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Review Article

Edible Plant Vaccines: A Step Towards Revolution in the Field of Immunology

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Abstract

Plants that express vaccine antigens are a viable strategy since recent advancements in immunology and molecular biology have resulted in the creation of novel vaccination tactics. There are a variety of systems to deliver vaccines but transgenic plants provide alternative means for the manufacture and distribution of vaccinations. It is less expensive, takes less time to transport, needs less refrigeration, and for a long time, it may be kept at room temperature to make in-plant edible vaccines. As a result, vaccines made from plants could be more broadly accessible in underdeveloped countries. Besides the advantages, there are a number of challenges in creating an edible vaccine, including dose, antigen choice, public image, the shelf life of the plants used as vaccines, and differentiation between "vaccine" fruit and regular fruit to prevent improper vaccination. Edible plant vaccines can increase both mucosal and systemic immunity. The best plants for making edible vaccines include tobacco, potato, tomato, and banana. Up to this point, plant-based vaccinations have been created to protect against illnesses including Cholera, Hepatitis, Malaria, Newcastle disease, Measles and the Rabies virus. Animal studies have shown that antigenic proteins produced from plants can delay the development of illness, and human therapeutic trials have demonstrated their safety and efficacy. There are currently very few approved vaccines made from edible plants that are being tested on both humans and animals. This paper examines recent developments, manufacturing techniques, and applications for edible vaccines.

Keywords: Edible vaccines; Antigen; Tobacco; Hepatitis; Mucosal immunity; Plants

Introduction

Immunization is a procedure that fortifies the body so that it can defend itself from any upcoming pathogenic invasion. A sort of immunization, vaccination is the process of giving out and delivering vaccines (Malik *et al*. 2011). Vaccines are intended to strengthen the immune system without actually transmitting disease. Conventional vaccinations contain disease-causing organisms that have been killed or diminished, making them not entirely safe. Their downsides include antigenic diversity among species, a low level of immunogenicity, a propensity for gene transfer in wild-type strains and a high probability of turning virulent again (Mishra *et al*. 2008; Chan and Daniell 2015). A large poliomyelitis outbreak was started in northern Nigeria in 2005 by a type 2 circulating vaccine-derived poliovirus (cVDPV2), which spread to the rest of Africa in 2006 and is still active today. In order to improve current immunizations, new strategies are required (Famulare and Hu 2015).

Edible plant vaccines

Previous research has demonstrated that transgenic plants may be used to generate and transport vaccinations. Antigens originating from numerous diseases can be produced in plants in high quantities and in their natural forms (Streatfield *et al.* 2001; Pyrski *et al.* 2019). Edible vaccines primarily include antigens and do not contain whole pathogen-forming genes. Since plants may produce more than one transgene, they can provide several antigens for recurrent inoculations. Edible vaccinations for illnesses such as cholera, foot and mouse disease, measles, hepatitis B and C6 are now being developed. The first study of the edible vaccine (a surface protein from *Streptococcus* found in tobacco) was reported as a patent application in 1990. under the worldwide patent cooperation treaty (Mishra *et al.* 2008). Several present restrictions are eliminated by plants with subunit vaccinations. Plant-based vaccinations are affordable since they simply require sunlight, water, and nutrients to grow and thrive. The likelihood of contamination is reduced with plant-based vaccines, which

also offer oral doses and a thermally stable environment, removing the potential for injection-related problems (Walmsley and Arntzen 2000; Stander *et al.* 2022). Orally administered edible plant vaccines stimulate the immune system and offer disease protection. Future vaccination delivery methods may undergo a revolution thanks to recombinant vaccines. Vaccine production will be more affordable through vaccinations made from plant tissues and the consumption of plant-based meals. Consumers can eat 1000 times more antigens than would be available by injection for the same cost. Scientists are also looking at utilizing maize grain as a delivery technique for consumable immunizations. These include vaccinations for the swine transmissible gastroenteritis virus and certain strains of *Escherichia coli* (ETEC) (TGEV) (Haq *et al.* 1995; Streatfield *et al.* 2001).

