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PLANT BASED EDIBLE VACCINES: A REVIEW

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ABSTRACT

Plant-based Edible vaccines are cost effective, easy to administer, storable and widely acceptable as a vaccine delivery system especially in the poor developing countries. Human trials conducted by the National Institute of Allergy and Infectious Diseases (NIAID), US Department of Health and Human Services, USA show that edible vaccines are feasible. Prodi Gene, a biotech company, has a patent for vaccine against, viral diseases of hepatitis and transmissible gastroenteritis virus. Plant-based Edible vaccines are subunit vaccines that introduce selected genes into the plants and facilitate the production of encoded protein. Plant-based Edible vaccines are currently being developed for number of human and animal diseases. There is an increasing demand of transgenic crops in both industrial and developing countries. This review is focused on the conception of plant based edible vaccines along with mechanism and production of plant based edible vaccines.

KEYWORDS: Plant-based Edible vaccine, transgenic crops, mucosal targeted vaccines, plant expression host.

INTRODUCTION

The term “edible vaccine” was initially used by Charles Arntzenwith Hugh Mason and colleagues in 1990. They are mucosal targeted vaccines, which causes stimulation to both systemic and mucosal immune response, one of the safe and effective way to control various infectious diseases like measles, cholera and hepatitis-B and many more are in progress. Edible vaccine may also help to suppress autoimmune disorders like diarrhea, Type 1 diabetes, rheumatoid arthritis, etc.[1] Theidea of plant molecular farming was developing quickly when the proof of concept for recombinant plant-derived pharmaceutical proteins was reported and published and the idea has since expanded to the production of many industrial and agricultural recombinant enzymes.[2] The most important purpose of this technology is to produce an edible vaccine that gives protection against many diseases by inducing specific immune response after oral delivery and uptake of plant-based edible vaccine.[3]

In 1986, the first transgenic tobacco was introduced to express human growth hormone Later another development of transgenic tobacco in 1989 expressing an antibody with proper assembly of functional complex glycoproteins by another research group.[4] The structural confirmation of recombinant protein was proved in 1992 by expressing the hepatitis B virus (HBV) surface antigen (HBsAg) in tobacco plant by Mason et.al.[5]

Creating plant-based edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. This process is known as transformation, and the altered plants are called transgenic plants. Plant-based Edible vaccines activate both mucosal and systemic immunity, as they come in contact with the digestive tract lining. This dual effect would provide first-line defense mechanism against pathogens attacking through mucosa, like Mycobacterium tuberculosis and agents causing diarrhea, pneumonia, STDs, HIV, etc. Scientists place high priority on combating the diarrheal agents in Norwalk virus, Rotavirus, Vibrio cholerae and enterotoxigenic E. coli (ETEC) responsible for about 3 million infant deaths per year, mainly in developing countries.[6]

Plant-based Edible vaccinesadministering tothe would-bemothers might be successful in immunizing the fetus-in-uterus by transplacental transfer of maternal antibodies or the infant through breast milk. In the presence of maternal antibodies edible vaccines are seroconversion, thus having a potential role in the protection of infants against diseases like group-B Streptococcus, respiratory syncytial virus etc., which are under investigation. Plant-based Edible vaccines would also be suitable against rare diseases like dengue, hookworm, rabies, etc. They may be integrated with other vaccine approaches, and multiple antigens may also be delivered. Banana, potato, tomato, lettuce, rice, etc. are the various foods which are under study. Edible vaccines arecurrently being developed for a number of human and animal diseases, including measles, cholera, hepatitis B, C and E.[7]

Types of edible vaccines[8]

1. Algae based edible vaccine
2. Insect based edible vaccine
3. Lactic acid bacteria based edible vaccine
4. Whole-cell based edible vaccine
5. Plant based edible vaccine

Advantages of plant-based edible vaccines[8]

