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Review Article

Edible Vaccines-The Current Scenario

***Karpagam .N[1], Sathiyanathan. M[1], Gopalakrishnan .M[1]**

Kavina .J[1] and Sivanandham .M[2]

[1] Department of Botany, Pachaiyappa's College,
Chennai 600 030, India.
[2]Department of Biotechnology, Sri Venkateswara College of Engineering,
Sriperumbudur 602 105, India.
*Corresponding Author Email id: mkarpagammurali@gmail.com

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Abstract:
A vaccine in which an antigenic protein is engineered into an edible plant, which after ingestion can be recognized by the immune system, is called an edible vaccine. Edible vaccine was a concept conceived about a decade ago which then seemed to be a fantasy. A decade later, the fantasy is rapidly becoming a reality. Edible vaccines now find applications from prevention of autoimmune diseases, in birth control and cancer therapy though it was initially thought that it could only be used as a preventive mechanism against the ever increasing number of communicable diseases. Edible vaccines have great potential to become an excellent alternative to conventional vaccines but it remains to be a subject of debate even now. Though the concept of edible vaccines has an appreciably large number of benefits it also has a number of disadvantages to overcome and innumerable critics to be silenced before being universally accepted. Edible vaccine provides easy production, maintenance, transportation, storage as well as a successful method to deliver the promised action of the vaccine of choice. The concept is a boon for many developing and under-developed countries which face numerous problems with the lack of proper storage facility, poor transport means, lack of economic support and the innumerable diseases. This review attempts to study the concept of edible vaccines and their development, their mode of action, the choice of expression system, the choice of host plant, the environmental concerns as well as the scope for future products.

Keywords: Immunogen, Edible vaccine, *Agrobacterium* based transformation, biolistics, viral vector systems

Introduction:

The immune system is a dynamic structure in our body that protects us from various pathogens. Immune system continuous tracking of molecules which circulate within the body to detect substances which negatively affect our health. Once the foreign bodies (pathogens) are identified, the immune system attacks to neutralize them with the help of antibodies [99].The idea of vaccination against diseases was conceptualized by Dr. Edward Anthony Jenner in

the year 1796, when he found that people who were inoculated with the cow pox virus did not catch the dreadful small pox disease which was causing thousands in deaths in Europe. An outbreak occurs when several cases of a particular disease increase more than expected in a specific region over a specific period. In the last three decades numbers of highly transmissible or pathogenic infectious disease like Zika, Ebola, monkeypox, SARS, measles, polio, cholera diphtheria cases have increased in many parts of the globe [94].Though the concept of vaccination is over two centuries old not many diseases have been wiped out from the face of the Earth, small pox being one of the few exceptions. The main reason for this is the fact that with the rise in the globalization phenomena the world is becoming a smaller place and this leads to the spread of many dreadful diseases. Developed countries, with their outstanding system of public health departments and immensely efficient and accurate immunization programs have little to worry about this issue. But Developing and the underdeveloped countries are the ones who are affected because most of these countries lack the proper facilities, knowledge, finances and economic needs required to immunize its people against the dreadful pathogens besides the poor sanitary and hygiene also add to their woes. The outcome is that more are more people are being infected with many diseases, there are newer diseases which have arisen each day and thousands of people are dying all over the world. There are innumerable vaccines-of various types, that are available today which include the attenuated vaccines, live vaccines, subunit vaccines, toxoids and the conjugate vaccines being the immensely popular ones with many others like DNA vaccines, dendritic cell vaccines and recombinant vaccines that are being tested. The contemporary vaccination is a solution but it has its drawbacks; the developing countries and the underdeveloped countries cannot bear the cost of these medicines, besides there are no proper storage facilities that are available to store the vaccines besides lacking trained professionals and proper transport means. To overcome all these problems edible vaccines can be used as an effective alternative.

