Academic Journal of Nutrition 4 (1): 40-49, 2015
ISSN 2309-8902

DOI: 10.5829/idosi.ajn.2015.4.1.956

Review on Edible Vaccine

[1]Hafiz Esmael and [2]Eyob Hirpa

[1]Wollega University, College of Healthy and Medical Science,
School of Veterinary Medicine, Nekemte, Ethiopia
[2]Wollega University, School of Veterinary Medicine,
Veterinary Microbiology, Nekemte, Ethiopia

Abstract: As prevention is better or cheaper than cure scientist develop vaccine as a means of prevention, which is prepared from etiologic agent, given to organisms to prevent future encountered diseases. A variety of delivery systems have been developed. Vaccinating animals/humans with edible plants is a new emerging idea that appears to hold great promise. Introduced as a concept about a decade ago, it has become a reality today. It involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. Edible vaccines hold great vow as a cost-effective, easy-to-administer, easy-to-store, fail-safe and sociocultural readily acceptable vaccine delivery system, especially for the poor developing countries. Therefore; it play great role, especially in Third World countries where transportation costs, poor refrigeration and needle use complicate vaccine administration. Initially thought to be useful only for preventing infectious diseases. It has also found application in prevention of autoimmune diseases, cancer therapy, etc.

Key words: Edible Vaccine · Immunity · Prevention

INTRODUCTION

All organisms are prone to one or more kinds of infectious and non infectious diseases throughout their life. To prevent these infection human beings discovered vaccine which is an immune-biological substance, used for specific protection against both infectious and non-infectious diseases [1]. It involves deliberate exposure to antigen under conditions where disease should not result and inducing active immunity in an individual, so that subsequent contact with the microorganism following natural infection induces strong protective immune response [2].

The main limitation with vaccines is their dependence on cold chain system, which is used to store and transport the vaccine under strict controlled conditions [3]. Other limitations are risk of adverse reactions such as reactions inherent to inoculation, reactions due to faulty techniques etc [4]. Thus, for the implementation of a successful global vaccination strategy, a well designed subunit oral vaccine system should satisfy the following criteria; Produce sufficient quantities of desired antigen, preserve the expressed antigen for a long time at room temperature; induce protective immunity; and be protected from enzymatic digestion in the gastrointestinal tract [5].

Therefore, there is an urgent need to search for vaccines which are easy to administer, easy to store, cost effective, easy to transport and possess readily acceptable delivery system. Hence, there is a lot of scope in developing plant derived vaccine [6]. As Hippocrates said, "Let thy food be thy medicine," scientists suggest that plants and plant viruses can be genetically engineered to produce vaccines against diseases [7]. Accordingly some study shows Plant derived vaccines significantly increase availability of vaccines in places where maintenance of cold chain system is difficult [8].

Advances in transgenic research have made use of crop plants to serve as bioreactor for the production of recombinant molecules. This means that transgenic plants are used to express antigen proteins induced by plant transgenic vectors and to produce certain special vaccines with high anti-disease ability [9].

Corresponding Author: Eyob Hirpa, Wollega University, College of Healthy and Medical Science, School of Veterinary Medicine, Veterinary Microbiology, Nekemte, Ethiopia.

During the last decade, different types of efficient plant based expression systems have been studied and more than 100 different types of recombinant proteins including plant-derived vaccine antigens have been successfully expressed in different types of plant tissues [10]. Positive effects of edible vaccines include decrease in potential hazards such as toxic compounds, responses to allergy and risk of attenuated strains reverting to pathogenic strains associated with established, production technologies that use bacteria, yeast and mammalian cells [11].Through recombinant DNA technology, different level of antigen expression for each independent line has been observed in plants [12]. Although edible vaccine has many functions for either individual animals or humans by providing long lasting immunity without risk of relapse reaction and faulty techniques, there exists lack of information's regarding to their production and action mechanisms. Therefore, the aim of this seminar paper is to review on edible vaccine production and application.

Objective: Review on Edible Vaccine.

In Review on Materials and Methods: This review work was carried out by undertaking a systematic review of the published research papers available online. Accordingly, about 80 published scientific research papers conducted on edible vaccine production and application reviewed and their extracted major findings were presented. The conclusion and recommendation of the reviewed papers and point out way forwards.

Edible Vaccine: The concept of edible vaccines was developed by Arntzen in the 1990s. The earliest demonstration of an edible vaccine was the expression of a surface antigen from the bacterium *Streptococcus mutans* in tobacco [13]. Edible Vaccines are prepared by introducing selected desired genes into plants and inducing these genetically modified plants (GMP) to manufacture the encoded proteins. This process is known as "Transformation" and the altered plants are called "Transgenic plants" [14]. The crop food product contains the proteins which are derived from some disease causing pathogen. Animals or People eat the plants, the food is digested and some of the protein makes its way into the bloodstream. This immune response would now neutralize the pathogen should the person ever encounter it in the future [15].