Mucosal and systemic immunity are both boosted when vaccinations consumed come into contact with the lining of the digestive tract. A first line of defense against diseases of the mucous membrane would be provided by their combined effect. The M cells in the ileum (Payer's patches) and the lymphoid tissue connected to the gut both take up the antigens generated in the intestines (GALT) (Rossi *et al.* 2015*)*. The vaccination antigen is preserved from deterioration by plants and also acts as a delivery mechanism for the vaccination into the git. This technique allows the vaccine antigen to successfully enter the mucosal immune system (Hefferon 2013).

Initial advancements in plant-based vaccines

By effectively synthesizing SpaA (*Streptococcus* mutans surface protein antigen a) in tobacco plants, Curtiss and Cardineau demonstrated the first instance of plant vaccine expression in 1990. When transgenic tobacco tissues were provided in place of the pathogen, antibodies were shown to be physiologically active against *S. mutans*. Eventually, as a result of the use of this technology, plants generated many antigens, including the hepatitis antigen in tobacco and lettuce, the rabies antigen in tomato, the cholera antigen in tobacco and potatoes, and the human CMV antigen in tobacco. In 1993, it was discovered that the cowpea mosaic virus surface included an FMDV epitope (CPMV). The utilization of chimeric plant viruses as proteins that transmit carcinogens was proven in 1994 by rabbits' immune reactions to pure chimeric (CPMV) particles expressing epitopes from the human rhinovirus 14 (HRV-14) and HIV-1 (Walmsley and Arntzen 2000; Pyrski *et al*. 2019). Scientists asked participants to consume anti-diarrheal transgenic potatoes in 1997, marking the first of edible vaccination testing on people. All participants produced antigens in their systems after eating potatoes, precisely as they do after standard anti-diarrheal immunization, with no negative effects. Volunteers are also testing a Hepatitis B antigen expressed in raw potatoes. After witnessing such a positive outcome from this trial, the NIAID encouraged the researchers to use this approach to develop vaccines for additional illnesses (Doshi *et al.* 2013).

Current advancements in vaccines produced from plants

The use of these vaccinations in both humans and plants is the focus of the current investigation on the synthesis of plant vaccines. Despite the fact that there aren't any plant-based vaccinations on the market right now, some of them have regulatory approval. They include a secretory antibody vaccine that received EU authorization, a chicken Newcastle disease vaccine that received USDA approval and a tobacco-based hepatitis B virus vaccine that received Cuban authorization (Tacket and Mason 1999). Many plant-based vaccines are now undergoing human trials, including one based on potatoes that defend against the rabies virus and one that defends against the hepatitis B virus. Data on the dose, ideal administration method, response type, strength, and duration have been generated for illnesses such as *Vibrio cholera*, HIV, *Pseudomonas aeruginosa*, *murine hepatitis virus*, and foot-and-mouth disease virus. Hence, the introduction of plant-consumable vaccines is anticipated within next several years. Increasing transgene expression in transgenic plants is one of the current main objectives. In plants, a number of reported codes or sequences may have resulted in improper processing or premature genotoxic eradication. Synthetic genes were produced by removing these directives. As a result, antigen accumulation increased 314 times in leaves and tubers. The results of the research involving the feeding of mice were eliminated. Antigen accumulation increased 3–14 times in leaves and tubers as a result. Studies on mouse feeding provided evidence for the findings (Walmsley and Arntzen 2000).

Selection of plants for the manufacture of an edible vaccine

Banana: The vaccination is given in the form of banana trees and tomato plants that were genetically engineered at the Boyce Thompson Institute for Plant Research. Bananas are viewed as a promising source of vaccine manufacture since they are widely available, can be eaten raw, and are popular with children in several emerging global areas (Langridge 2000). Banana is also regarded as the greatest host for vaccine manufacturing since it provides benefits such as digestibility and palatability to newborns. They are readily available all year round in any area of the world where strong antibodies are needed to vaccinate a sizable section of the populace. The edible banana is ideal for quality control since it doesn't have seeds and develops from a tree's stem, making it challenging to detect transgenes (Kumar *et al.* 2005). The disadvantage of using bananas is that the fruit degrades quickly after reaching maturity whereas the banana plant takes several years to mature. *Agrobacterium transformation*, particle bombardment and electroporation are just a few techniques that may be used to genetically

modify bananas.