The mechanism of the vaccine as in any vaccine is the external induction of immunity so as to protect against the infectious diseases. The process involves administering the person with a minimal amount of any form of the immunogen and waiting for the triggering of the immune systems response. This is effectively priming of the immune system. Once the immune system is triggered, there is production of antibodies against the antigen besides there being memory cells being produced which can recognize the targeted immunogen in the event of another attack thereby immunity is provided against the particular immunogen. Hence a new alternative approach comes into the picture nowadays that is “edible vaccine” refer to the use of edible plant/vegetable parts or probiotics (live microorganisms) as a vaccine, which is taken orally as whole or parts of plant/vegetable. The concept of “edible vaccine” first given by “Charles Arntzen” in 1990[93].

The concept of edible vaccines:

In 1990, Dr. Arntzen introduced the concept of using transgenic plants to produce and deliver subunit vaccines. This idea of Arntzen proved thatthe edible vaccine can annihilate the restrictions in the production of traditional vaccines. In tobacco plant, (Streptococcus mutants) a surface antigen is expressed from hepatitis B by Mason et al. is the milestone in edible vaccine production[97].In parallel to the production of edible vaccine in tobacco, they also started the production of hepatitis B and heat-labile toxin B in potato and potato plants [97]. These vaccines have an indefensible advantage of over traditional conventional vaccines. Particularly in the developing world, edible vaccine offer exciting possibilities of reducing

the burden of diseases such as hepatitis b and diarrhoea where storing and administering vaccine are often major problems. For production of edible vaccines or antibodies, it is desirable to select suitable plants, algae, yeast, insect cells and lactic acid bacteria whose products are consumed raw to avoid degradation [97,98].

In 2018, around140,000 measles deaths were observed globally, mostly among children under the age of five, even after the availability of a safe and cost effective vaccine. The oral efficacy of measles oral live attenuated vaccine is absent and when maintained on a cold chain of refrigeration they are destroyed. Due to the presence of maternal antibody, the effectiveness is reduced. The advances in genetic engineering in the 1980's led to the introduction of first field trials to be conducted in 1986, with herbicide tolerance used as a marker gene in tobacco, in the USA and France[1]. Since then it was thought that genetic engineering of plants could bring about various benefits and could introduce many new techniques to breed transgenic plants and also introduce various genes into the plants. It was therefore a great step when in 1989 Hiatt and co-workers[2] attempted to produce antibodies in plants which could serve the purpose of passive immunization. In 1992, the concept of using tobacco plants which were genetically transformed with the gene encoding hepatitis B surface antigen (HBsAg) linked to a nominally constitutive promoter was carried out successfully. The presence of recombinant HBsAg in the genetically modified tobacco which showed the properties-antigenically and physically similar to those HBsAg particles derived from human serum and recombinant yeast, which are used as vaccines, it was concluded that the transgenic plants could be used as an effective alternative to the vaccines[3]. The production of the edible vaccine using tobacco hence made geneticists believe that plants could be used to produce edible vaccines for many other diseases. Antigens or antibodies expressed in plants can be administered orally as any edible part of the plant, or by parenteral route (such as intramuscular or intravenous injection) after isolation and purification from the plant tissue. The edible part of the plant to be used as a vaccine is fed to prevent possible denaturation during cooking, and avoided cumbersome purification protocols.

Second generation edible vaccines are the latest trend and this involves multicomponent vaccines (vaccines that provide action against two or more pathogens). The multicomponent vaccines were tested on mice where the vaccine was based on epitope fusion to subunits of the cholera toxin. The cholera toxin acts as a scaffold for presentation of rotavirus and Enterotoxic *E.coli* epitopes and acts as a vaccine and also gives adjuvant activity[4]. 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 plants[5].Oral tolerance essentially allows us to eat without detrimental immunological reaction to components of our food. Once a mechanism to overcome oral tolerance is found, it is envisioned that fruits and vegetables from our diet will be used to produce the vaccines. It is envisioned that plant material will be dried and packaged in capsules for oral delivery. It is hoped that the edible vaccines will not require refrigeration and will be significantly cheaper to produce[92].

Plant hosts for edible vaccines:

The plants used for edible vaccine production include tobacco, lettuce[6], alfalfa[7], potato[8], tomato[9], maize[10], rice[11]and soya bean[12].