They are cheaper, heat-stable, do not require cold -chain maintenance, can be stored near the site of use, Non –requirement of syringes and needles, showed exhibit good genetic stability, grown locally using standard methods and do not require capital intensive pharmaceutical manufacturing facilities [16].

Even though edible vaccines are stable and easily accessible there are some limitations which restrict its development. For example, one could develop immune-tolerance to the vaccine peptide or protein. Little research has been done on this Korban *et al.* [17]. Another concern with whole fruit or vegetable vaccines is the consistency of dosage from fruit to fruit, plant to plant and generation to generation. Another limitation is storage of edible vaccines [18].

The first patented edible vaccine to demonstrate efficacy in animal trials was against the transmissible gastroenteritis virus (TGEV) in pigs and was under planning to be made commercially available [19]. Vaccines against porcine reproductive and respiratory syndrome (PRRS) and other diseases like Foot and Mouth diseases virus are being investigated [20]. Various transgenic animal feeds are currently undergoing clinical trials in pigs [21]. Other candidates are diseases of pets, animals in swine and poultry industries, draft and wild animals [22]. The trial carried out by prodiGene Institution showed for the first time that an oral vaccine produced in plants could protect live stock against virulence challenge [23]. The first product to reach market could be a poultry vaccine developed by Dow Agro Sciences [24], has been proposed for market release sometime in 2006.

Edible Vaccine Production: To produce edible vaccine, desired gene of interest is introduced in the selected plants to manufacture proteins encoding for the same gene [25]. Introduction of foreign DNA into plant's genome can be done through one of the following methods.

Agrobacterium Tumefaciens: It is a naturally occurring soil bacterium, which has the ability to get into plants through some kind of wound (Scratch, etc.). It possesses a circular "Ti plasmid" (Tumor inducing), which enables it to infect plant cells, integrate into their genome and produce a hollow tumor (Crown gall tumor), where it can live. This ability can be exploited to insert foreign DNA into plant genome [26]. But prior to this, the plasmid needs to be disarmed by deleting the genes for auxin and

cytokinin synthesis, so that it does not produce tumor. Genes for antibiotic resistance are used to select out the transformed cells and whole plants, which contain the foreign gene; and for expressing the desired product, which can then be regenerated from them [27]. The DNA integrates randomly into plant genome, resulting in a different antigen expression level for each independent line, so that 50-100 plants are transformed together at a time, from which one can choose the plant expressing the highest levels of antigen and least number of adverse effects [28].

Chimeric Viruses: Plant viruses are engineered to carry the desired genes and used to infect their natural hosts such as the edible plants where the cloned genes are expressed to varying degrees in different parts of the plant, including their edible portions [29]. Certain viruses can be redesigned to express fragments of antigenic proteins on their surface, such as CPMV (Cowpea mosaic virus), alfalfa mosaic virus, TMV (Tobacco mosaic virus), CaMV (Cauliflower mosaic virus), potato virus and tomato bushy stunt virus. Technologies involved are overcoat and epicoat technology [30].Overcoat technology permits the plant to produce the entire protein, whereas epicoat technology involves expression of only the foreign proteins [31].

Biolistic Method: The gene containing DNA coated metal (e.g. gold, tungsten) particles are fired at the plant cells using gene gun. Those plant cells that take up the DNA are then allowed to grow in new plants and are cloned to produce large number of genetically identical crop. This method is quite attractive because DNA can be delivered into cells of plant which makes gene transfer independent of regeneration ability of the species. But the chief limitation is the need for costly device particle gun [32].

Electroporation: Here there is introduction of DNA into cells by exposing them for brief period to high voltage electrical pulse which is thought to induce transient pores in the plasma lemma. The cell wall presents an effective barrier to DNA. Therefore, it has to be weakened by mild enzymatic treatment so as to allow the entry of DNA into cell cytoplasm [33].

Second Generation Edible Vaccine: Successful expression of foreign genes in plant cells and/or its edible portions has given a potential to explore further and expand the possibility of developing plants expressing more than one antigenic protein. Multicomponent vaccines can be obtained by crossing two plant lines harboring different antigens. Adjutants may also be co-expressed along with the antigen in the same plant [34]. This feature can bring several different antigens to M cells at one time - for example, a trivalent edible vaccine against cholera, Enter toxigenic *E. coli* and rotavirus could successfully elicit significant immune response to all three. Global alliance for vaccines and immunization (GAVI) accords very high priority to such combination vaccines for developing world [35].

