International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR |Volume 5 | Issue 2 | Apr - Jun - 2021
www.ijpcr.net

Research article *Clinical research*

ISSN: 2521-2206

Edible vaccines: Modern Approach for Immunization

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ABSTRACT

Edible vaccines offer cost-effective, easily administrable, storable and widely acceptable as bio friendly particularly in developing countries. Oral administration of edible vaccines proves to be promising agents for reducing the incidence of varied diseases like hepatitis and diarrhea especially within the developing world, which face the problem of storing and administering vaccines. Edible vaccines are obtained by incorporating a specific gene of interest into the plant, which produces the desirable encoded protein. Edible vaccines are specific to supply mucosal activity alongside systemic immunity. Various foods that are used as alternative agents for injectable vaccines include cereals (wheat, rice, corn) fruits (bananas) and vegetables (lettuce, potatoes, tomatoes). Thus, edible vaccines overcome all the issues related to traditional vaccines and convince be best substitutes to traditional vaccines.

Keywords: Edible vaccines; Transgenic plant; Traditional vaccines

INTRODUCTION

Vaccines have proved to be boon for the prevention of infectious diseases. In spite of the worldwide immunization programme for youngsters against the six devastating diseases, 20% of infants still remain unimmunized which cause approximately two million unnecessary deaths per annum, particularly within the faraway and poor parts of the planet . This is due to the restrictions on vaccine production, distribution and delivery. This problem must resolve so as to stop the spread of infections and epidemics by un-immunized populations within the immunized, safe areas . Immunization surely infectious diseases, either don't exist or they're unreliable or very expensive like; immunization via DNA vaccines is substitute but is an upscale method, along with some undesirable immune responses. Besides being expensive, these vaccines pose the matter of storage and transportation, as many of them require refrigeration. Hence, there's look for easily administrable, storable,safe and widely acceptable bio friendly vaccines and their delivery systems especially in developing countries. Therefore, as substitutes need to be produced for traditional vaccines, it had been envisaged that plants might be promising agents for efficient production system for vaccines, which successively gave rise to the novel concept of edible vaccines.

- The evolution of vaccines has led to the discovery of new forms of vaccination that are effective and cover a wider array of disease.
- **Live-attenuated vaccines:** these are considered the original and 1st vaccines. Here, the weakened form of a live infectious organism is used as a vaccine.
- **Inactivated vaccines: t**hese are vaccines where the debris of the dead organism is used as a vaccine.

- **Toxoid vaccines:** the toxin generated by the organism is used as the vaccine. Toxoid vaccines focus on preventing the ill effects from the infection rather than the infection itself.
- **Biosynthetic vaccines:** as the name suggests, these vaccines are man-made and have very similar shape and properties to the infectious organism.
- **DNA vaccines:** plasmid DNA with sequences encoding the antigen. This plasmid DNA is then introduced directly to a specific muscle or tissue where it is expressed.
- **Recombinant vaccines:** vaccines where a recombinant plasmid with the gene encoding the antigen is expressed in bacteria. This protein is then purified and used as vaccine.
- **Edible vaccines:** the edible part of a plant is genetically modified to express antigens, thus eliciting an immune response upon consumption.

Concept of Edible Vaccines

Development of edible vaccines involves the method of incorporating the chosen desired genes into plants then enabling these altered plants to supply the encoded proteins. This process is understood as transformation, and therefore the altered plants are referred to as transgenic plants. Edible vaccines like traditional subunit vaccines contains antigenic proteins and are barren of pathogenic genes. Despite this advantage, traditional subunit vaccines are unaffordable and technology-intensive, require purification, refrigeration and produce poor mucosal response. Unlikely, edible vaccines would eliminate the necessity for trained medical personnel required for oral administration particularly in children. Production of edible vaccines is effective process and may be easily scaled up. Edible vaccines offer numerous advantages like they posses good genetic and heat stability and do not need cold-chain maintenance. Edible vaccines are often stored at the location of use thus avoiding long-distance transportation. Syringes and needles also are not required, thus reduces the incidence of varied infections[3]. Important advantage of edible vaccines is elimination of contamination with animal viruses-like the mad cow disease, which may be a hazard in vaccines developed from cultured mammalian cells, as plant viruses cannot infect humans. Edible vaccines act by stimulating the mucosal also as systemic immunity, as soon they meet the alimentary canal lining. This dual mechanism of action of edible vaccines provide first-line defense against pathogens attacking via mucosa, like tubercle bacillus and carriers causing diarrhea, pneumonia, STDs, HIV etc. Oral administration of edible vaccines to mothers might convince be useful in immunizing the fetus-in-utero by transplacental movement of maternal antibodies or the infant through breast-feeding. Edible vaccines enable the method of seroconversion within the presence of maternal antibodies, thus playing a possible role in protecting children against diseases like group-B Streptococcus, respiratory syncytial virus (RSV), etc. At present edible vaccines are produced for various human and animal diseases (measles, cholera, foot and mouth disease and hepatitis B , C and E). They can even be wont to prevent exceptional diseases like dengue, hookworm, rabies, etc. by combining with other vaccination programmes enabling multiple antigen delivery. Various foods under investigation to be used in edible vaccines include banana, potato, tomato, lettuce, rice, etc.