Though the host being chosen is dependent on the level of expression, the vaccine can have in that plant and the extent of ease with which a host can be transformed; one of the major criteria for choice of the host is the edibility besides

having high nutritive and protein content. 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[91].A list of host plants, which are used in the production of edible vaccines, is given in Table 1. From the table, potato, for example, can be relatively easily transformed using Agrobacterium mediated methods but the fact that potato is practically difficult to be eaten raw makes it a bad host[13].Tobacco was the first used plant to produce plant vaccine for Hepatitis B surface antigen, but tobacco too is difficult to be taken via oral route[3]. Lettuce[14] and tomato[15] are good candidates for the vaccine production. Banana is the top contender for the production of edible vaccines not only because banana provides good transformation efficiency but also due to the fact that banana is edible raw and also because banana is a fruit that can be grown in almost in all the developing countries[16]. Analyzing from an economic point of view, alfalfa, soybean and corn are preferred because the yield to planting area ratio is high for these plants besides the requirements for these plants are minimal and they can easily be grown in green houses[17].

Hence the choice of a suitable host depends on various aspects like the transformation, the nature of the part (edible or non edible) of the plant under consideration, the ease of growth, the time of growth cycle, the environmental requirements and economic constraints[18].Edible vaccine might be solution to get rid of various ailments as it has more advantages compared to traditional vaccine. Edible plant-derived vaccine may lead to a future of safer and more effective immunization[19]. Edible vaccine holds a great promise to the field of molecular biology and genetic engineering. It has given a new concept by creating genetically engineered plants, whose edible parts contain proteins that can function as oral vaccines or food vaccines. These oral vaccines are produced by molecular farming techniques with the help of genetic engineering. They can be one of the alternatives over conventional vaccines to overcome many autoimmune diseases[20].

Table 1: Host Plants For Edible Vaccines With Advantages And Disadvantages[19].

PLANT	ADVANTAGES	DISDVANTAGES
Tobacco	Easy transformation, easy culture, large volumes of tissues can be got, very good yield and quite easy to cultivate.	Not palatable, toxic alkaloids also hinder the use of the crop as edible vaccine.
Lettuce	Edible raw, easy transformation, easy to culture and easy to grow.	Low yields of the recombinant protein.
Tomato	Edible raw, easy transformation and easy to green house culture.	Acidic nature of the fruit hinders their use and poor protein yield.
Banana	Can be cultivated in most countries, edible raw.	Low yield, difficult to transform, lack of fruit specific promoters and difficult to grow in green houses.
Potato	Easy transformation grows in most conditions and good protein storage capacity.	Not edible raw. Cooking alters the protein.
Alfalfa	Very high yield, easy transformation and edible raw.	No major disadvantages.
Cereals	Easy cultivation, stable protein content and high yield.	May be difficult to transform. Difficult to be eaten raw by humans.

Methods of expression in plants:

Two different expression methods can be used to produce antigen vaccines. They are stable genetic transformation where there is production of a genetic line propagated using vegetative propagation or from seeds in the case of sexual reproduction, and transient transformation where

a recombinant plant virus is used as a mode to carry the gene for the antigen and can be expressed in the plant via infection[20].

It is to be noted that there are three compartments in the plant that stores genetic information; they are, the nucleus that follows Mendelian rules of inheritance, mitochondria and plastids that follow Non-Mendelian characteristics[21].

Stable transformation involves integration of the recombinant DNA (with the vaccine antigen gene) into the host genome (chloroplast or nucleus). Meanwhile, in the case of transient transformation there is no integration of the gene with the host genome. The gene may exist independently with its own replication cycle[22].