Tomato: Scientists at Loma Linda University conducted numerous studies and came to the conclusion that antigens may be generated by tomato plants. Tomato cultivation or growth is done quickly and in big quantities, however it is not that rapid (Langridge 2000). The HBsAg factor-containing tomato that transforms and regenerates has been considered (Cortina and Culiáñez-Macià 2004; Wang and Li 2008). Bock and his colleagues have reported on a tomato plastid transformation in which plastid transgene in tomato plants may attain a noteworthy amount of protein. Their research led to the discovery that an edible vaccine may be created by transforming tomato plastids. The only distinct transplastomic plants identified as viable and capable of passing on transgenes to subsequent generations are the tomato plants described by Bock and colleagues (Maliga 2001).

Potatoes: Because they don't require refrigeration for long-term storage, potatoes are regarded as being suitable for the creation of vaccines. Another seen was the consumption of uncooked potatoes in South America. The protein in potatoes denatures when heated, yet surprisingly, it was discovered that the antigen in the potatoes was not fully destroyed (Langridge 2000).

Tobacco: Charles Arntzen's cluster at Lone Star State A & M in the United States was the first to discover the tobacco plant's expression of the hepatitis B surface antigen (Tregoning *et al.* 2004). The buildup of recombinant SARS CoV prickle protein in the cytosol and chloroplasts of tobacco plants is one of the various recombinant virus vaccines produced recently in transgenic plants (Li *et al.* 2006) and T.B. antigen overexpression in tobacco leaves (Dorokhov *et al.* 2007).

Additional plants used in edible vaccines

Lettuce, rice, wheat, soybeans, and corn are also used to make edible vaccinations. Vaccines against enterotoxigenic strains of *Escherichia* (ETEC) and swine-transmissible gastroenteritis virus have been generated using corn grains (TGEV) (Streatfield *et al*. 2001). Arabidopsis and rice are likewise genetically altered, but they cannot pass genes on to future generations (Maliga 2001). Transgenic tobacco plants, tobacco cells, lettuce and ligneous plant, carrot, and potato are the primary sources of hepatitis B antigen production (HBsAg) (Kumar *et al.* 2005; Karaman *et al.* 2006). Numerous different plants that express VP1 include cress, alfalfa, potato, and a variety of others (Kim *et al.* 2009). Because alfalfa is known to be more effective at encouraging the expression of the FMDV VP1 protein. Transgenic apples have also expressed the HBV expansion surface antigen PRS-S1S2S quality (Lou *et al.* 2005).

Strategies for expression system

The following are the plant expression system strategies:

***Agrobacterium-mediated* transformation:** There are other techniques to modify plants, but *Agrobacterium*-mediated transformation is best for developing edible vaccines. The host becomes infected after *Agrobacterium* inserts a DNA fragment (TDNA) into its genome. By employing this technique, we insert our target gene into the plant genome (Walmsley and Arntzen 2000). plant pathogen *A. tumefaciens* successfully introduces DNA into the host plant, where it fuses with several chromosomal sites in the nucleus. In certain *Agrobacterium* strains, the virulence genes that cause plant tumor development have been eliminated, but the genes that encourage efficient gene transfer have been maintained. The target gene (antigen) is supplied and after integrating with the plant's nucleus, it is produced and normally is handed down through generations (Fig. 1).

Biolistic method: Several important plant species, such as soybean and the bulk of cereal grains, cannot be transformed by *Agrobacterium*. For gene transfer in such plants, a biolistic technique is used. In comparison to *Agrobacterium* transformation, the biolistic approach has certain advantages since it causes the incorporation of foreign genes in large copy numbers, which improves expression. The genome of the chloroplast is expanded using transgenes. High chloroplast genome copy number increases recombinant protein expression in plant cells. Plant phenotype is less impacted by nuclear-mediated expression of recombinant proteins than chloroplast-mediated expression. Chloroplast transformation has been utilized to express 25 different foreign genes, despite the fact that this is a relatively uncommon process (Sharma and Sood 2011).

Electroporation: This process involves briefly exposing plant cells to a high-voltage electrical pulse in order to introduce DNA into the cells. It results in momentary plasma membrane gaps. DNA must get through the strong cell wall to enter the cell cytoplasm. To do this, we first weaken the cell membrane with moderate enzymatic treatment and then enable DNA to slip through (Singh 2002).