Mechanism of action of edible vaccines

Edible vaccines are required to induce the activation of the mucosal immune response system (MIS). The MIS is the first line of defense as it is where human pathogens initiate their infection. Mucosal surfaces are found lining the digestive tract, respiratory tract, and urino-reproductive tract. There are multiple ways by which the antigen can enter the gut mucosal layer, namely by M cells and macrophages. Macrophages are usually activated by interferon gamma. This activation leads to the macrophages presenting fragmented peptides to the helper T cells that further produce antibodies[1]. M cells are another way by which the antigens are transported to the T cells. The antigenic epitopes are then present on the APC surface with the assistance of helper T cells, which then activate B cells. Activated B cells then migrate to the mesenteric lymph nodes where they mature into plasma cells, which then migrate to mucosal membranes to secrete immunoglobulin A (IgA). IgA then forms the secretory IgA, which is then transported into the lumen. Production of secretory IgA is another complex event since 50% of secretory IgA (sIgA) in gut lumen is produced by B1 cells in the lamina propria in a T-cell-independent fashion. These sIgA are polyreactive and usually recognize the foreign antigens. In the lumen, the sIgA neutralizes the invading pathogen by reacting with the specific antigenic epitopes[1]. The most common problem most oral vaccines/ therapeutics face is the tolerance towards the vaccine in the gut. This problem can be overcome by some methods:

- Immune suppression by using triamcinolone. However, this has to be done in small amounts so as prevent any major health concerns or even fatality.
- Increasing the dosage of the vaccine significantly can often lead to jump starting the immune response.
- Multiple doses over a specific period of time as suggested by Silin and Lyubomska[3].

Developing an Edible Vaccine

The selected gene obtained from the microbes encoding specific antigen are often handled in two different ways:
1. Suitable virus is genetically engineered to supply the specified peptides/proteins. The recombinant virus is then incorporated into the plant, which enables it to supply an enormous number of latest plants from which chimeric virions are isolated and purified. The consequential edible plant vaccine can then be used for immunological applications.
2. In another method, the desirable gene is incorporated with plant vector by transformation. Many other approaches are utilized which may be categorized into following groups:

Agrobacterium mediated gene transfer

In this method, the acceptable gene (recombinant DNA) is incorporated into the T-region of a disarmed Ti plasmid of Agrobacterium; a plant pathogen, which is co-cultured with the plant cells, or tissues that must be transformed. This approach is slow with lower yield however; it showed satisfactory leads to dicotelydenous

plants like potato, tomato and tobacco. Researches in some fields have proven this approach good in expressing the desirable traits by selected genes in several experimental animals and plants.

Table 1: Transformation techniques in plants, microalgae, and bacteria

Transformation method	Plant	Microalgae	Bacteria	Reference
Agrobacterium mediated gene transfer	✓	✓	-	(4–6)
Biolistic method/ Gene gun	✓	✓	-	(7–9)
Electroporation	✓	✓	✓	(10–13)
Glass beads	-	✓	-	(14,15)
Electrospray	-	-	✓	(16)
Heat-shock method	-	-	✓	(17)

Biolistic method

This sophisticated method involves the utilization of gene gun that fires the gene containing DNA coated metal (e.g. gold, tungsten) particles at the plant cells. Plant cells are then permitted to grow in new plants, which are afterward cloned to supply ample number of crop with similar genetic composition. This approach is very attractive thanks to its undependability on regeneration ability of the species as DNA is directly incorporated into cells of plant. However, requirement of pricy device particle gun adds to the main drawback to the present method.

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.

Major Plant Species Used as Vaccine Models

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[18]. An edible vaccine against mink enteritis virus attack was developed in potatoes. Potato edible vaccine also tried against rabbit hemorrhagic virus in wild rabbits. The main benefit of producing edible vaccine from potato is the ease of transformation and propagation. There is no need of refrigerators for storing and one of the main disadvantages is cooking leads to denature of antigens[19].

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 amount 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[19, 20].

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[21].

Tomato

An effective vaccine against acute respiratory syndrome, SARS caused by corona virus 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[22]. Tomatoes have also been used to express HBsAg. An effective vaccine against the Alzheimer's disease was developed in this plant by the expression of beta-amyloid proteins. The vaccines for pneumonia, septicaemia, and bubonic plagues were developed from tomatoes. It grows quickly and can cultivate broadly. High content of Vitamin A in tomatoes may boost immune response. But it
Readily spoils[23, 24]

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[25, 26]

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[27-29].

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 were developed to express Eeg95-EgA31 of *Echinococcus ganulosus* [29].

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[30, 31].

Table 2: Edible plant vaccines for various diseases in human clinical trials.

