects, economic aspects and more importantly public perceptions on the use of genetically modified organisms – GMOs [40]. While no plant based vaccine have proceeded past introductory clinical trials and in addition to that no complete explanation of the manufacturing and monitoring strategies of such vaccines have been found so far, investigators and researchers in this arena still continue largely to be hopeful that they will develop to the benefit of society. With the advancement of this genetic technology, cumulative emphasis will be hired on the monitoring agenda that gearshifts and supports these resources. However, National regulatory authorities must ponder the hazards and recompences of producing these transgenic vaccines especially in a food-grade structure for application as a pharmaceutical substance. Although food stuffs like eggs and yeast have been already used to harvest vaccines but, in this case, the extremely measured manufacturing condition is considerably different to the plant-based vaccines as they are going to include the whole agricultural sector. The plant-based vaccines which are currently under development will employ natural manufacturing and harvesting measures, which are identical to those used in the regular agricultural segment for food and feed making. The major risks that have been identified as possible apprehensions for the exclusive features and manufacturing approaches for transgenic plant vaccines are:

1. Even if from specific tactical point of view, edible vaccines area lower-cost option but in real this statement is not firmly true basically after the production and development of these vaccines. In addition to that, purification, control and biosafety are the various particulars that are mandatory to be concerned of by the pharmaceutical

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

companies, which hold extra costs and generate obstacle in the production and manufacture of such vaccines by small and medium size pharmaceutical companies [41,42].

2. Another important restraint of edible vaccines is the indistinctness connected to the calculation of precise proficient oral dose to be administered to any individual. Moreover, the uniformity of dosage form fruit to fruit, plant to plant and generation-to-generation is also doubtful [43].
3. Over consumption of these plants bearing antigens may easily lead to the development of immune tolerance to such vaccines. Apart from that, certain foods like potato, spinach, rice which cannot be eaten raw; cooking may weaken or sometimes destroy the antigenic property [44,45].
4. Another significant point is that, although the use of an edible vaccine is based on the ingestion of a section of a plant, this practice shows remarkable difficulties in standardizing the antigen concentrations in different plant tissues [44, 45].
5. Among regulatory challenges, the most important obstacle lies within to gain regulatory approval from the USDA, FDA and/or EPA apply to plant-based vaccines [46].
6. In addition, issues related to separating of a pharmaceutical product from the original food crop embattled for the food chain have turn out to be gradually more vital as concerns over adventitious existence of medicinal products in the food supply have surfaced in recent years [46, 47]. Hence, if the manufacturing costs stay elevated and an appropriate inference of essential antigen concentration relics unresolved, the prospect of edible vaccines will remain uncertain [48-50].

7. Negative environmental impacts: Usual damage and deficiency of cellular components including DNA and proteins, within the environmental structure, or assimilation by non-target species may have unidentified poisonousness consequences. There is every possibility of the immigration of the antigen to the conventional nutrition chain through genomic crossbreeding or by any means of contamination and this could lead to the development of tolerance.

8. Worker exposure: Poignant or gasping of plant vaccine ingredients throughout manufacture may lead to oral tolerance and allergenicity.

Conclusion

This review article concludes that plant vaccines are one of the budding types of vaccines having considerable advantages over traditional vaccines. Yet, there is still a long way to go to mechanize these vaccines across the globe. Roughly, suitable gene delivery methods need to be developed and improved for efficient and optimum vaccine production. More importantly, the bioethical issues need to be solved. The customs to endorse the speedy expansion of transgenic plant vaccines include intensification of high-tech innovations and creating public awareness about the compensations of transgenic plants to eradicate the distress. The approval and commercialization of