Candidates Plants: Edible parts of different plant species like the grains or fruits are utilized for the expression of desired antigen of interest. Cereals like rice and maize, fruits like banana, leaves of many plants (Tobacco, alfalfa, peanut leaves)[36], tubers like potatoes, tomatoes, soybean seeds, cowpea, pea, carrot, peanut and lettuce have been extensively used for high levels of antigenic protein expression[37]. Points to shine in mind while choosing the vehicle for vaccine: Plant should be hardy, it should be palatable and well relished, it should be indigenous and easily available and Transformation can be done easily [38, 39].

Several things have to be kept in mind when selecting an expression host like gene of interest to be expressed in leaves germinating seedlings chloroplast [40] or in dry tissues like cereals based on the final part to be used for the vaccination purpose. The advantages of using grains as an expression host are many like it can store proteins for years, is cost effective, large volumes of desired products can be produced in short span of time and can be easily harvested and processed [41].

The most common plant used for expression of protein is tobacco because of its transforming ability. The ultimate goal of using transgenic plants as production systems for animal and human vaccine antigens is to facilitate easier delivery of immunizing antigen so that mass immunization programmed against various infectious diseases can be achieved in a time bound fashion [42, 43].

Banana: Tropical climate is suitable for growing bananas. Most third-world countries are found in this climate. As a result most studies are leaning towards the use of bananas as the vector of edible vaccines. A merit of banana isit grows in tropical climates, it can be eaten raw as compare to potato or rice that need to be cooked while its demerits are tree take 2-3 years to mature, transformed tree take about 12months to bear fruit, spoils rapidly after ripening [44].

Fig. 1: Banana for vaccine [45]

Fig. 2: Edible vaccine using genetically modified rice [47]

Fig. 3: Edible vaccine using maize [48]

Rice: Edible vaccine using genetically modified rice used in cholera. Cholera vaccine is exist but provides short lived protection and requires refrigeration. After Japanese researchers have created a strain of rice that can act as a vaccine and last for more than a year and half at room temperature. It does not require needles, purification or refrigeration, but Grow slowly, requires specialized glasshouse condition [46].

Maize: Maize plants produce a protein used to make the Hepatitis B virus vaccine. It is cheaper and not need to be refrigerated. Not require skilled person and needles to deliver the vaccine. Their demerits are need cooking to use and take a time to reach [7].

Potato: A potato based vaccine to combat the Norwalk virus (Stomach virus), which is spread by contaminated food and water. The virus causes severe abdominal pain and diarrhea. The Norwalk virus and closely related members of the same virus family account for more than 90% of non-bacterial gastroenteritis and cause of infant mortality. Eating of potato was effective way to develop immunity to the E. coli toxin. Advantages are safe and stimulate antibodies, Ease, affordable and effective. Disadvantages are Needs cooking which can denature antigen and decrease immunogenicity [49].

Tomato: It is possible to produce the vaccines against Anthrax, Rabies and HIV/AIDS by using tomato as a vector. It has merits like grow quickly, cultivated broadly, taste good and but, spoils easily [51].

Mechanism of Action: Plant derived vaccines have demonstrated the ability to induce both systemic and mucosal immune responses [54]. The major obstacle to oral vaccination is the digestion of the antigenic protein in the stomach. The antigens in transgenic plants are delivered through bio-encapsulation, i.e, the tough outer wall of plant cells, which protects them from gastric secretions and finally break up in the intestines. The antigens are released, taken up by M cells in the intestinal lining that overlie peyer's patches [55]. Peyer's patches (PP) are an enriched source of secretory immunoglobulin (IgA) producing plasma cells and have potential to populate mucosal tissue and serves as mucosal immune effectors sites. The breakdown of edible vaccine near PP, consisting of lymphoid nodules on the outer surface of the intestine and contain follicles, which on antigenic stimulation develops the germinal center. Through these follicles antigen penetrates into epithelium of intestine and accumulates antigen within organized lymphoid tissues [56, 57].

Fig. 4: Edible vaccine using potato [50]

Fig. 5: Edible vaccine using Tomato [52]

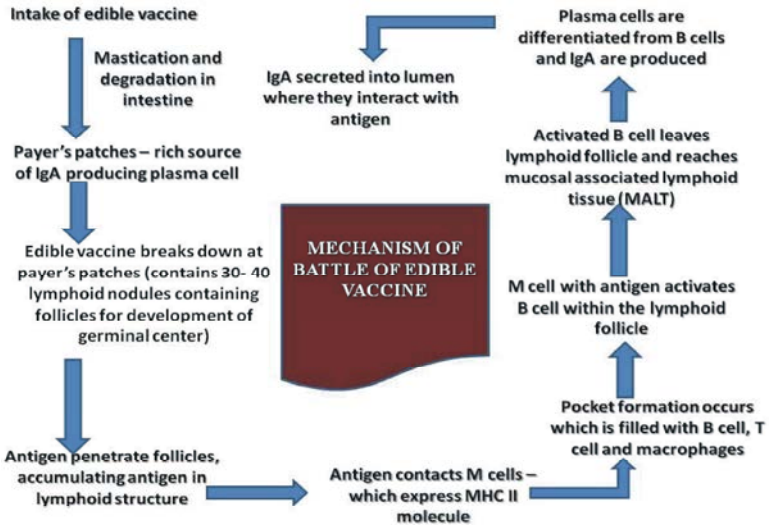

Fig. 6: Immunological mechanisms of action of plant based edible vaccines [53]