Disease	Host plant	Reference
Hepatitis B	Lettuce	(32)
	Potato	(33)
Cholera	Rice	(34,35)
Influenza	*Nicotina benthamiana*	(36)
	Nicotina benthamiana	(37)
	Nicotina benthamiana	(38,39)
Rabies	Spinach	(40)
ETEC	Potato	(41)
	Maize	(42)

Table 3: Other therapeutic applications in current research

Disease condition	Plant used for expression	References
Auto-immune Type I diabetes	Potato and tobacco	Ma *et al*., 1995
Enterotoxic *E. coli* heat labile enterotoxin (LT□B)	Potato	Mason *et al*., 1998
Measles	Tobacco	Huang *et al*., 2001
Cancer	Rice, Tobacco	Ma *et al*., 1998; Torres *et al*., 1999
Dental caries	N. tabacum	Ma *et al*., 1995, 1999
Hepatitis B	Potato	Domansky, 1995; Richter *et al*., 2000
Colon cancer	T. benthamiana	Verch *et al*., 1998
Herpes virus	Soybean	Zeitlin *et al*., 1998
Norwalk virus	Banana, tomato	Carter *et al*., 2002
Anthrax	Tomato, spinach	Sciencedaily.com
Respiratory syncytial virus (RSV)	Tomato, potato	Sandhu *et al*., 2000

Table 4: Currently developing edible vaccines against viral diseases of human beings and animals

Virus	Plant used for expression	Target species	Route of administration	References
Enterotoxigenic E.coli	Tobacco	Humans	Oral	Joensuu *et al*., 2004
Enterotoxigenic E.coli	Potato	Humans	Oral	Tacket *et al*., 1998
Enterotoxigenic E.coli	Maize	Humans	Oral	Streatfield *et al*., 2003
Vibrio cholera	Potato	Humans	Oral	Arakawa et al., 1997
HIV	Potato	Humans	Oral	Horn *et al*., 2003
Hepatitis-B virus	Potato	Humans	Oral	Thanavala *et al*., 1995
Hepatitis-B virus	Tomato	Humans	Oral	Richter *et al*., 2000
Hepatitis-B virus	Lettuce	Humans	Oral	Prakash *et al*., 1996
Norwalkvirus	Tobacco	Humans	Ora	Mason *et al*., 1996
Norwalkvirus	Potato	Humans	Ora	Tacket *et al*., 2000
Rabies virus	Tomato	Humans	Intact glycoprotein	Prakash *et al*., 1999
Rabies virus	Tobacco	Humans	Oral	Brown.edu.com
Human cytomegalovirus	Tobacco	Humans	Immunological protein	Wright *et al*., 2001
Rabbit hemorrhagic disease virus	Potato	Rabbit	Injection	Castaon *et al*., 1999
Transmissible gastroenteritis corona virus (TGEV)	Maize	Swine	Oral	Lamphear *et al*., 2004
TGEV	Tobacco	Swine	Injection	Sciencedaily.com
TGEV	Arabidopsis	Swine	Injection	Sciencedaily.com
FMD	Arabidopsis	Bovine	Injection	Wigdorovitz *et al.1999*
FMD	Alfalfa	Bovine	Oral or injection	Dus Santos *et al* 2004
Bovine viral diarrhea virus	Alfalfa	Bovine	Oral	Aguirreburualde *et al*., 2013
Bovine rotavirus	Alfalfa	Bovine	oral	Wigdorovitz, *et al*., 2004
Peste des petits ruminants virus (PPRV)	Pigeon pea	Small ruminants	Oral	Prasad *et al*., 2004

Table 5: Live bacterial edible vaccines.

Carrier organism	Disease	Reference
Listeria monoctogenes	Influenza	(55)
	HIV	(56)
Streptococcus gordonii	HIV	(57)
Lactobacillus casei	Anthrax	(58)

Table 6: Edible algal vaccines for various diseases.

Disease	Host algae	Reference
Malaria	*Chlamdomonas reinhardtii*	(43–46)
Hepatitis B	*Dunaliella salina*	(47)
	Phaeodactylum tricornutum	(48)
Foot and mouth disease	*Chlamydomonas reinhardtii*	(49)
Classical swine flu	*Chlamydomonas reinhardtii*	(50)
White spot syndrome	*Chlamydomonas reinhardtii*	(51)
Staphylococcus aureus	*Chlamydomonas reinhardtii*	(52)
Human papilloma virus	*Chlamydomonas reinhardtii*	(53)
Hypertension (angiotensin II)	*Chlamydomonas reinhardtii*	(54)

Applications of Edible Vaccines

(i) Malaria: Malaria remains one of the most significant causes of human morbidity and mortality worldwide, with 300 to 500 million new cases of infection annually resulting in 1.5 to 2.7 million deaths. Three antigens are currently being investigated for the development of a plant-based malaria vaccine, merozoite surface protein (MSP) 4 and MSP 5 from *Plasmodium falciparum*, and MSP 4/5 from *P. yoelli*.

(ii) Hepatitis B: The hepatitis B virus is estimated to have infected 400 million people throughout the globe, making it one of the most common human pathogens. The hepatitis B surface antigen (HbsAg) is used as a vaccine against Hepatitis B.

The HbsAg subtype ayw was cloned into CaMv plasmid and the regenerated plants from the transformed cells were shown to produce HbsAg. Furthermore, expression of the antigen was found to be higher in roots of the transgenic potato than in leaf tissues.

(iii) Measles: Measles is a highly contagious viral disease caused by the *Paramyxo* virus spread by air and includes symptoms such as high fever, skin rash and spots. Each year, almost one million children die from the measles and many of the survivors are weakened by pneumonia or encephalitis or become deaf.

Recent studies report expression of the *Paramyxo* virus surface protein haemagglutinin in tobacco, potato, rice and lettuce with satisfying results.