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

transgenic plants vaccine against Newcastle disease in 2006 by the United States Department of Agriculture (USDA) is a successful landmark example in this regard. It is expected that this instance will positively play a major role in encouraging the growth and commercialization of supplementary transgenic plant vaccines. With the founding and optimization of standardized GM machineries, it is projected that regulatory endorsement will be settled eventually to transgenic plant vaccines to support in the worldwide global disease control.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Aher, V. D., Banerjee, S., & Mahaur, K. K. (2011). Vaccine: An ultimate way of immunization. *Research J Pharm Tech*, *4*(3), 369-74.
2. Visweswaran, V., Binoy, A., Sreenivas, A., Abhinand, B., & Vijayan, M. (2017). Vaccines-pillars of preventive health. *Research Journal of Pharmacy and Technology*, *10*(9), 3205-3210.
3. Jan, N., Shafi, F., Hameed, O., Muzaffar, K., Dar, S., & Majid, I. (2016). An overview on edible vaccines and immunization. *Austin J Nutri Food Sci*, *4*(2), 1078.
4. Ramsay, A. J., Kent, S. J., Strugnell, R. A., Suhrbier, A., Thomson, S. A., Ramshaw, I. A., ... & Ramshaw, I. A. (1999). Genetic vaccination strategies for enhanced cellular, humoral and mucosal immunity. *Immunological reviews*, *171*(1), 27-44.
5. Famulare, M., & Hu, H. (2015). Extracting transmission networks from phylogeographic data for epidemic and endemic diseases: Ebola virus in Sierra Leone, 2009 H1N1 pandemic influenza and polio in Nigeria. *International health*, *7*(2), 130-138.
6. Maheswari, K. (2015). Knowledge of mothers regarding newer vaccines and vaccines preventable diseases. *International Journal of Advances in Nursing Management*, *3*(2), 107-108.
7. Patil SM, Maske AP, Sapkale GN, Kure AB. Unique Approaches to Vaccine Development Formulation and Delivery. Res J Pharmacol Pharmacodyn 2010; 2: 99-102
8. Alam N, Agrawal OP, Rimpi, Alam P, Agrawal S, Kaushik, M Dhari JS, Sharma OP. Natural Immunoenhancers. Res J Pharm Tech 2011; 4: 1526-1532
9. Faith, V. H., Debnath, S., Lavanya, D., Jerusha, V., & Madhuri, M. V. R. (2015). Food as a Vaccine. *Research Journal of Pharmaceutical Dosage Forms and Technology*, *7*(2), 161.
10. Holaskova, E., Galuszka, P., Frebort, I., & Oz, M. T. (2015). Antimicrobial peptide production and plant-based expression systems for medical and agricultural biotechnology. *Biotechnology advances*, *33*(6), 1005-1023.
11. Naderi, S., & Fakheri, B. (2015). Overview of plant-based vaccines. *Res J Fish Hydrobiol*, *10*(10), 275-89.
12. Korban, S. S. (2005). Opportunities and challenges for plant-based vaccines. NABC.
13. Jaffe, G. (2004). Regulating transgenic crops: a comparative analysis of different regulatory processes. *Transgenic Research*, *13*(1), 5-19.
14. Gadhave, D. U., Gaikwad, P. S., Pimpodkar, N. V., & Udugade, S. B. (2015). A hope full ray in Immunology. *Asian Journal of Research in Pharmaceutical Science*, *5*(2).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

15. Altpeter, F., Baisakh, N., Beachy, R., Bock, R., Capell, T., Christou, P., ... & Visser, R. (2005). Particle bombardment and the genetic enhancement of crops: myths and realities. *Molecular Breeding*, *15*(3), 305-327.
16. Ma, H., & Chen, G. (2005). Gene transfer technique. *Nature and Science*, *3*(1), 25-31.
17. Taylor, N. J., & Fauquet, C. M. (2002). Microparticle bombardment as a tool in plant science and agricultural biotechnology. *DNA and cell biology*, *21*(12), 963-977.
18. Mercenier, A., Wiedermann, U., & Breiteneder, H. (2001). Edible genetically modified microorganisms and plants for improved health. *Current Opinion in Biotechnology*, *12*(5), 510-515.
19. Chikwamba, R., Cunnick, J., Hathaway, D., McMurray, J., Mason, H., & Wang, K. (2002). A functional antigen in a practical crop: LT-B producing maize protects mice against Escherichia coli heat labile enterotoxin (LT) and cholera toxin (CT). *Transgenic research*, *11*(5), 479-493.
20. Arakawa, T., Yu, J., Chong, D. K., Hough, J., Engen, P. C., & Langridge, W. H. (1998). A plant-based cholera toxin B subunit–insulin fusion protein protects against the development of autoimmune diabetes. *Nature biotechnology*, *16*(10), 934-938.
21. Jan, N., Shafi, F., Hameed, O., Muzaffar, K., Dar, S., & Majid, I. (2016). An overview on edible vaccines and immunization. *Austin J Nutri Food Sci*, *4*(2), 1078.
22. Schillberg, S., Twyman, R. M., & Fischer, R. (2005). Opportunities for recombinant antigen and antibody expression in transgenic plants—technology assessment. *Vaccine*, *23*(15), 1764-1769.
23. Mishra, N., Gupta, P. N., Khatri, K., Goyal, A. K., & Vyas, S. P. (2008). Edible vaccines: A new approach to oral immunization.
24. Streatfield, S. J. (2006). Mucosal immunization using recombinant plant-based oral vaccines. *Methods*, *38*(2), 150-157.
25. Pascual, D. W. (2007). Vaccines are for dinner. *Proceedings of the National Academy of Sciences*, *104*(26), 10757-10758.
26. Sala, F., Rigano, M. M., Barbante, A., Basso, B., Walmsley, A. M., & Castiglione, S. (2003). Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. *Vaccine*, *21*(7-8), 803-808.
27. Curtiss, R., & Cardineau, G. A. (1990). World intellectual property organization. *PCT/us*, *89*, 03799.
28. Russell, M. W., Harrington, D. J., & Russell, R. R. (1995). Identity of Streptococcus mutans surface protein antigen III and wall-associated protein antigen A. *Infection and immunity*, *63*(2), 733-735.
29. Aliahmadi, A., Rahmani, N., & Abdollahi, M. (2006). Plant derived human vaccines; an overview. *Int J Pharmacol*, *2*(3), 268-279.
30. Richter, L., & Kipp, P. B. (2000). Transgenic plants as edible vaccines. *Plant Biotechnology*, 159-176.
31. Tacket, C. O., Mason, H. S., Losonsky, G., Clements, J. D., Levine, M. M., & Arntzen, C. J. (1998). Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. *Nature medicine*, *4*(5), 607-609.
32. Tacket, C. O. (2007). Plant-based vaccines against diarrheal diseases. *Transactions of the American Clinical and Climatological Association*, *118*, 79.
33. Teunis, P. F., Moe, C. L., Liu, P., E. Miller, S., Lindesmith, L., Baric, R. S., ... & Calderon, R. L. (2008). Norwalk virus: how infectious is it?. *Journal of medical virology*, *80*(8), 1468-1476.
34. Campos, C. J., & Lees, D. N. (2014). Environmental transmission of human noroviruses in shellfish waters. *Applied and environmental microbiology*, *80*(12), 3552-3561.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:54 Issue:07:2021
DOI 10.17605/OSF.IO/BNJSU