Both stable and transient techniques have both advantages and disadvantages. Stable transformation is generally obtained using Agrobacterium mediated processes. The advantage of stable transformation is that the gene of interest is fused into the nucleus and thus it can be assured that the gene would be expressed even in the future generations[19]. A stable transformation can initiate easier scale-up and lesser problems of selection every time to check if the plant is transformed. The disadvantage of stable transformation is that in many cases the yield of the protein is quite low when in comparison with the transient cases[19]. Besides, there are chances that the stable plant can lead to cross pollination of other non-transformed plants causing unwanted complications.[20]

In transient transformation that is got generally via biolistic methods or using viruses in some cases[21], the advantage is that the expression is very high when compared to the stable transformed plants. The disadvantages are a large number of transformations are required to get substantial amount of the product as the recombinant protein will not be prevalent in the future generations. Though nuclear genome is generally preferred and widely studied for transformation, the chloroplast genome can also provide an alternative. Mitochondrial genome is not generally preferred owing to their small genome size and difficulty in doctoring the genome to suit the needs of transformation. Plastids of higher plants are cellular organelles with circular, double-stranded genomes of 120–160 kilobases in size. The genome of each plastid encodes approximately 120 genes: each cell contains up to 10000 identical copies of each plastid gene[23]. Chloroplasts though not used for transformation in the early edible vaccine development years now there is interest in researchers to try this alternative. A detailed review of using plastids for transformation was done by Bock Ralph in 2001 and since then the idea of using plastids for transformation was thought by researchers to be a good alternative[24, 76, 77]. To obtain a genetically stable plant, all genome copies have to be uniformly transformed. Plastid transformation is obtained through the following steps: first, the introduction of transforming DNA that encodes a selectable marker (e.g. an antibiotic resistance gene) by the biolistic process or by polyethylene glycol treatment; second, the integration of the transforming DNA by two homologous recombination events and third, the gradual elimination of wild type genome copies during repeated cell divisions on a selective medium[25, 78]. Advantages of incorporating the transgenes in the plastid genome are: containment of transgenes due to the lack of pollen transmission (due to Non Mendelian characteristics of plastids); expression of multiple genes in operons; high expression levels; possibility of expressing unmodified bacterial genes and human cDNAs; and lack of gene silencing and position effects. The disadvantage is that the proteins are not generally exported to the cytoplasm[26].

Bacterial vectors for gene transformation:

The most commonly used technique to achieve transformation is using Agrobacterium bacteria

to cause chromosomal integration. The Agrobacterium mediated method involves utilizing the natural ability of the species to infect the plants and cause tumor in the plant of interest. It infects the wounded sites of the plant and causes the tumor[27]. Virulent strains of A. tumefaciens and A. rhizogenes, when interacting with an injured dicotyledonous plant cells, induce crow gall and hairy roots, respectively[28].

The best example for bacterial carriers is Listeria monocytogenes. Listeria monocytogenes is a Gram-positive bacterium that mediates cell response against its own proteins. What makes L. monocytogenes special is its ability to breach into the cytoplasm of the host, thus allowing the recombinant protein into the antigen-processing pathway. This makes it very effective in clearing bacterial, viral, and parasitic pathogens, and tumors [95]. Mutations in the virulence-related genes are exploited to make suitable vaccine carriers. They are also known to protect against tumors by producing tumor associated antigens.

Agrobacterium tumefaciens has the ability to transfer the T-DNA fragment of the tumor-inducing (Ti) plasmid into the genome of the infected plant and cause crown gall disease in the plant. The T-DNA cannot be used as a whole to cause the transformation as it has two types of genes: the oncogenic genes that encode auxins and cytokinins promoting enzymes that are responsible for tumor formation and the genes that encode opines[29]. Outside the T-DNA of the Ti plasmid are the genes for opine catabolism, the genes of T-DNA transfer from the bacterium to the plant cell and the genes for plasmid conjugative transfer. The bacterium however is engineered to not cause unwanted tumor growth in the infected plant when used for laboratory purposes. When in use for transformation, the Agrobacterium as such is not very effective in giving transformation as desired by the researcher. Ti plasmid is engineered as a cointegrate vector or a binary vector for effective transformation[30-35].

Agrobacterium mediated transformation remains the method of choice for dicots like soyabean[36, 37, 79]. There are also works being carried out to induce Agrobacterium mediated transformation in monocots[38].