(iv) Stopping Autoimmunity: In the past 15 years, investigators have identified several cell proteins that can elicit autoimmunity in people predisposed to Type I diabetes. The development of plant based diabetes vaccine in potato was attempted.

The development of transgenic potato and tobacco plants when fed to non-obese diabetic mice showed increased levels of IgG, an antibody associated with cytokines that suppress harmful immune response. Feeding of the vaccines to mouse strain that becomes diabetic helped to suppress the autoimmune attack and to prevent the delay of high blood sugar.

Advantages of Edible Vaccines

Potential advantages of plant-based vaccines are

i. Edible means of administration.
ii. Reduced need for medical personnel and sterile injection conditions.
iii. Economical in mass production and transportation.
iv. Therapeutic proteins are free of pathogens and toxins.
v. Storage near the site of use.
vi. Heat stable, eliminating the need for refrigeration.
vii. Antigen protection through bio-encapsulation.
viii. Subunit vaccine (not attenuated pathogens) means improved safety.
ix. Seroconversion in the presence of maternal antibodies.
x. Generation of systemic and mucosal immunity.
xi. Enhanced compliance (especially in children).
xii. Delivery of multiple antigens.
xiii. Integration with other vaccine approaches.
xiv. Plant derived antigens assemble spontaneously into oligomers and into virus like particles.

Challenges of Edible Vaccines

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[59]. These therapeutics were produced in various transgenic plants such as insulin in transgenic safflower (SemBioSys), growth factor in transgenic barley (ORF Genetics), taliglucerase alfa in transgenic carrot (Protalix BioTherapeutics), avian influenza vaccine in transgenic tobacco (Medicago), and Ebola Vaccine in transgenic tobacco (Mapp Biopharmaceutical)[59,60]. 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. The issues that need to be addressed could start from the upstream processes to the implementation of the vaccines. Generally, three main challenges are the selection of antigen and plant expression host, consistency of dosage, and manufacturing of vaccines according to Good Manufacturing Practice (GMP) procedures.

1. Selection of Antigen and Plant Expression Host. The first issue is the selection of an antigen and the right plant expression host[61,62]. This stage is very important in developing a vaccine that is able to fulfill all the requirements needed because not all antigens are compatible with the selected host plants[62].The proper and careful selections will not only help to determine the safeness of the vaccine produced, it can also be used to produce thermal-stable vaccine[62]. Meanwhile, identification of antigen candidate of poorly characterized pathogen with promising characteristics can be done by applying genomics or proteomics approaches[63].

2. Consistency of Dosage. The consistency of dosage is another challenge that the researchers have to face as dosage produced may vary within the plants of the same species, from fruit to fruit and from generation to generation due to the size and ripeness of the fruits or plants[64]. The transgenic plants show intrinsic variability in the antigen expression due to the position and pleiotropic effects caused by nonspecific integration of the transgene into the host plant genome .On top of that, it is also quite difficult to evaluate the required dosage for every patient. Levels of innate and adaptive immune responses generated in different individuals may vary based on the types of antigens being exposed in the body. Between two patients with different body weight as well as their age, the dosage of plant-based vaccine required will be different. If this issue is not monitored carefully, an immunological tolerance will be induced when the patient is overdosed while reduction in antibody production will occur when the patient is under dosed . Besides that, gene silencing might be induced due to the accumulation of Mrna in the transgenic plant cells as the growth of the plants is stopped and the fruit formation is reduced while the antigen content is increased[84]. In such case, consumption of plant based vaccines may induce allergic reaction and few side effects such as toxicity on central nervous system, cytokine induced sickness, and autoimmune diseases .

3. Manufacturing of Vaccines according to GMP Procedures. The ultimate goal of plant-based vaccines is to produce stable transgenics vaccines which are safe for consumption while reducing the production cost. Besides all the underlying issues that may affect the efficacy of plant-based vaccines, the regulatory guideline regulates by U.S. Department of Agriculture (USDA) and FDA especially the growth of transgenic plants, production and purification of plant-based vaccines, and all phases of clinical trial until marketable stage shall be strictly implemented[63]. Therefore, the manufacturers shall ensure their responsibility to follow the Good Agricultural Practices (GAP) and Good Manufacturing Practice (GMP)so that the upstream to downstream production of plant-based vaccines is strictly controlled for quality management.