The component of immune system like B -cell, T-cells and macrophages are accumulated in these pockets. M -cells expressing class II MHC molecules and antigens transported across the mucous membrane by M-cells can activate B-cells within these lymphoid follicles [58]. The activated B -cells leave the lymphoid follicles and migrate to diffuse mucosal associated lymphoid tissue (MALT) where they differentiate into plasma cells that secrete and generating serum IgG, IgE responses, local IgA response and memory cells, which would promptly neutralize the attack by the real infectious agent [59].

Schematic representation of the mechanism of action of plant based edible vaccines is depicted in Figure 5.

Some Diseases Prevented by Edible Vaccine

Anthrax: Tobacco leaves bombarded with pag gene (Anthrax protective antigen - PA) using a gene gun could express a protein structurally identical to the major protein present in existing vaccine. Billions of units of anthrax antigen could be produced. In addition, this vaccine was devoid of edema factor and lethal factor, responsible for the toxic side effects. The same anthrax antigen is now being put in tomato plants [60].

Hepatitis B Virus: HBs Ag was expressed in transgenic potato plant and tested in mice for production of antibodies [61]. Hepatitis B virus has large surface antigen that expressed in transgenic tomato plant [62]. Transgenic lettuce plant carrying recombinant hepatitis B Virus antigen HBs Ag was demonstrated in Brazil [63].

Cancer: Plants can make monoclonal antibodies for cancer therapy in sufficient quantities. Soybean has been genetically engineered to make monoclonal antibody as a vehicle for targeting doxorubicin for breast, ovarian, colon and lung tumors [64].

Autoimmune Disease: Among the autoimmune disorders that might be prevented or ceased are type 1 diabetes, multiple sclerosis, rheumatoid arthritis, transplant rejection [65]. Potatoes expressing insulin and a protein called GAD (Glutamic acid decarboxylase), were able to suppress immune attack in a mouse strain that would become diabetic and could delay the onset of high blood sugar [66].

Marketing Edible Vaccine

Accessibility: Accessibility of edible vaccine is simple than any other types of traditional ways of vaccinating organisms. We can produce them locally by using plants that found in our areas. Different countries that found in the tropical region of the world are highly potential and greatly promised with a new emerging biotechnology. So as a chance many third world countries like Africa especially found in this region. Different plants that found here are can fulfill what the world is crying for; that means mass immunization either for animal or for human which promised by the only means of edible vaccine [67].

Affordability: The attraction of plant-based systems is that they exhibit good genetic stability and are cheaper to develop and easier to scale up for commercial production.

They are better as compared to the traditional vaccines when mass production, distribution and delivery are concerned. One of the key goals of the edible vaccine pioneers is to reduce immunization costs. The theory goes that edible vaccines would be far cheaper than current inject able vaccines since they would not have to undergo the expensive purification and refrigeration of traditional vaccines and shipping costs would be much reduced [68].

Distribution: One of the big draws for edible vaccines is the potential to drastically reduce or eliminate transport costs. Some researchers imagine vaccines being produced in national or regional greenhouses, which would be an improvement on flying the vaccines in from overseas, but this could probably better be achieved by establishing a conventional vaccine plant in country [69].

Opportunities and Advantages of Plant Systems

Storage/Shelf Life/Purification: Plant-based expression systems raise the possibility that antigens or antibodies can be produced in a form that is stable during storage and is amenable to extraction and purification procedures [70]. Dried or lyophilized leafy biomass, as well as plant storage tissues, such as seeds, retains unchanged levels of accumulated recombinant proteins for years at normal room temperatures, thus reducing stowing costs and facilitating distribution without need for a cold chain [71].

Such a production system would allow stockpiling of the transgenic plant material (Lyophilized leaf biomass or seeds) for manufacturing of rapid-response veterinary biologics targeted against pathogens of epidemiological relevance. The time to product would then only be dependent on the speed of extraction and downstream processing, if purification is required, or immediate, if formulated lyophilized biomass or whole seeds can be administered in feed [72].

Vaccine Bio-Encapsulation and Delivery: Although plants have been shown to manufacture biologically-active therapeutics in amounts sufficient for oral administration to livestock, plant-derived antigens require a formulation to protect them from the hostile environment of the gastro-intestinal tract, without interfering with the immunogenicity of the antigen. Depending on the plant species and the plant tissue in which the subunit proteins are expressed, the plant matrix could provide some protection against these harsh conditions [73]. Not only does this eliminate the need for costly purification, but the provision of a durable matrix offers a protective and stabilizing effect beyond harvest and upon mucosal administration. Any plant cell matrix can be effective to enhance resistance against digestion, thereby increasing exposure of a vaccine to immune effectors cells, but the protective effect can be further enhanced by incorporation of the vaccine proteins into storage organelles [74].