1. Edible means of administration.
2. No need of medical personnel and syringes.
3. Sterile injection conditions are no more required.
4. Economical in mass production by breeding compared to an animal system.
5. Easy for administration and transportation
6. Effective maintenance of vaccine activity by controlling the temperature in plant cultivation.
7. Therapeutic proteins are free of pathogens and toxins.
8. Storage near the site of use.
9. Heat stable, thus eliminating the need of refrigeration.
10. Antigen protection through bioencapsulation.
11. Subunit vaccine (not attenuated vaccine) means improved safety.
12. No serious side effect problems have been noticed until now
13. Enhanced compliance (especially in children).
14. Delivery of multiple antigens.
15. Integration with other vaccine approaches.
16. Plant-derived antigens assemble spontaneously into oligomers and into virus like particles.

Disadvantages of plant-based edible vaccines[8]

1. Consistency of dosage from fruit to fruit, plant to plant, lot to lot, and generation to generation is not similar.
2. Stability of vaccine in fruit is not known.
3. Evaluation of dosage requirement is tedious.
4. Selection of best plant is difficult.

5. Certain foods like potatoes are generally not eaten raw and cooking the food might weaken the medicine present in it.
6. Not convenient for infants as they might spit it, eat a part or eat it all, and throw it up later. Concentrating the vaccine into a teaspoon of baby food may be more practical than administering it in a whole fruit.
7. There is always possibility of side effects due to the interaction between the vaccine and the vehicle.

Limitations of plant-based edible vaccines[9,10,11]

1. Since edible vaccines are still in their infancy, there are still many unknowns left to discover.
2. The adequate dosage amount and how long it lasts is still undetermined. Thedosage varies due to many factors including: the plant generation, the individual plant, the protein content, the ripeness of the fruit and how much of it is eaten.
3. The dosage also varies due to the difficulty in standardizing the concentration of the antigen in the plant tissue; it can be tedious to produce both consistently and large scale. The antigen concentration can also vary significantly between individual fruits on a plant, individual plants, and between plant generations.
4. Low doses result in the consumption of less antibodies but a high dose result in establishing an oral and immune tolerance to the vaccine proteins. The logistics of controlling dosage, quality, and consistency still needs to be determined and verified.
5. Being a new concept, the long-term effects are still unknown. Additionally, the effects and risk of using pesticides on the plants could be negative towards both the plant vaccine and the consumer.
6. There is also the risk of transgenic escape into the surrounding environment; however, this could be reduced by regulating growing practices and locations. Manyplants are not eaten raw and the cooking could weaken or destroy the proteins in the vaccine.

Mode of action of plant-based edible vaccines

1. Plant-basedEdible vaccine mainly stimulates mucosal immunity. This configuration contains both the immune system's innate and adaptive arm (T and B cells).
2. The composition is well structured and these so-called lymphoid mucosal-associated tissues (MALT). IgA also plays a key role in protecting mucosal surfaces from adhesion for both microbes and toxin activity.

3. The creation of new platforms for the delivery of pathogens or toxin-specific IgA and systemic IgG is the key to improve vaccine efficacy
4. Microfold (M) cells are one of the major routes of the capture of the antigen at the intestinal level. M cells are a small quantity of follicular-associated enterocytes (FAE) which are mainly found in the gastrointestinal tract.
5. These cells capture a wide range of macromolecules from lumens in the small intestines to antigen submucosal cells (APCs) on Peyer's patches effectively.[14] Of many APCs, dendritic (DC) cells appear to be the most powerful antigenic cells to trigger an adaptive immune reaction in the priming naive T cells.[15]
6. In an immediate phase, DC is found in a stable state, marked by strong endocytic activity and low capacity for primary naive T cells. DCs, however, mature, increase co-stimulatory molecules and migrate to T-cell areas in lymph nodes under inflammatory situations.[16]