Generally, to produce a plant product that could meet the quality standard, the biomanufacturing facilities must be well equipped so that complete processing cycles of the plant vaccines could be accomplished. The facilities include equipment for plant and bacterium cultivation, infiltration, plant harvest, and protein purification[64]. Takeyama et al. also summarized a few GMP plants that produce various vaccines such as influenza HA antigen, Norovirus capsid protein subunit vaccine, and rice-based cholera vaccine[65]. Concurrently, Kashima et al. reported that in order to produce a plant vaccine that meets the governmental regulatory requirements, a lot of steps and precautions need to be taken into consideration. During the production of a rice-basedoral cholera vaccine, MucoRice-CTB, the biomanufacturing agency successfully established specific techniques to maintain the seed of MucoRice-CTB. The agency further evaluated the seed's propagation and stored seeds were renewed periodically to maintain the good quality. Furthermore, cultivation of the plant using a closed hydroponic system helps to minimize the variations in vaccine production. The rice produced was polished, powdered, and packaged to make the MucoRice-CTB drug substance. Final check on the identity, potency, and safety of Muco Rice-CTB product must be conducted and only the products that met the quality requirements will be released[66]. It remains a great challenge to maintain the GMP standard for the product in plant-based vaccine industry. Besides the equipment, facilities, and method used to produce the vaccine, other considerations that have to be taken into account are those stated in the GMP guideline published by WHO[67, 68]. GMP for biological products guideline stated that some particular precautions are necessary for the manufacture, control, and administration of biological products as procedures and processes used in the production usually lead to high variation in the quality of products. Thus, the precautious steps should start from the very beginning of the production processes. However, in-process control is also important during the manufacturing of the biological products. Skillful staff is required to run the production processes and thus the biomanufacturing agency should provide necessary training to the staff. Buildings for the vaccine production must be designed in a way that operations can be carried out smoothly. A special design is required for plant vaccine production, in which the seed lots should be stored separately from other materials. Some other general rules of GMP shall be followed to maintain the quality standard of the vaccine products. These include the facts that standard operating procedures shall be implemented for all manufacturing operations, all products shall be clearly labeled, lot processing and distribution records shall be properly kept, and quality assurance and control shall be in place in monitoring the product quality.

CONCLUSION

Edible vaccine might be solution to get rid of various ailments as it has more advantages compared to traditional vaccine. It would production, distribution and delivery and could be incorporated into the immunization plans. It would be more beneficial and profitable to populations of developing world. But still there is lack of production and investment in this new technology but it will be likely conquered to make plant derived vaccine more efficient and dependable. Edible vaccines are much safer and cheaper alternatives to traditional vaccines. As any edible plant/algae, they can make scaling up so much easier. The problem with edible vaccines is the notion that genetically modified crops are bad, which prevails in many developing nations. With the ever growing and evolving technologies, genetically modified crops are getting safer than ever. There have been reports of laboratory-synthesized meat that can act as replacements for normal meat. In the near future, such meat can also be modified to deliver vaccines of interest

upon consumption. With edible vaccines popularized properly and distributed around the world, many diseases can be eradicated and millions of lives can be saved. EVs are the milestone in the branch of biotechnology for developing inexpensive vaccines that are particularly useful in immunizing people in developing countries, where high cost, transportation and the need for cold storage conditions, are hampering effective vaccination programs. Edible plant-based vaccine may lead to a future of safer and more effective immunization. The expectation is that EVs may be fully grown in many of the developing countries where they would actually be used.