The use of seeds is particularly suitable to achieve bioencapsulation of recombinant proteins in specialized storage organelles that are derived from the endomembrane system and allow proteins to accumulate within a protective matrix [75]. Clear benefits have been demonstrated in seed based production systems as recombinant proteins expressed in rice or pea seeds were better protected from degradation than their purified counterparts upon oral delivery. Nevertheless, plant based production could also negatively influence the immunogenicity of the antigen by influencing antigen folding, glycosylation and/or by interaction with the antigen, interfering with its capacity to target the mucosa [76].

Scale-up and Speed: Scaling up production of recombinant proteins in transgenic plants is much easier and less costly than similar scaling up in mammalian or microbial cells for the simple reason that each plant could be considered as a bioreactor and all that is required is sowing a seed and providing light, water and fertilizer, as opposed to an expensive upfront investment in infrastructure and bioreactor facilities for cell cultures and the formulation of complex cell culture media. Therefore, scaling up or down can be achieved quickly with no major investment. A single tobacco plant can produce over 150 000 seeds, allowing fast scale-up within a few months. Tobacco biomass yields can reach up to 100 tons/ha in a single field season [77].

For this, transient production in Nicotine benthamiana is ideal with a timeline from cloning of the genes to vaccine production of only a few weeks. As well, this system obviates the need for production of transgenic plants, thus easing the regulatory framework for scale up. In this system, wild type Nicotine benthamiana plants are grown in greenhouses and infiltrated with Agrobacterium cultures containing the expression construct required for recombinant protein production [78].

Status of Edible Vaccine in Developing Countries: In the Third World countries where transportation costs, poor refrigeration, needle use complicate vaccine administration and also where healthcare workers are scarce, edible vaccine are very important means of diseases prevention. In such countries large percents of their child's, their animals and also themselves are under the risk of many disease problems. But much more of those diseases are preventable but not cure as they caused by virus and other agents. Since "Prevention is better than cure" (WHO) we easily prevent them by introducing edible vaccine which is food as well as therapeutics [79].

Twelve countries (Ethiopia, Ghana, Kenya, Madagascar, Mozambique, Namibia, Nigeria, Rwanda, Senegal, Uganda, Tanzania and Zambia) have partially met the provisions of the commission research and development activities involving GM crops in containment and confinement but not to commercially release GM crops. Most of these countries have moved on to develop policies and legislation on GM crops but this process is yet to receive full parliamentary and/or cabinet endorsement in the respective countries. In Ethiopia there is National Biotechnology Steering Committee and Sectoral Biotechnology Committees in place. Guidelines for regulating GM crops are non-existent and no field trials or commercial release of GMOs have been approved by Ethiopia recently [80].

CONCLUSION AND RECOMMENDATIONS

Edible plant derived vaccine may lead to a future of safer and more effective immunization. They would overcome some of the difficulties associated with traditional vaccines, like production, distribution and delivery and they can be incorporated into the immunization plans. Even if it is mass immunization mechanism that can solve poor countries problem; however it is not yet well known and expanded to every corner of these countries due to lack of advanced technology and well coordinated research to satisfy means of edible vaccine as needed and has the challenges of dose calculations. Therefore based on the above conclusion the following recommendations are forwarded:

- Creation of awareness about edible vaccine practice and use.
- Research has to be done continuously which can be disclosing the secret of dose calculation, protein expression levels and prevention of proteolysis in plastids.
- Further investigation have to be conducted to develop this vaccine in plants like teff coffee and natural grasses which much of human population and animal population uses in our country and elsewhere.