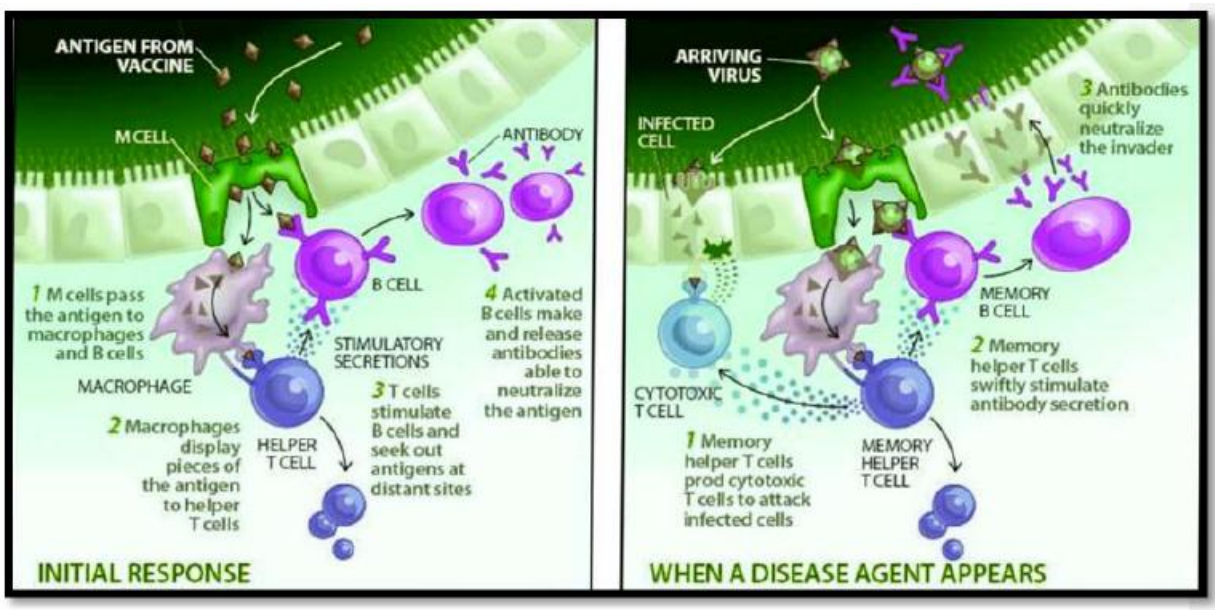

Fig. 1: Mode of Action of Plant-based Edible Vaccines.[17]

Plant-based edible vaccines

Unlike the production of biomolecule, however, the edible vaccine formulations require no pre-administration treatment or purification, which further lowers the cost involved with production. The most studies have utilized cultivated potatoes but, as cooking or boiling can weaken most of antigenic proteins, potatoes might not be the best choice in edible vaccines.[14] More than a generalized application, but a successful design and development of genetic transformation methods, plants including tomatoes, maize, tobacco, bananas, carrots and peanuts will have a brighter future as edible vaccines.[18]

Major plant species used as vaccine models

Tobacco

Tobacco is not an edible plant. It is used as a model for the development of edible vaccines. A vaccine was developed in tobacco for Norwalk virus in 1996 that causes gastroenteritis. Transgenic tobacco expresses VP1 protein against chicken infectious anemia. Tobacco has the ability to express a polypeptide related to hepatitis B. It is also used to develop vaccine against coccidiosis.[19-21]

Potato

Potato is an appropriate model for producing vaccines against tetanus, diphtheria, hepatitis B and Norwalk virus. The first attempt to develop edible vaccine in potato is for enteritis caused by E. coli strain. Potato may also have a role as an oral strengthening to the hepatitis B vaccines in humans.[22]

Rice

Rice is the other plant species used for the development of edible vaccines. Advantages over other plants were commonly used in baby food and high expression of antigen. But it grows slowly and requires glasshouse condition. In 2007, a study conducted in transgenic rice called Oryza sativa persuades significant number of antibodies against E coli. Functional expression of HBsAg in rice seeds was confirmed in 2008. Vaccines developed from rice plant will have a massive power on the public health where rice is the major source of food.[23,24]