REFERENCES

1. Johansen FE, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C, Brandtzaeg P. Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component– deficient mice. J Exp Med. 1999;190(7):915-22. doi: 10.1084/jem.190.7.915, PMID 10510081.
2. Walmsley AM, Arntzen CJ. Plants for delivery of edible vaccines. Curr Opin Biotechnol. 2000;11(2):126-9. doi: 10.1016/S0958-1669(00)00070-7, PMID 10753769.
3. Silin DS, Lyubomska V. Overcoming immune tolerance during oral vaccination against Actinobacillus pleuropneumoniae. J Vet Med B Infect Dis Vet Public Health. 2002;49(4):169-75. doi: 10.1046/j.1439-0450.2002.00546.x, PMID 12069268.
4. Silin DS, Lyubomska V. Overcoming immune tolerance during oral vaccination against Actinobacillus pleuropneumoniae. J Vet Med B Infect Dis Vet Public Health. 2002;49(4):169-75. doi: 10.1046/j.1439-0450.2002.00546.x, PMID 12069268.
5. Fischer R, Emans N. Molecular farming of pharmaceutical proteins. Transgenic Res. 2000;9(4-5):279-99; discussion 277. doi: 10.1023/A:1008975123362, PMID 11131007.
6. Goddijn OJM, Pen J. Plants as bioreactors. Trends Biotechnol. 1995;13(9):379-87. doi: 10.1016/S0167-7799(00)88985-4.
7. De Muynck B, Navarre C, Boutry M. Production of antibodies in plants: status after twenty years. Plant Biotechnol J. 2010;8(5):529-63. doi: 10.1111/j.1467-7652.2009.00494.x, PMID 20132515.
8. Daniell H, Singh ND, Mason H, Streatfield SJ. Plant-made vaccine antigens and biopharmaceuticals. Trends Plant Sci. 2009;14(12):669-79. doi: 10.1016/j.tplants.2009.09.009, PMID 19836291.
9. Koya V, Moayeri M, and Leppla SH, Daniell H. Plant-based vaccine: mice immunized with chloroplast-derived anthrax protective antigen survive anthrax lethal toxin challenge. Infect Immun. 2005;73(12):8266-74. doi: 10.1128/IAI.73.12.8266-8274.2005. PMID 16299323.
10. Arakawa T, Chong DK, Merritt JL, Langridge WH. Expression of cholera toxin B subunit oligomers in transgenic potato plants. Transgenic Res. 1997;6(6):403-13. doi: 10.1023/A:1018487401810, PMID 9423288.
11. Doshi V, Rawal H, Mukherjee S. Edible vaccines from GM crops: current status and future scope. J Pharm Sci Innov. 2013;2(3):1-6. doi: 10.7897/2277-4572.02321.
12. Xi JN, Graham DY, Wang KN, Estes MK. Norwalk virus genome cloning and characterization. Science. 1990;250(4987):1580-3. doi: 10.1126/science.2177224, PMID 2177224.
13. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am J Public Health. 1982;72(12):1329-32. doi: 10.2105/AJPH.72.12.1329, PMID 6291414.
14. Jiang X, Wang M, Graham DY, Estes MK. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol. 1992;66(11):6527-32. doi: 10.1128/JVI.66.11.6527-6532.1992, PMID 1328679.
15. Green KY, Lew JF, Jiang X, Kapikian AZ, Estes MK. Comparison of the reactivities of baculovirus-expressed recombinant Norwalk virus capsid antigen with those of the native Norwalk virus antigen in serologic assays and some epidemiologic observations. J Clin Microbiol. 1993;31(8):2185-91. doi: 10.1128/jcm.31.8.2185-2191.1993, PMID 8396590.
16. Mozo T, Hooykaas PJ. Electroporation of megaplasmids into Agrobacterium. Plant Mol Biol. 1991;16(5):917-8. doi: 10.1007/BF00015085, PMID 1859872.
17. Froger A, Hall JE. Transformation of plasmid DNA into E. coli using the heat shock method. J Vis Exp. 2007;6(6):253. doi: 10.3791/253, PMID 18997900.
18. Concha C, Cañas R, Macuer J, Torres MJ, Herrada AA, Jamett F, Ibáñez C. Disease prevention: an opportunity to expand edible plant-based vaccines? Vaccine. 2017;5(2):14-23. doi: 10.3390/vaccines5020014, PMID 28556800.
19. Mason HS, Ball JM, Shi JJ, Jiang X, Estes MK, Arntzen CJ. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. Proc Natl Acad Sci U S A. 1996;93(11):5335-40. doi: 10.1073/pnas.93.11.5335, PMID 8643575.
20. Oszvald M, Kang TJ, Tomoskozi S, Tamas C, Tamas L, Kim TG, Yang MS. Expression of a synthetic neutralizing epitope of porcine epidemi c diarrhea virus fused with synthetic b subunit of Escherichia coli heat labile enterotoxin in rice endosperm. Mol Biotechnol. 2007;35(3):215-23. doi: 10.1007/BF02686007, PMID 17652785.
21. Qian B, Shen H, Liang W, Guo X, Zhang C, Wang Y, Li G, Wu A, Cao K, Zhang D. Immunogenicity of recombinant hepatitis B virus surface antigen fused with preS1 epitope sex pressed in rice seeds. Transgen Res. 2008;17(4):621-31. doi: 10.1007/s11248-007-9135-6, PMID 17882531.
22. Kumar GBS, Ganapathi TR, Revathi CJ, Srinivas L, Bapat VA. Expression of hepatitis B surface antigen in transgenic banana plants. Planta. 2005;222(3):484-93. doi: 10.1007/s00425-005-1556-y, PMID 15918027.
23. Zhang X, Buehner NA, Hutson AM, Estes MK, Mason HS. Tomato is a highly effective vehicle for expression and oral immunization with Norwalk virus capsid protein. Plant Biotechnol J. 2006;4(4):419-32. doi: 10.1111/j.1467-7652.2006.00191.x, PMID 17177807.