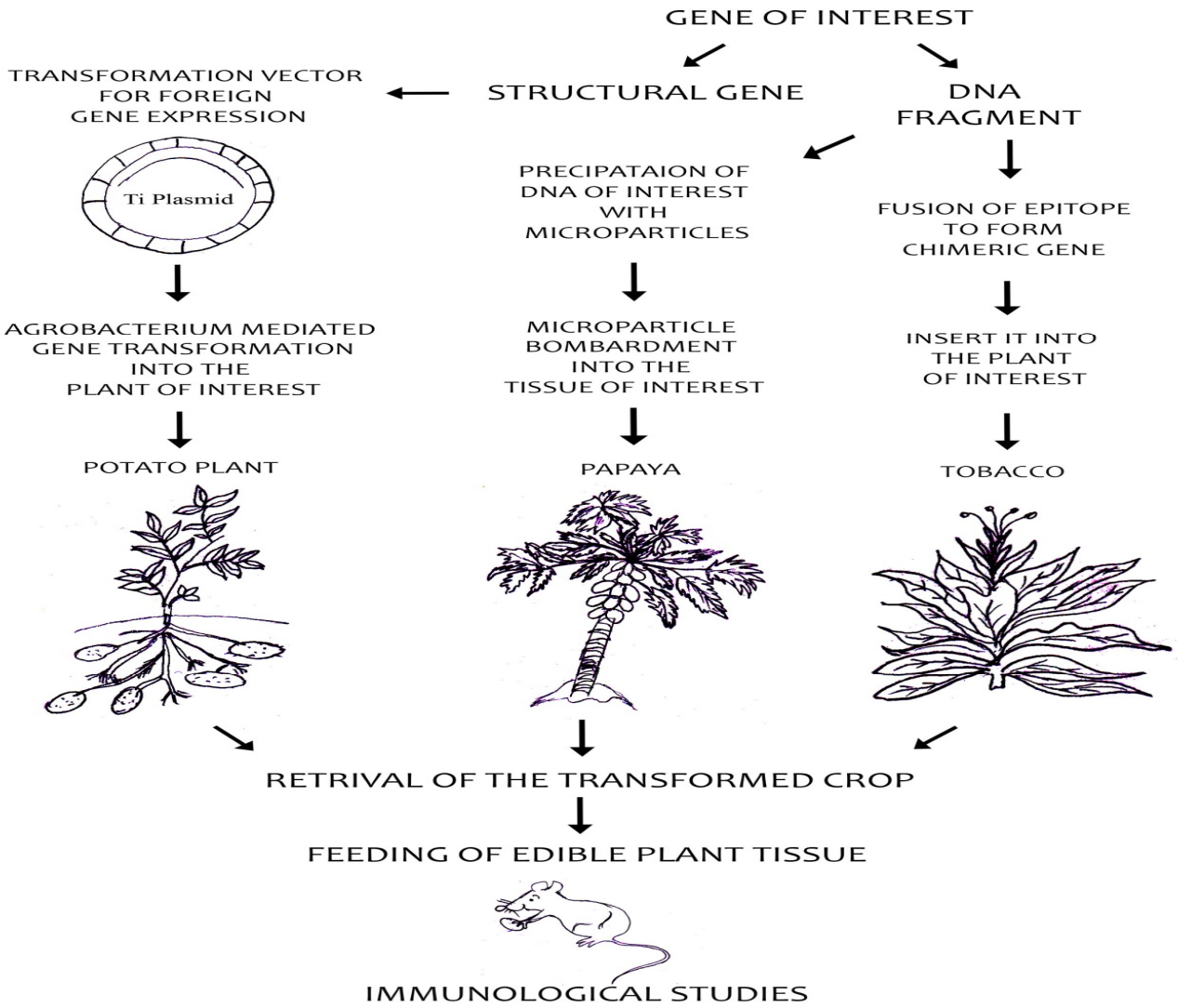
Plant derived edible vaccines have been engineered using *Agrobacterium tumefaciens* using lettuce as the plant host against Hepatitis B virus[15]. Other examples for Agrobacterium mediated edible vaccines include potatoes against cholera toxin[39] and Streptococcus mutants surface antigen against dental carries using tobacco plant[40].