Banana

Banana is the commonly used plant species in the production of edible vaccine. It does not need cooking. Proteins were not destroyed even after cooking. Inexpensive when compared to other plants. Banana plants express HBsAg. The leaf contains antigen. The main disadvantage is it takes 2–3 years to mature and spoils fast after ripening.[25]

Tomato

An effective vaccine against acute respiratory syndrome, SARS caused by coronavirus was first established in tomato. It produces better effect against Norwalk virus than vaccines produced from potato. The leaves, stem, fruits, and other tissues has the ability to express CT-B proteins from Vibrio cholera B toxin.[26]

Lettuce

This plant is an effective model system against enteric diseases in both animals and humans caused by E coli. Glycoprotein E2 expressed lettuce for classical swine fear hog pest virus was developed. This plant is mainly used up in the raw form and it produces beneficial effects against hepatitis B virus. It is the utmost effective plant that can be used as an edible vaccine.[27,28]

Alfalfa

Alfalfa is the plant used to develop edible vaccines mainly for veterinary purposes. Transgenic alfalfa containing hog pest virus glycoprotein E2 was developed in 2005. Alfalfa plants was developed to express Eeg95-EgA31 of Echinococcusganulosus.[21]

Carrots

Carrots were not only healthy and delicious but also can be consumed in the form of edible vaccines. Vaccines against HIV, E coli, Helicobacter pylori shows potential effects when it is produced in transgenic carrots. People having weak immune system gets proper benefit by consuming this type of antigen containing carrot edible vaccine.[29,30]

Production of plants-based edible vaccines

1. Direct gene delivery method- Direct gene delivery is the simple method. In this the selected DNA or RNA is directly introduced in to the plant cell. The most commonly used direct gene delivery method is the biolistic method and it is also known as gene gun or micro-projectile bombardment method. This is a vector-independent method. This is done when gene transfer through agrobacterium species-mediated transformation is not possible.[31-33]