24. Lou XM, Yao QH, Zhang Z, Peng RH, Xiong AS, Wang HK. Expression of the human hepatitis B virus large surface antigen gene in transgenic tomato plants. Clin Vaccine Immunol. 2007;14(4):464-9. doi: 10.1128/CVI.00321-06, PMID 17314228.
25. Srinivas L, Sunil Kumar GB, Ganapathi TR, Revathi CJ, Bapat VA. Transient and stable expression of hepatitis B surface antigen in tomato (*Lycopersicon esculentum* L.). Plant Biotechnol Rep. 2008;2(1):1-6. doi: 10.1007/s11816-008-0041-z.
26. Kim TG, Kim MY, Kim BG, Kang TJ, Kim YS, Jang YS, Arntzen CJ, Yang MS. Synthesis and assembly of *Escherichia coli* heat-labile enterotoxin B subunit in transgenic lettuce (*Lactuca sativa*). Protein Expr Purif. 2007;51(1):22-7. doi: 10.1016/j.pep.2006.05.024, PMID 16919472.
27. Hahn B, Jeon I, Jung Y, Kim J, Park J, Ha S, Kim K, Kim H, Yang J, Kim Y. Expression of hemagglutinin-neuraminidase protein of Newcastle disease virus in transgenic tobacco. Plant Biotechnol Rep. 2007;1(2):85-92. doi: 10.1007/s11816-007-0012-9.
28. Gómez E, Zoth SC, Asurmendi S, Vázquez Rovere C, Berinstein A. Expression of hemagglutinin-neuraminidase glycoprotein of newcastle disease Virus in agro infiltrated Nicotiana benthamiana plants. J Biotechnol. 2009;144(4):337-40. doi: 10.1016/j.jbiotec.2009.09.015, PMID 19799942.
29. Pérez Filgueira DM, et al. Protection of mice against challenge with foot and mouth disease virus (FMDV) by immunization with foliar extracts from plants infected with recombinan tobacco mosaic virus expressing the FMDV structural protein VP1. Virology. 2002;264(1):85-91.
30. Yan-Ju YE, Wen-Gui LI. Immunoprotection of transgenic alfalfa (*Medicago sativa*) containing Eg95-EgA31 fusion gene of *Echinococcus granulosus* against Eg protoscoleces. *Journal of Tropical Medicine*. 2010;3:10-3.
31. Zhang H, Liu M, Li Y, Zhao Y, He H, Yang G, Zheng C. Oral immunogenicity and protective efficacy in mice of a carrot-derived vaccine candidate expressing UreB subunit against *Helicobacter pylori*. Protein Expr Purif. 2010;69(2):127-31. doi: 10.1016/j.pep.2009.07.016, PMID 19651219.
32. Sobrino F, Sáiz M, Jiménez-Clavero MA, Núñez JI, Rosas MF, Baranowski E, Ley V. Foot-and-mouth disease virus: a long known virus, but a current threat. Vet Res. 2001;32(1):1-30. doi: 10.1051/vetres:2001106, PMID 11254174.
33. Brown LE, Sprecher SL, Keller LR. Introduction of exogenous DNA into Chlamydomonas reinhardtii by electroporation. Mol Cell Biol. 1991;11(4):2328-32. doi: 10.1128/mcb.11.4.2328-2332.1991, PMID 2005916.
34. Suzuki JY, Bauer CE. Light-independent chlorophyll biosynthesis: involvement of the chloroplast gene chlL (frxC). Plant Cell. 1992;4(8):929-40. doi: 10.1105/tpc.4.8.929, PMID 1392602.
35. Sun M, Qian K, Su N, Chang H, Liu J, Shen G. Foot-and-mouth disease virus VP1 protein fused with cholera toxin B subunit expressed in Chlamydomonas reinhardtii chloroplast. Biotechnol Lett. 2003;25(13):1087-92. doi: 10.1023/A:1024140114505, PMID 12889819.
36. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11(2):97-107. doi: 10.1046/j.1365-2893.2003.00487.x, PMID 14996343.
37. Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature. 1982;298(5872):347-50. doi: 10.1038/298347a0, PMID 7045698.
38. McAleer WJ, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. Nature. 1984;307(5947):178-80. doi: 10.1038/307178a0. PMID 6318124.
39. Yano A, Maeda F, Takekoshi M. Transgenic tobacco cells producing the human monoclonal antibody to hepatitis B virus surface antigen. J Med Virol. 2004;73(2):208-15. doi: 10.1002/jmv.20077, PMID 15122794.
40. De Muynck B, Navarre C, Nizet Y, Stadlmann J, Boutry M. Different subcellular localization and glycosylation for a functional antibody expressed in Nicotiana tabacum plants and suspension cells. Transgenic Res. 2009;18(3):467-82. doi: 10.1007/s11248-008-9240-1, PMID 19140023.
41. Komarnytsky S, Borisjuk N, Yakoby N, Garvey A, Raskin I. Cosecretion of protease inhibitor stabilizes antibodies produced by plant roots. Plant Physiol. 2006;141(4):1185-93. doi: 10.1104/pp.105.074419, PMID 16896231.
42. Hempel F, Lau J, Klingl A, Maier UG. Algae as protein factories: expression of a human antibody and the respective antigen in the diatom Phaeodactylum tricornutum. PLOS ONE. 2011;6(12):e28424. doi: 10.1371/journal.pone.0028424. PMID 22164289.
43. Gozar MM, Price VL, Kaslow DC. Saccharomyces cerevisiae- secreted fusion proteins pfs25 and pfs28 elicit potent Plasmodium falciparum transmission-blocking antibodies in mice. Infect Immun. 1998;66(1):59-64. doi: 10.1128/IAI.66.1.59-64.1998, PMID 9423839.
44. Gozar MM, Muratova O, Keister DB, Kensil CR, Price VL, Kaslow DC. Plasmodium falciparum: immunogenicity of alum-adsorbed clinical-grade TBV25-28, a yeast-secreted malaria transmission-blocking vaccine candidate. Exp Parasitol. 2001;97(2):61-9. doi: 10.1006/expr.2000.4580, PMID 11281702.
45. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12-9. doi: 10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F. PMID 10451482.
46. Correia-da-Silva M, Sousa E, Pinto MMM, Kijjoa A. Anticancer and cancer preventive compounds from edible marine organisms. Semin Cancer Biol. 2017;46:55-64. doi: 10.1016/j.semcancer.2017.03.011, PMID 28392464.
47. Mclaughlin-Drubin ME, Münger K. The human papillomavirus E7 oncoprotein. Virology. 2009;384(2):335-44. doi: 10.1016/j.virol.2008.10.006, PMID 19007963.
48. Demurtas OC, Massa S, Ferrante P, Venuti A, Franconi R, Giuliano G. A chlamydomonas-derived human papillomavirus 16 E7 vaccine induces specific tumor protection. PLOS ONE. 2013;8(4):e61473. doi: 10.1371/journal.pone.0061473, PMID 23626690.