Transient expression: According to this method of expression, a plant virus that contains the vaccine gene infects the plant systemically (Mason *et al.* 2002). For the plant viral genome to create a significant number of copies of recombinant proteins, the plant virus must be able to independently replicate, transcribe, and translate. When the virus is replicating, foreign genes are continually produced, combined with the genes that make the plant virus's capsid protein, expressed as soluble proteins, and dispersed throughout the cytoplasm of the host plant cell (Guan *et al*. 2013). These plant viruses are manipulated by inserting a gene of interest. Plant viruses then infect the host, and genes are expressed in various areas of the plant, however, the amount of expression varies (Maliga 2002). Many viruses, such as the CPMV (Cowpea Mosaic Virus), alfalfa mosaic virus, TMV (Tobacco Mosaic Virus), CaMV (Cauliflower Mosaic Virus), potato virus, and tomato bushy stunt virus, may have their surfaces modified to produce antigenic protein snippets. The use of overcoat and epicoat

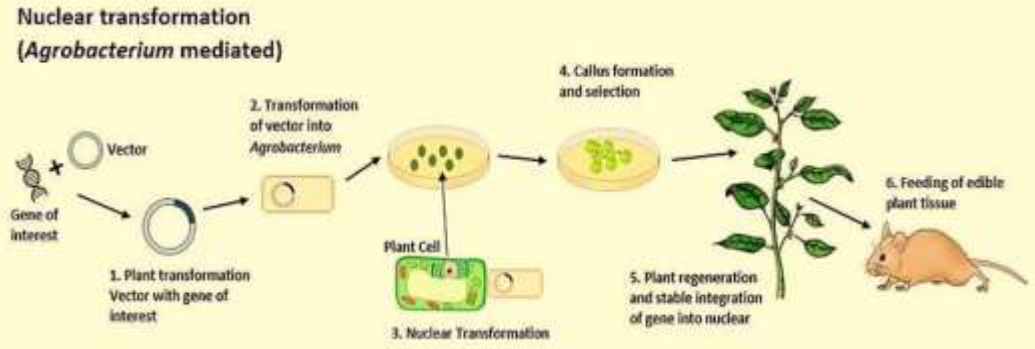

Fig. 1: Schematic representation of stable nuclear transformation process. A gene of interest is introduced into plant chromosomes via Agrobacterium-mediated transformation, followed by selection and regeneration. The edible plant tissue is then fed to the mouse to check the response of the edible vaccine

technologies is part of this strategy (Ramshaw and Ramsay 2000). The full protein may be produced by plants using overcoat technology, but just the foreign proteins may be expressed by them using epicoat technology (Karasev *et al.* 2005).

Application

Cholera: In the small intestine, enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholera* produce enterotoxins as colonies, which lead to severe watery diarrhea. Cholera toxin (CT) and heat-labile enterotoxin (LT) from *E. coli* are both protein toxins with similar immunochemical characteristics. The GM1 ganglioside on the surface of intestinal epithelial cells is where LT and CT interact through specific interactions with the B-subunit pentamer. To provide immunization against CT-B and avoid damage, a vaccination taken orally comprising CT-B subunits and testing was done on entirely dead *cholera* cells in Bangladesh. The host cells are prevented from receiving a component. Recombinant potato LT-B tubers were administered to mice in one experiment to induce blood and mucosal antibodies against LT-B, which had the intended result of providing protection against oral challenge with LT. The findings demonstrated that plant-expressed LTB can be administered as an adjuvant or part of an anti-ETEC injection and anti-cholera vaccines. Given the similarity between CT and LT, CT-B produced in plants would also provide protection. Another study discovered that the plant chemical CT-B increased the production of toxin-neutralizing antibodies in mice (Mason *et al.* 1998).