REFERENCES

1. Twyman, R., S. Schillberg and R. Fischer, 2012. The production of vaccines and therapeutic antibodies in plants, recent advances and future prospects. Springer Science and Business Media, New York, 5: 145-159.
2. Ahmad, P., M. Ashraf, M. Younis, A. Kumar, N. Akram and F. Al-Qurainy, 2012. Role of transgenic plants in agriculture and biopharming. Biotechnology Advantage 30(3): 524-540. Antigens in Chlamydomonas starch granules. Edible Malarial Vaccine, 5: 154-156.
3. Park, K., 2005. Park's Preventive Social Medicine. Banarsidas Bhanot Publisher, 56: 95-100.
4. Goldblatt, D. and M. Ramsay, 2003. Immunization in Domestic Animal. Oxford text book of medicine fourth edition. Oxford University Press, 32(4): 378-396.
5. Levine, M., 2006. Enteric infections and the vaccines to counter them. Future directions of Vaccine. New York National Medicine, 24: 3865-3873.
6. Yoshida, T., E. Kimura, S. Koike, J. Nojima, E. Futai, N. Sasagawa, Y. Watanabe and S. Ishiura, 2011. Transgenic rice expressing amyloid β-peptide for oral immunization. Journal of Biological Science, 7(3): 301-307.
7. Arakawa, T., D. Chong and W. Langridge, 1998. Transgenic plants for the production of edible vaccine and antibodies for immunotherapy. Nature Biotechnol., 16: 292-297.
8. Sharma, M. and B. Sood, 2011. A banana or a syringe: journey to edible vaccines. Journal of Microbiology and Biotechnology, 27(3): 471-477.
9. Nochi, T., H. Takagi, Y. Yuki, L. Yang, T. Masumura, M. Mejima, U. Nakanishi, A. Matsumura, A. Uozumi and T. Hiroi, 2007. Rice-based mucosal vaccine as a global strategy for cold-chain and needle-free vaccination. Proclamation for National Academic Science, 104: 10986-10991.
10. Tiwari, S., P. Verma, P. Singh and R. Tuli, 2009. Plants as bioreactors for the production of vaccine antigens. Biotechnology Advantage, 27(4): 449-467.

11. Pelosi, A., R. Shepherd and A.s Walmsley, 2012. Delivery of plant-made vaccines and therapeutics. Biotechnology Advantage, 30(2):440-4488.
12. Shih, S. and P.M. Doran, 2009. Foreign protein production using plant cell and organ cultures: Advantages and limitations. Biotechnology Advantage, 27: 1036-1042.
13. Krishna, G., V. Chaitanya and U. Jonnala, 2006. Edible Vaccines. sriramandra. Journal of Medicine, 1(1): 123-129.
14. Lal, V., R. Ramachandran, H. Goyal and K. Sharma, 2007. Edible vaccines; Current status and future. Journal of Indian Medical Microbiol., 25(2): 93-102.
15. Daniell, H., D. Singh and H. Mason, 2009. Plant-made vaccine antigens and biopharmaceuticals. Trends in Plant Science, 14: 669-679.
16. Webster, D., M. Thomas, R. Strugnell, I. Dry and S. Wesselingh, 2002. Appetizing solutions, An edible vaccine for measles. Journal of Medical Science, 176: 434-437.
17. Korban, S., S. Krasnyanski and D. Buetow, 2002. Foods as production and delivery vehicles for human vaccines. Journal of Animal Nutrition, 21: 212S-7S.
18. Richter, L. and P. Kipp, 1999. Topics in Microbiology and Immunology. Plant Biotechnology. New Products and Applications, 1: 159-176.
19. Lamphear, B., J. Jilka, L. Kesl, M. Welter, J. Howard and S. Streatfield, 2004. Acorn-based delivery system for animal vaccines. An oral transmissiblegastroenteritis virus vaccine boosts lactogenic immunity in swine. Vaccine, 22: 2420-2424.
20. Yang, C., J. Liao, C. Lai, M. Jong, C. Liang, Y. Lin, N. Lin, Y. Hsu and S. Liang, 2007. Induction of protective immunity in swine by recombinant bamboomosaic virus expressing foot-and-mouth disease virus epitopes. BMC Biotechnology, 7: 62.
21. Kolotilin, I., A. Kaldis, B. Devriendt, J. Joensuu, E. Cox and R. Menassa, 2012. Production ofa subunit vaccine candidate against porcine post-weaning diarrhea inhigh-biomass transplastomictobacco. PLoS, 7: 4240-4243.
22. Jacob, S., S. Cherian, T. Sumithra, O. Raina and M. Sankar, 2013. Edible vaccinesagainst veterinary parasitic diseases–current status and future prospects. Vaccine, 31: 1879-1885
23. Lamphear, B., S. Streatfield and J. Jilka, 2002. Delivery of subunit vaccines in maize seed. Control Release, 85(1-3): 169-80.
24. Ramachandra, S., 2006. Edible vaccine. Current status and future. Journal of Medicine Guru Teg, New Delh,1: 33-36 September 2006.
25. Sibila, J., M. Snjezana and B. Natasa, 2012. Production of biopharmaceuticals, antibodies and edible vaccines in transgenic plants. Current Studies of Biotechnology, 1: 121-127.
26. Streatfield, S., 2005. Mucosal immunization using recombinant plant based oral vaccines. Methods of Immunization, 38: 150-57.
27. Mercenier, A., 2002. Edible genetically modified microorganisms and plants for improved health. Curr Opin Biotechnol., 12: 510-515.
28. Haq, T., H. Mason, J. Clements and C. Arntzen, 2009. Oral Immunization with a Recombinant Bacterial antigen produced in transgenic plants. Science, 268: 714.
29. Maliga, T., 2002. Engineering the plastid genome of higher plants. Curr Opin Plant.
30. Ramshaw, I. and A. Ramsay, 2000. The prime-boost strategy. Exciting prospects for improved vaccination. Immunology Today, 21: 163-5.
31. Karasev, A., S. Foulke, C. Wellens, A. Rich, K. Shon and L. Zwierzynski, 2005. Plant based HIV-1 vaccine candidate. Tat Protein Produced in Spinach, 23: 1875-80.
32. Taylor, N. and C. Fauquet, 2002. Microparticle bombardment as a tool in plant science and agricultural biotechnology. DNA Cell Biology, 21: 963-977.
33. Singh, B., 2002. Biotechnolgy. Kalyani publishers. New Delhi, 1: 323.
34. Landridge, W., 2010. Edible vaccines. Scientific America, 283: 66-71.
35. Yu, J. and W. Langridge, 2001. A plant-based multicomponent vaccine protects mice from enteric diseases. National Biotechnology, 19: 548-52.
36. Yoshimatsu, K., N. Kawano, N. Kawahara, H. Akiyama, R. Teshima and M. Nishijima, 2012. Current status of applicat ion and commercialization of genetically modified plants forhuman and livestock health and phytoremediation. Yakugaku Zasshi, 132: 629-674.
37. Huy, N., S. Kim, M. Yang and T. Kim, 2012. Immunogenicity of a neutralizing epitope from porcine epidemic diarrhea virus: M cell targeting ligand fusion protein expressed in transgenic rice calli. Plant Cell Report, 31: 1933-1942.
38. Wang, Y., Q. Shen, Y. Jiang, Y. Song, L. Fang, S. Xiao and H. Chen, 2012. Immunogenicity of foot and mouth disease virus structural polyprotein P1 expressed in transgenic rice. Journal of Virology Methods, 181: 12-17.