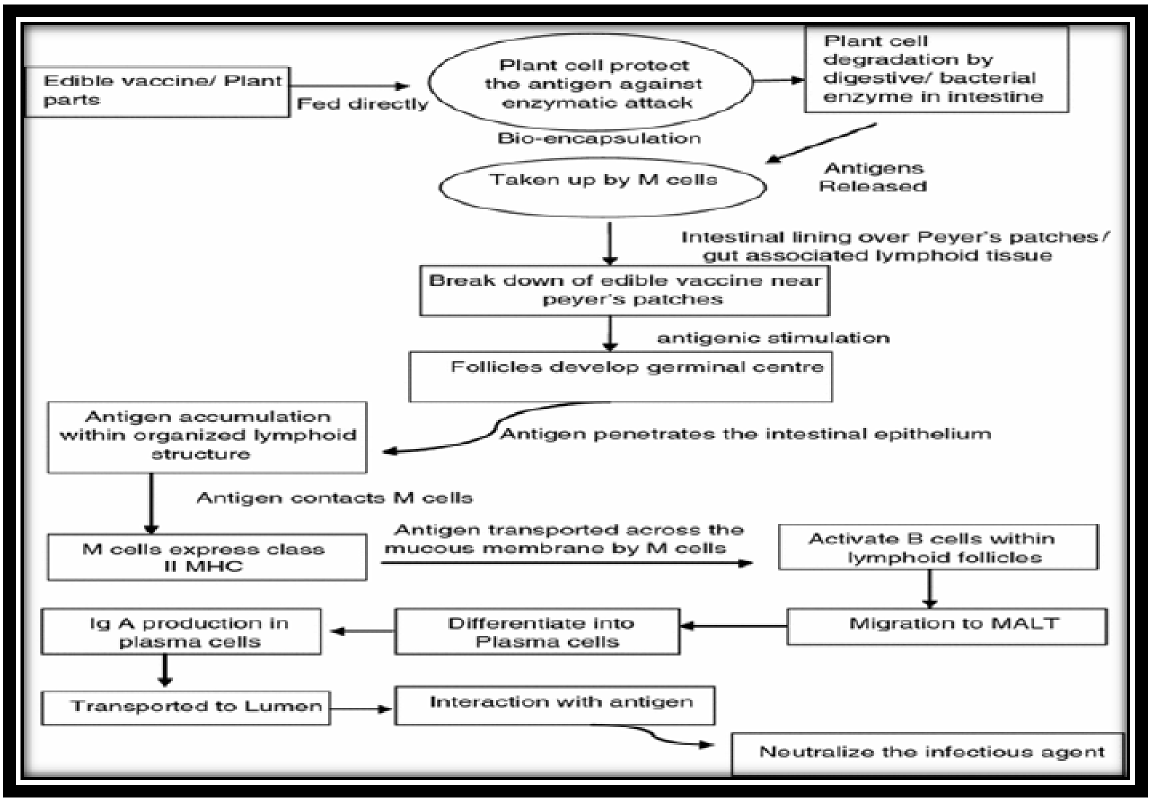

Fig. 2: Chart flow for mucosal immunity.[34]

Examples of vaccines produced by biolistic methods are cholera, Lyme disease, anthrax, tetanus, plague, Rota virus and canine parvovirus.[35]

2. Indirect delivery method - This is a vector-mediated gene delivery. In this method, the desired plant cells were infected with plant bacteria or plant virus to produce the protein of interest.[36]

Agrobacterium mediated Gene transfer- Agrobacterium is a gram-negative bacteria that attacks the plants and transfer their genes to plant nucleus. Agrobacterium tumefaciens and Agrobacterium rhizogenes were the two species that are commonly used. Agrobacterium tumefaciens carries tumor-inducing Ti plasmid and agrobacterium rhizogenes carries root-inducing plasmid Ri plasmid.[37]

Genetically engineered plant virus

This process modifies an appropriate plant virus to make a viral coat protein chimeric gene. It is therefore a vector for the delivery of genetic components in plant cells. In plants, this technique leads to transient antigen expression.[38,39] The recombinant virus is a product of viral replication throughout viral infection in plants that expresses the intended protein or peptide. Furthermore, vaccine epitopes can be synthesized and accumulated by changing viral capsid proteins.[39,40]

Plant Virus expression system mainly includes engineered viruses such as RNA Virus (CMV).[41] Such viruses do not reproduce in mammalian cells and are, therefore, an acceptable alternative vector for human and veterinary production.

Ideal properties of plant-basededible vaccines[42]

1. It should not be toxic or pathogenic, i.e., it should be safe.
2. It should have very low levels of side effects in normal individuals.
3. It should not cause problems in individuals with impaired immune system.
4. It should produce long-lasting humoral and cellular immunities.
5. The vaccination technique should be simple.
6. The vaccine should be less expensive.
7. Contamination of the environment should not happen.
8. It should be effective and affordable.

Challenges in production of plant based edible vaccines[43]

Although many plant-based vaccines that have been produced are still in phase 1 clinical trials, some vaccines have proceeded or completed phases II and III trials. These therapeutics were produced in various transgenic plants such as insulin in transgenic safflower (Sem Bio Sys), growth factor in transgenic barley (ORF Genetics), taliglucerase alfa in transgenic carrot (Protalix Bio Therapeutics), avian influenza vaccine in transgenic tobacco (Medicago), and Ebola Vaccine in transgenic tobacco (Mapp Biopharmaceutical). Nevertheless, up till today, there is no plant made vaccine that has been approved to be marketed for human consumption. Thus, it is worthwhile to note that even though the production of plant-based vaccines had been initiated almost two decades since 1989, a few challenges still have to be overcome in order to develop them into highly efficacy vaccines.