Hepatitis B: One of the diseases that concern people the most in poor countries is hepatitis B, with around 350 million chronic carriers. The illness can be treated with vaccinations; however, the recombinant vaccine made from yeast is exceedingly costly in impoverished countries. It could be more affordable to produce the hepatitis B vaccine in food plants and fruits. Transgenic plants, including tobacco, carrot, potato, lettuce, and lupin, have been shown to express the hepatitis B surface antigen (HBsAg). The capacity to immunize huge populations, infant digestibility and palatability, year-round availability, absence of transgenic segregation and ability to produce palatable hepatitis vaccinations make banana a great candidate. Using techniques like *Agrobacterium*-mediated transformation, particle bombardment, or electroporation, banana has successfully undergone genetic modification, enabling the transfer and expression of advantageous genes (Kumar *et al*. 2005). Using transgenic potato tubers, preclinical animal experiments developed recombinant hepatitis B surface antigens (HBsAg). A main immunological response was observed in mice given transgenic HBsAg potato tubers (Richter *et al*. 2000).

Newcastle Disease: Shariari and his colleagues sought to produce a recombinant vaccination in hairy roots in 2015. The pBI121 expression vector was used to clone the epitopes for the haemagglutinin neuraminidase (HN) and Newcastle disease virus fusion (F). The vector was subsequently introduced into tobacco leaf discs using Agrobacterium rhizogenic (*Nicotiana tabacum* L.). PCR was used to validate the heterologous gene's incorporation into the hairy root genome. To measure gene expression, dot-blot and ELISA tests, real-time PCR, and ELISA assays at the translational and transcriptional levels were applied. They all verified that the recombinant protein was made and that the heterologous gene was expressed (Shahriari *et al.* 2015).

Measles: Measles causes 800,000 fatalities globally each year, with the majority of those afflicted developing encephalitis or going deaf. During the immunization period, those above the age of 18 months produced 95% seroconversion. A live-attenuated measles vaccination can be damaged by heat, which does not need oral immunization and just requires refrigeration for storage. Moreover, maternal antibodies reduce the vaccination response to the vaccine. Tobacco plants were genetically modified utilizing plasmid/vector *A. tumefactiens* to produce the measles virus hemagglutinin (MV-H) antigen for an edible vaccine (Huang *et al.* 2001). After oral treatment of a plant expressing MVH, serum antibodies

were generated that were capable of neutralizing wild-type MV while yet retaining its immunogenicity. As a result, mice given plant-derived MV-H orally exhibited IgA antibodies in their feces (Ishiwada *et al.* 2001). A transgenic carrot plant that may be used to disseminate the virus' antigens was shown to be developing a measles vaccine in another investigation (Marquet-Blouin *et al.* 2003). In one experiment, transgenic bananas were administered to healthy animals, and antibodies were monitored in blood samples directed against hemagglutination. The study showed that experimental animals exposed to antigenic hemagglutination proteins generated from banana plants might trigger immunological responses (Diane *et al.* 2002).

Rabies virus vaccine: For a large portion of the world's population, the rabies virus poses a substantial risk to both human and animal health. Antigens from the rabies virus glycoprotein (G protein) and nucleoprotein (N protein) can be used to create vaccines. The N protein activates T lymphocytes specific to the rabies virus, whereas the G protein is the primary antigen that triggers protective immunization. Both support other immunological processes as well as neutralize antibody production. Tobacco and spinach plants were created using chimeric peptides that were cloned and synthesized and contained rabies virus glycoprotein and nucleoprotein antigenic components. As a result, recombinant virus-carrying mice showed resistance to infection. Infected spinach leaves were ingested by three out of five human participants, who afterward experienced an allergic response to the peptide antigen (Yusibov *et al.* 2002). In both transgenic tomato plants and *N. benthamiana* plants, the full-length nucleoprotein gene of the rabies virus was generated using agroinfiltration. 1–5% of the total soluble protein was expressed in both tomato and *N. benthamiana* (Perea *et al.* 2008). In a different experiment, chimeric proteins comprising synthetic CT-B attached to rabies surface glycoprotein at their C-termini were produced in tobacco plants (G protein). About 80.3 kDa fusion polypeptide was expressed at 0.4% of the total soluble protein in the leaves of the selected transgenic lines (Roy *et al.* 2010).

Anti-autoimmune illness vaccinations: To cure autoimmune illnesses, auto-antigens are generated in transgenic plants. The immune system perceives bodily proteins as invading substances. Type 1 diabetes, myasthenia gravis, arthritis and multiple sclerosis are all illnesses. When administered orally, an autoantigen made from plants will halt the progression of the autoimmune disease (Langridge 2000). GAD (Glutamic acid decarboxylase) and insulin-producing potatoes have been shown to lessen diabetic immunological assaults and the prevention of the initiation of high blood sugar (Arakawa *et al.* 2007).