39. Loza, E. and E. Rojas, 2010. Vaccine production in plant systems. An aid to the control of viral diseases in domestic animals. Acta Veterinaria Hungarica, 58: 511-522.
40. Dauvillee, D., S. Delhaye, S. Gruyer, C. Slomianny, S. Moretz, C. Hulst, C. Long, S. Ball and S. Tomavo, 2010. Engineering the Chloroplast Targeted Malarial Vaccine.
41. Streatfield, S., J. Jilka, E. Hood, D. Turner, M. Bailey, J. Mayor, S. Woodard, K. Beifuss, M. Horn, D. Delaney, I. Tizard and J. Howard, 2001. Plant-based vaccines: unique advantages. Vaccine, 19: 2742-2748.
42. Santi, L., 2009. Plant derived veterinary vaccines. Veterinary Research Communication, 33: 61-66.
43. Rybicki, E., 2010. Plant made vaccines for humans and animals. Journalof Plant Biotechnology, 8: 641-643.
44. William, S., 2002, A review of the progression of transgenic plants used to produce plant bodies for human usage. Journal of Young Investigators, 4: 56-61.
45. Giddings, G., 2012. Transgenic plants as factories for biopharmaceuticals. journal of Nat Biotechnol, 2000, 18: 1151-5.
46. Thanavala, Y., Y. Yang, P. Lyons, H. Mason and C Arntzen, 1995. Edible vaccine from GM crop; Current and future status. National Academic Science USA, 92: 358-361.
47. Karyn, D., 2011. Using transgenic plants as bioreactors to produce edible vaccines. Bioreactor vaccine. Journal of Biotechnology Res., 4: 92-99.
48. Mason, H., 2009, Expression of hepatitis B surface antigen in transgenic plants. Proclamation of National Academic Science USA, 89: 11745-11749
49. Arakawa, T., D. Chong and W. Langridge, 1997. Expression of Choleratoxin B subunitoligomers in transgenic potato plants, Transgenic, 6: 403-413.
50. Rajendra, 2012. Review on Edible Vaccine. Journal of International pharmaceutical Research and Bioscience.
51. Doshi, V., H. Rawal and S. Mukherjee, 2013. Edible vaccine from crop. Current and future scope. Journal of Pharmacology Science Innovation, 2(3): 1-6.
52. William, H., 2000. Edible Vaccines by using potato. Altenatives to injectable vaccine.Scientific American, 12(1): 67.
53. Dhama, 2013. Journal of Experimental Biology and Agricultural Sciences, 1: 23.
54. Kong, Q., L. Richter, Y. Thanavala, F. Yu, C. Arntzen and H. Mason, 2001. Oral immunisation with hepatitis B surface antigen expressed in transgenic plants. PNAS, 98: 11539-44.
55. Lossl, A. and M. Waheed, 2011. Chloroplast-derived vaccines against human diseases: achievements, challenges and scopes. Journal of Plant Biotechnology, 9: 527-539.
56. Streatfield, S., 2006. Mucosal immunization using recombinant plant based oral vaccines. Methods of Immunization, 38: 150-57.
57. Takahashi, I., T. Nochi, J. Kunisawa, Y. Yuki and H. Kiyono, 2010. The mucosal immune system for secretory IgA responses and mucosal vaccine development. Inflammation and Regeneration, 30: 40-47.
58. Hefferon, K., 2010. The mucosal immune response to plant derived vaccines. Pharmaceutical Research, 27: 2040-2042.
59. Yuki, Y. and H. Kiono, 2003. New generation of mucosal adjuvants for the induction of protective immunity. Reviews in Medical Virology, 13: 292-310.
60. Koya, V., M. Moayeri, H. Leppla and H. Daniell, 2005. Plant-based vaccinemice immunized. With chloroplast-derived anthrax protective antigen survives anthrax lethal toxin challenge. Infectious Immunity, 73: 8266-8274.
61. Richter, L., Y. Thanavala, C. Arntzen and H. Mason, 2000. Production of hepatitis B surface antigen in transgenic plants for oral immunization. National Biotechnology,18: 1167-1171.
62. Lou, X., Q. Yao, Z. Zhang, R. Peng, A. Xiong and H. Wang, 2007. Expression of the human hepaptitis B virus large surface antigen gene in transgenic tomato plant. Clinical Vaccine Immunology, 14(4): 464-469.
63. Marcondes, J. and E. Hansen, 2008. Transgenic lettuce seedlings carrying hepatitis B virus antigen. Brazil. Journal of Infectious Disease,12(6): 469-471
64. Prakash, C., 2013. Edible vaccines and antibody producing plants. Biotechnology Develop Monitor, 27: 10-13.
65. Landridge, W., 2010. Edible vaccines. Scientific America, 283: 66-71.
66. Arakawa, T., J. Yu, D. Chong, J. Hough, P. Engen and W. Langridge, 2007. A plant based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes. National Biotechnology, 16: 934-8.