35. Takeyama, N., Kiyono, H., & Yuki, Y. (2015). Plant-based vaccines for animals and humans: recent advances in technology and clinical trials. *Therapeutic advances in vaccines*, *3*(5-6), 139-154.
36. Landry, N., Pillet, S., Favre, D., Poulin, J. F., Trépanier, S., Yassine-Diab, B., & Ward, B. J. (2014). Influenza virus-like particle vaccines made in Nicotiana benthamiana elicit durable, poly-functional and cross-reactive T cell responses to influenza HA antigens. *Clinical Immunology*, *154*(2), 164-177.
37. Zhou, J. Y., Cheng, L. Q., Zheng, X. J., Wu, J. X., Shang, S. B., Wang, J. Y., & Chen, J. G. (2004). Generation of the transgenic potato expressing full-length spike protein of infectious bronchitis virus. *Journal of biotechnology*, *111*(2), 121-130.
38. Joensuu, J. J., Verdonck, F., Ehrström, A., Peltola, M., Siljander-Rasi, H., Nuutila, A. M., ... & Niklander-Teeri, V. (2006). F4 (K88) fimbrial adhesin FaeG expressed in alfalfa reduces F4+ enterotoxigenic Escherichia coli excretion in weaned piglets. *Vaccine*, *24*(13), 2387-2394.
39. Loza-Rubio, E., Rojas-Anaya, E., López, J., Olivera-Flores, M. T., Gómez-Lim, M., & Tapia-Pérez, G. (2012). Induction of a protective immune response to rabies virus in sheep after oral immunization with transgenic maize, expressing the rabies virus glycoprotein. *Vaccine*, *30*(37), 5551-5556.
40. Divyadarshini V. Genetically modified foods – A review. Res. J. Pharm. Tech., 2014; 7, 292-295
41. Shah, C. P., Trivedi, M. N., Vachhani, U. D., & Joshi, V. J. (1990). Edible vaccine: A better way for immunization. *pharmaceuticals*, *4*, 5.
42. Juarez, P., Virdi, V., Depicker, A., & Orzaez, D. (2016). Biomanufacturing of protective antibodies and other therapeutics in edible plant tissues for oral applications. *Plant biotechnology journal*, *14*(9), 1791-1799.
43. Chaitanya, V., & Kumar, J. (2006). Edible vaccines. *Sri Ramachandra J. Med*, *1*, 33-34.
44. Kilany, W. H., Arafa, A., Erfan, A. M., Ahmed, M. S., Nawar, A. A., Selim, A. A., ... & Abdelwhab, E. M. (2010). Isolation of highly pathogenic avian influenza H5N1 from table eggs after vaccinal break in commercial layer flock. *Avian diseases*, *54*(3), 1115-1119.
45. Howard, J. A. (2005). Commercialization of biopharmaceutical and bioindustrial proteins from plants. *Crop Science*, *45*(2), 468-472.
46. Korban, S. S. (2002). Targeting and expression of antigenic proteins in transgenic plants for production of edible oral vaccines. *In Vitro Cellular & Developmental Biology-Plant*, *38*(3), 231-236.
47. Streatfield, S. J., & Howard, J. A. (2003). Plant production systems for vaccines. *Expert review of Vaccines*, *2*(6), 763-775.
48. Buetow, D. E., & Korban, S. S. (2000). Transgenic plants producing viral and bacterial antigens. *AgBiotechNet*, *2*(ABN 045), 1-6.
49. Takeyama, N., Kiyono, H., & Yuki, Y. (2015). Plant-based vaccines for animals and humans: recent advances in technology and clinical trials. *Therapeutic advances in vaccines*, *3*(5-6), 139-154.
50. Twyman, R. M., Schillberg, S., & Fischer, R. (2005). Transgenic plants in the biopharmaceutical market. *Expert opinion on emerging drugs*, *10*(1), 185-218.