Biolistic methods for gene transformation:

Biolistics is another method generally used to mediate transformation in plants. This method is highly preferred for Agrobacterium recalcitrant crops like corn[36] and for chloroplast transformation in tobacco[37]. Papaya transformation using biolistic methods is another big achievement of the gene gun method where stable transformation was achieved in papaya[38]. Biolistic is a combination of words like biological and ballistics and it refers to biological ballistics. The biolistic approach is based on the microprojectile bombardment method[20]. An Overview of gene insertion methods for edible vaccine production is shown in Figure 1, where the technique involves precipitation of DNA sequences on metal microparticles particles like gold and bombarding them against the tissue of interest with the help of a particle gun that accelerated the microparticle. 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 possible90. The particles deliver the gene of interest to the plant tissue.

The method can lead to insertion of the gene into the nuclear or chloroplast genome or can also exist as an independent extrachromosal subunit. The nature of the mechanism by which the insertion occurs is not known very well. The biolistic method gives higher copy-number integration of the gene of interest in certain cases than Agrobacterium, which can in certain instances give enhanced expression[34]. High level expression or enhanced expression of nuclear genes can cause gene silencing and give low expression levels. Hence it is imperative to select transgenic lines carefully[40]. The biolistic method of transformation has been successfully achieved in tobacco by using the chloroplast genome to enhance the insert to express *pagA* gene coding for anthrax immunogen in a better way than the nuclear insert[41]. The biolistic method is also used to integrate the gene of interest into the chloroplast genome[42].

Figure 1. Overview of gene insertion methods for edible vaccine production.



Viral vector for gene expression:

Transient gene expression is generally obtained using viral vectors. Some of the viral vectors, their proteins, the target plants and their nature of

expression are shown in Table 2. Initially, plant viral vectors with DNA as genetic material were preferred to insert the gene of interest into the plant but it ended in failure. The problem majorly involved complexity in the replication cycles of these viruses with the relatively big inserts[43]. The vast majority of plant viruses have genomes that consist of one or more molecules of positive-sense RNA. These viruses can grow in a wide range of hosts, and some can reach extremely high values. The ability of the genomes of positive-sense RNA plant viruses to be directly translated into the respective proteins on entering a plant cell has made them a very attractive choice to express recombinant proteins in plants[44].

Human papilloma virus. The human papilloma virus is responsible for almost 6.1% of allcancer cases worldwide. Of those, 99.7% are agents responsible for cervical cancer. More than half the cases are caused by HPV16 [96].

Two basic types of expression system based on RNA plant viruses have been developed for the production of vaccines in plants; they are epitope presentation systems and polypeptide expression systems[20].

In epitope presentation systems, the genes of interest is fused with the coat proteins and expressed on the surface and these proteins can be readily purified[45]. If epitopes within the antigens can be identified the gene fragment encoding the epitopes can be utilized to produce chimeric viruses by fusion with a protein coat gene of a plant virus. Cowpea mosaic virus (CPMV) was the first plant virus to be developed as an epitope presentation system[46]. The other viruses used include Tobacco mosaic virus (TMV)[47], Tomato bushy stunt virus (TBSV)[48], Plum pox virus (PPV)[49], Potato virus X (PVX)[50]and alfalfa mosaic virus[51].

In polypeptide expression systems, express a whole unfused recombinant protein that accumulates within the plant[51, 52]. This method does not make it necessary to know the epitope of interest but the whole polypeptide is expressed. This is not a hinderance to the process because in the case of edible vaccine the whole fruit or the plant part is eaten.The transient transformation method also has various advantages like the viral genomes as being small and easily manipulated[80], simpler infection of plants with modified viruses and quicker regeneration of transformed plants, and the sequence inserted into a virus vector will be highly amplified[53].