67. Muffat, A., 2012. Exploring transgenic plants as a new vaccine source. Science, 268: 658-660.
68. Rukavtsova, E., E. Chebotareva, N. Rudenko and Y. Buryanov, 2011. Immunogenicity of biologically safe potato tubers synthesizing hepatitis B surface antigen. Doklady Biological Sciences, 437: 110-112.
69. Rybicki, E., 2010. Plant-made vaccines for humans and animals. Journal of Plant Biotechnology, 8: 620-637.
70. Peter, J. and E. Stoger, 2011. Transgenic crops for the production of recombinant vaccines and antimicrobial antibodies. Human Vaccine, 7: 367-374.
71. Stoger, E., J. Ma, R. Fischer and P. Christou, 2005. Sowing the seeds of success:pharmaceutical proteins from plants. CurrOpin Biotechnology, 16: 167-173.
72. Lakshmi, P., D. Verma, X. Yang, B. Lloyd and H. Daniell, 2013. Low cost tuberculosis vaccine antigens in capsules: expression in chloroplasts, bio-encapsulation, stability and functional evaluation in vitro. PLoS One, 8: 54708.
73. Takaiwa, F., 2013. Update on the use of transgenic rice seeds in oral immunotherapy. Immunotherapy, 5: 301-312.
74. Khan, I., R.M. Twyman, E. Arcalis and E. Stoger, 2012. Using storage organelles for the accumulation and encapsulation of recombinant proteins. Biotechology, 7: 1099-1108.
75. Hofbaver, A. and E. Stoger, 2013. Subcellular accumulation and modification of pharmaceutical proteins in different plant tissues. Curr. Pharm. Des., 19: 5495-5502.
76. Zimmermann, J., I. Saalbach, D. Jahn, M. Giersberg, S. Haehnel, J. Wedel, J. Macek, K. Zoufal, G. Glunder, D. Falkenburg and S. Kipriyanov, 2009. Antibody expressing pea seeds as fodder for prevention of gastrointestinal parasitic infections in chickens. BMC Biotechnology, 9: 79.
77. Woodleif, W., J. Chaplin, C. Cambell and D. Dejong, 2006. Effect of variety and harvest treatments on protein yield of close grown tobacco. Tobacco Science, 25: 83-86.
78. Aoust, M., M. Couture, N. Charland, S. Trépanier, N. Landry, F. Ors and L. Vézina, 2010. The production of hemagglutinin-based virus-like particles in plants: a rapid, efficient and safe response to pandemic influenza. Plant Biotechnol. J., 8: 607-619.
79. Pawar, K., G. Patel and S. Wani, 2010. promotion of global health via oral Immunotherapy using Edible vaccine. Pharmaceutical Review. New York USA, 8(1): 28-32.
80. Francis, N., 2014. The status of Regulations for Genetically modified crops in countries of Sub-Saharan Africa. African Agricultural Technology Foundation. Biology, 5: 164-172.