1. Selection of Antigen and Plant Expression Host
2. Consistency of Dosage
3. Manufacturing of Vaccines according to GMP Procedures

Table no. 1: Edible vs Traditional Vaccines.[44,45]

Traditional vaccine	Edible vaccine
Too expensive to beused on large scale	Comparatively less expensive
Lack of physical Infrastructure makes it impossible to disseminate the vaccine	May be easily available
Required trained personnel to administer injections	Do not require any trained personnel to administer
Required elaborate production facilities, purification, sterilization, packaging	No purification strategies required
Can't directly stimulate the immune system	Vaccines when taken orally, can directly stimulate the immune system
Required cold chained	Not required cold chained
Required refrigeration	Heat stable
Syringe needles are required	Syringe needle are not required

Applications of Plant-based Edible vaccines[46]

1. Malaria
2. Measles
3. Hepatitis
4. Autoimmune diseases
5. Diarrheal Diseases

Current Status and Future aspects of plant based edible vaccines[47]

Plant-basedEdible vaccines hold great potential, especially in developing countries where transportation costs; poor refrigeration and needle use complicate vaccine administration. While research is also being conducted using laboratory animals, diabetics may someday benefit from an edible form of insulin. National sanitation foundation and other Government-agency and industry-funded researchers have developed technologies that allow the introduction of a hybrid gene, which produces human insulin in potatoes. For diabetics, insulin-containg potatoes may help train thebody's defenses to stop reacting to insulin as if they were a foreign material. Plant-basedEdible vaccines might overcome some of the difficulties of production, distribution and delivery associated with traditional vaccines. Significant challenges are still to be overcome before vaccine crops can become a reality. However, while access to essential healthcare remains limited in most of the world and the scientific community is struggling with complex diseases such as HIV and malaria, plant-derived vaccines represent an appetizing prospect. Strategies to improve the recombinant protein yield in plants include the development of novel promoters, the improvement of protein stability and accumulation through the use of signals that target the protein to intracellular compartments, and the improving of downstream processing technologies.

Regulatory, Ethical aspects and challenges

Plant-derived vaccines should be clinically tested under US investigational new drug application, and also must follow all the regulatory and GMP requirements.[20,21]

The future ofplant-basededible vaccine depends on many criteria. It should be well approved by the population so that it is necessary to make aware the society on the use and benefits of edible vaccines. In some areas, it is believed that genetically modified plant and products were a threat like evil spirits and destroy the world so there is a crucial role to awake the people from this myth of evil spirit by the authorities. The next important benchmark to check is the stability of the genetically modified plants and proper isolation of the plant is essential.[22,23] Sometimes the transgene causes allergies. Plant-made oral vaccines might induce allergic reactions during post-translational modifications, and oral tolerance when co-administered with oral adjuvants to mostly activate the mucosal immune system may provoke hypersensitive responses to other proteins contained in the daily food.

CONCLUSION

Plant-based Edible vaccines are produced by introducing the selected desired genes into plants and inducing the plants to produce the altered desired protein. Plant derived edible vaccines may lead to a future of safer and more efficacious immunization. From the literature-based review. It seems to be affordable, stable under various conditions and it has a novelty approach to oral immunization. Plant-based Edible vaccines successfully embraced the obstacles encountered in risingvaccine technology. Despite restrict in global access to health care and much attention still being paid towards complex diseases like HIV, malaria, etc. IN due course of time there is a need for an economical, safer and effective delivery system to be developed at a large scale in the form of edible vaccines The ray of hope is based on assuming that edible vaccines may be grown mostly in the developing countries which is basically a fact as in reality they would be used in these countries. Hence, plant-basededible vaccines provide a greater opportunity in the near future when no longer injectable needles be used but a fruitful path may be available where an individual get protect him from diseases by only eating a fruit.

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