Mode of action:

To understand the impact of adjuvant strategies on the immuneresponse to lactic acid bacteria (LAB) mucosal vaccine, it's essential to explore the endogenous immune activating mechanisms possessed by LAB [100]. The features that make LAB particularly attractive to be used as mucosal vaccine vector is that it ability to stimulate innate immunity response through its Gram-positive cell wall of lipoteichoic acid and peptidoglycan that activate pattern-recognition receptors such as nucleotide-binding oligomerization domain (NOD)-like receptor(NLR) family, toll-like receptor (TLR) 2, and C-type lectin receptors. Different LAB species can activate TLR9, TLR6, and TLR3 as well as stimulate interferon responses [100]. In addition, some LAB stains can bond to intestinal mucus and mucosal epithelium or microfold (M) cells leading to mucosal colonization and increase uptake and transport to the mucosal immune induction sites such as Peyer's patches and tonsil arcrypts. Lactic acid bacteria can interact with APCs such as induced IgG and SIgA and dendritic cells [90]. The mechanism of activation of dendritic cells and the resulting immune response depends on the lactic acid stain. For example, the response of murine DCs can respond differently depending on LAB stains and is further complicated by the fact that these responses can be different even between DC subtypes. It shows the complexity

of choosing a suitable LAB stain as a vaccine vector.

One of the most prominent entry routes for pathogens is through the mucosal surface lining in the respiratory, digestive and the urino-reproductive tracts. The mucosal immune system is the first line of defense and is hence the most suited site for vaccination against those pathogens that try to gain entry to the host through the mucosal system[54].

The antigens in transgenic plants are delivered through bio-encapsulation, the tough outer wall of plant cells acts as a covering which protects them from gastric secretions, and finally break up in the intestines for effective delivery of the antigen in the intestine[55]. The antigen uptake mechanism involves the small intestine which acts primarily via nonspecific of the contents in the gut lumen (where the food gets transported to) by M cells on the Peyer's Patches (lymphoid organ) and other lymphoid components of the gut-associated lymphoid tissue (GALT)[56]. Thus there is lympohocyte activation and generation of IgA immune response and in addition there is also the sensitization of IgG and IgE suppressor cells that accompanies the suppression of serum IgG and IgE responses[57].

In the case of Cholera toxins[58] and E.coli[13], heat labile enterotoxins are potent antigens but when they are coupled with other antigens they act as adjuvants and enhance the immunogenicity because these toxins can assure better antigen uptake and presentation which in turn helps the system to provide better response[81]. These adjuvant systems can be classified under the second generation edible vaccines.

Table 2: Edible vaccines, their carriers and their nature of expression.

Application	Protein	Plant	Carrier	Expression	Genome of Insert	Ref
Influenza	Hemagglutinin	Tobacco	TMV	Stable	Nuclear	59
Hepatitis B	Recombinant HbS antigen	Tobacco	TMV	Stable	Nuclear	60
Cancer	C-myc	Tobacco	TMV	Stable	Nuclear	59
Rabies	Rabies virus glycoprotein	Spinach	AIMV	Stable	Nuclear	9
HIV	HIV epitope(gp41)	Cow pea	CPMV	Stable	Nuclear	61
Malaria	Malarial B-cell epitope	Tobacco	TMV	Stable	Nuclear	62, 63
Dental Carries	Streptococcus mutans surface protein SpaA	Tobacco	AMT	Stable	Nuclear	40
Rhinovirus	Human rhinovirus epitope(HR14)	Blackeyed bean	CPMV	Stable	Nuclear	39
Cholera	Vibrio cholera toxin CtoxA and CtoxB subunits	Potato	AMT	Stable	Nuclear	39
Foot and mouth disease	Foot and mouth virus epitope (VP1)	Blackeyedbean	CPMV	Stable	Nuclear	59

Advantages and Limitations of Edible Vaccines:

Edible vaccines have been the subject of research for various research groups and many of them have been successfully patented. Further research is undergoing in order to make them commercially available, on a large scale. Some of the patent holders, their names and the vaccine functions are given in Table 3. Edible vaccines, though they are thought to have given a scientific breakthrough in the field of medicine for their various advantages, they still have some disadvantages which have to be considered in detail.

Advantages of Edible Vaccines:

The major advantages of edible vaccines are:

- Ease of delivery:The vaccine is delivered orally hence there is no need for experts in delivering the vaccine[81, 82]. Besides, the presence of the cell wall in plant parts guarantees proper delivery to the stomach and slow release of the antigen into the body.