49. Alonso LG, García-Alai MM, Nadra AD, Lapeña AN, Almeida FL, Gualfetti P, Prat-Gay GD. High-risk (HPV16) human papillomavirus E7 oncoprotein is highly stable and extended, with conformational transitions that could explain its multiple cellular binding partners. Biochemistry. 2002;41(33):10510-8. doi: 10.1021/bi025579n, PMID 12173938.
50. Smal C, Alonso LG, Wetzler DE, Heer A, de Prat Gay G. Ordered self-assembly mechanism of a spherical oncoprotein oligomer triggered by zinc removal and stabilized by an intrinsically disordered domain. PLOS ONE. 2012;7(5):e36457. doi: 10.1371/journal.pone.0036457, PMID 22590549.
51. Hormaeche CE, Joysey HS, Desilva L, Izhar M, Stocker BA. Immunity induced by live attenuated Salmonella vaccines. Res Microbiol. 1990;141(7-8):757-64. doi: 10.1016/0923-2508(90)90107-2. PMID 2101466.
52. Vilar MM, Barrientos F, Almeida M, Thaumaturgo N, Simpson A, Garratt R, Tendler M. An experimental bivalent peptide vaccine against schistosomiasis and fascioliasis. Vaccine. 2003;22(1):137-44. doi: 10.1016/S0264-410X(03)00300-1.
53. Medina E, Guzmán CA. Use of live bacterial vaccine vectors for antigen delivery: potential and limitations. Vaccine. 2001;19(13-14):1573-80. doi: 10.1016/S0264-410X(00)00354-6, PMID 11166877.
54. Yap KL, Ada GL, McKenzie IF. Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza virus. Nature. 1978;273(5659):238-9. doi: 10.1038/273238a0, PMID 306072.
55. Yap KL, Ada GL, McKenzie IF. Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza virus. Nature. 1978;273(5659):238-9. doi: 10.1038/273238a0, PMID 306072.
56. Shirai M, Pendleton CD, Ahlers J, Takeshita T, Newman M, Berzofsky JA. Helper-cytotoxic T lymphocyte (CTL) determinant linkage required for priming of anti-HIV CD8+CTL in vivo with peptide vaccine constructs. J Immunol. 1994;152(2):549-56. PMID 8283036.
57. Zegers ND, Kluter E, van Der Stap H, van Dura E, van Dalen P, Shaw M, Baillie L. Expression of the protective antigen of Bacillus anthracis by Lactobacillus casei: towards the development of an oral vaccine against anthrax. J Appl Microbiol. 1999;87(2):309-14. doi: 10.1046/j.1365-2672.1999.00900.x, PMID 10475978.
58. Oggioni MR, Medaglini D, Romano L, Peruzzi F, Maggi T, Lozzi L, Bracci L, Zazzi M, Manca F, Valensin PE, Pozzi G. Antigenicity and immunogenicity of the V3 domain of HIV Type 1 glycoprotein 120 expressed on the surface of Streptococcus gordonii. AIDS Res Hum Retroviruses. 1999;15(5):451-9. doi: 10.1089/088922299311204, PMID 10195755.
59. Faye L, Gomord V. Success stories in molecular farming-a brief overview. Plant Biotechnol J. 2010;8(5):525-8. doi: 10.1111/j.1467-7652.2010.00521.x, PMID 20500680.
60. McCarthy M. US signs contract with ZMapp maker to accelerate development of the Ebola drug. BMJ. 2014;349:Article ID g5488. doi: 10.1136/bmj.g5488, PMID 25189475.
61. Rigano MM, Walmsley AM. Expression systems and developments in plant-made vaccines. Immunol Cell Biol. 2005;83(3):271-7. doi: 10.1111/j.1440-1711.2005.01336.x, PMID 15877605.
62. Sharma M, Sood B. A banana or a syringe: journey to edible vaccines. World J Microbiol Biotechnol. 2011;27(3):471-7. doi: 10.1007/s11274-010-0481-9.
63. Streatfield SJ. Plant-based vaccines for animal health. Rev Sci Tech. 2005;24(1):189-99. doi: 10.20506/rst.24.1.1559, PMID 16110888.
64. Mishra N, Gupta PN, Khatri K, Goyal AK, Vyas SP. Edible vaccines: a new approach to oral immunization. Indian J Biotechnol. 2008;7(3):283-94.
65. Takeyama N, Kiyono H, Yuki Y. Plant-based vaccines for animals and humans: recent advances in technology and clinical trials. Ther Adv Vaccines. 2015;3(5-6):139-54. doi: 10.1177/2051013615613272, PMID 26668752.
66. Kashima K, Yuki Y, Mejima M, Kurokawa S, Suzuki Y, Minakawa S, Takeyama N, Fukuyama Y, Azegami T, Tanimoto T, Kuroda M, Tamura M, Gomi Y, Kiyono H. Good manufacturing practices production of a purification-free oral cholera vaccine expressed in transgenic rice plants. Plant Cell Rep. 2016;35(3):667-79. doi: 10.1007/s00299-015-1911-9, PMID 26661780.
67. World Health Organization. Good manufacturing particles for biological products. World Health Organ Tech Rep S 822. 1992.
68. World Health Organization. Report WHO informal consultation on scientific basis for regulatory evaluation of candidate human vaccines from plants. WHO quality assurance and safety of BioLogicals, 2005.

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