- Storage:The plant parts of interest do not require any special storage conditions like a cold storage in the case of classical vaccines.
- Low cost of growing plants: The plants are easier and cost efficient to grow when in comparison with the bacterial or viral systems which have major economical problems like cost for preventing contamination and the need for special equipments.
- Immune response:Edible vaccines are primarily designed to trigger the mucosal immune system,thus preventing pathogen entry at mucosal surfaces; they also elicit serum and cytotoxic responses.
- Ease of scale up: It is relatively very easy to scale-up because there is no need to design special equipments or formulation of medium; the only needed action is planting the crop in a larger area[83].
- Optimized production in organs: Plants may be engineered to accumulate the antigen in convenient intracellular compartments. This can also induce easy recovery as per the organelle in which the protein is stored.
- Safety: Lack of contamination with mammalian pathogens unlike in the case of traditional vaccine production.
- Ideal for veterinary use: Cost affordable when in comparison with traditional vaccines.
- Elimination of need of purification: There is no need to purify the product out if the vaccine is to be taken as a whole plant part-fruit, leaf, tuber, etc.

Limitations of Edible Vaccines:

The major limitations edible vaccines are:

- Difficult to zero on a dosage: It is quite an impossible task as of now to identify the correct dosage a particular plant product can give.
- The stability of the vaccine: The exact mechanism the vaccine might be subjected to the event of adverse effects like temperature or humidity is unknown.
- Low accumulation levels of recombinant proteins: The accumulation levels of the proteins in the plants generally tend to be low even though in some cases promoters may be used to boost up the production rate[84].
- Insufficient information on post translational events: The post translational events in the plants are different from the other systems.
- Lack of data on downstream processing: Not much has been reported on the downstream processing mechanisms to be followed to get the desired product-vaccines but this is not a problem if the whole plant part of interest is consumed.

Table 3: Major Patents in Plant Vaccines.

Patent Holder	Patent Claim	Ref
University of Yale	Vaccines against invertebrates	64
Rubicon Lab	Retrovirus expressed in plant cells useful as virus or cancer vaccine	65
Applied Phytologics	Gene constructs for vaccine production in cereals	66
Biosource	Plant viral vector with potential as anti AIDS vaccine	67

Environmental and Social Concerns:

The release of the genetically modified plants into the environment is a major concern. It is highly possible that the transformed plants may cross pollinate another plant and cause 'contamination' of the plant produce[68, 85]. This cross pollination may essentially cause major environmental problems with the extreme case of disturbing the ecosystem to a large extend[55, 71]. There can also be problems like the vaccine leeching out into the ground or pollute the ground water[72]. This can cause major health problems to the people inhabiting the areas where the vaccine-plant is being grown[73]. The grazing animals may also be tempted to graze on the plants[74]. The only way to overcome these potentially deadly problems is by growing the transformed plants in strictly restricted places

like a greenhouse and also artificially inducing sterility in the plants[69, 70, 75]. A large number of edible species, such as lettuce, tomato, potato, papaya, carrot, quinoa and tobacco has been converted into vaccine antigens. In animal models the appropriate folding and improved expression of the antigens obtained in these processes have been screened[92]. Vaccination is a huge medical advancement that has saved a countless number of lives and could potentially save many more.

Conclusions:

Edible vaccines have immense potential in them to become a big phenomenon due to their distinct advantages like cost, ease of production, storage, distribution and delivery besides the possibility of incorporating the vaccines into immunization plans. They may also help overcome the problems posed by traditional vaccines like: toxicity, the antigen reverting back to a virulent state and allergic reactions. The edible vaccines have a major role to play in the under-developed and developing countries especially in the event of an epidemic outbreak. The ease of storage, transport and administration are also major advantages of edible vaccines. Even with immense advantages there are very few edible vaccines commercially available in the market though there are a large number of trials being conducted on these vaccines. It is likely that all the challenges projected in developing plant based vaccines would be overcome in the coming decade and hopefully there would be many vaccines in the market.

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