Vaccines against human tumors: Several tumors, including melanoma and breast cancer, have specific proteins on their cell surfaces. Antibodies against these antigens that are passively supplied, naturally acquired, or actively produced have eradicated circulating tumor cells and micrometastasis. Since tumor antigens are also auto-antigens, developing a cancer vaccine becomes more difficult. During the past 10 years, immunologists have discovered and classified epitopes unique to a variety of human tumor types. For instance, a polyepitope-containing naked recombinant plasmid DNA was recovered from a human melanoma tumor. Mice were injected with the plasmid DNA, which causes a cytotoxic T-cell response that is epitope specific. With the purpose of developing this DNA, a melanoma vaccine obtained from plants, is now being integrated into the nuclear and chloroplast DNA of tobacco (Sala *et al*. 2003).

Advantages

As edible plant vaccines may be used as a dietary supplement and activate the humoral, cell-mediated and mucosal immune systems, they offer several benefits. Compared to normal methods, transgenic plants' production costs are much less. When expressed in plants' natural storage tissues, foreign proteins become more stable and don't need to be refrigerated, shipped, or stored as much. Plant tissues, components and without the need for any additional equipment, all extracts can be stored at ambient temperature. Only a minor portion of a pathogen's antigenic component is generated in plants, which lowers the danger of infection and contamination with mammalian viruses because plants are not hosts for animal viruses. The antigen is shielded by the plant cell walls from digestion and eventually released into the lymph and blood. Several transgenic lines combined with seeds can produce multicomponent vaccines that express various proteins (Streatfield *et al.* 2001; Shahriari *et al*. 2015). Purification is not required because the plant includes recombinant proteins in edible form, such as potatoes. Sexual crossing of plants can introduce new or numerous transgenes. Transgenic animal ethical issues are avoided (Malik *et al*. 2011).

Disadvantages

Although the concept of edible vaccination is appealing, its execution may be difficult. While developing a plant-based vaccine, there are several factors to take into account, such as system efficacy, dosage, antigen choice, administration, safety, public perception and quality control, plant choice, and licensing. The antigen of choice needs to work with the type of plant in which it will be expressed. The patient's weight, age, size and the fruit or plant's ripeness all have a role in determining the dosage of an edible vaccine. Under dosing, which would lead to reduced antibody production, or overdosing, which would lead to tolerance, might arise from this. It is difficult to maintain dosage constancy from fruit to fruit, plant to plant, and generation to generation. The shelf life of the fruits, herbs, and vegetables used as vaccines is crucial. To prevent infection or sickness from degrading, these vector plants must be kept in good

condition. The ability to distinguish "vaccine" fruit from conventional fruit in order to reduce improper vaccination delivery may be another concern. Plant-based vaccines may result in allergic reactions or negative side effects, such as autoimmune diseases, central nervous system injury, or cytokine-induced sickness (Sharma and Sood 2011).

Mucosal immune system

Most infections enter the body through mucous membranes. Parenteral administration of candidate antigens cannot sufficiently activate the mucosal immune system, in contrast to the humoral immune system. Oral vaccines are extremely important because they are effective in stimulating mucosal immunity at the intestine's surface. Edible plant vaccines provide good protection against a wide range of diseases that come into touch with the body's mucosal surfaces via the oral, respiratory, urinary and vaginal pathways. They may be used independently or in combination with other vaccination techniques. Since antigens must be presented directly to the mucosal surface, oral vaccinations are the best technique to develop mucosal immunity. Subunit vaccines have yet to achieve economic value through any method of manufacture. The primary issue with using orally given immunogenic proteins is that they degrade after ingestion and may not be recognized efficiently in the stomach. Plant cell walls protect vaccinations and can overcome degradation problems, such as liposomes and microcapsules. The thick surface of the lower digestive tract is covered by a large amount of plant cell wall, allowing for a delayed release of the antigen (Streatfield *et al.* 2001; Habibi-Pirkoohi and Mohkami 2015).

Mechanism of action

Mucosal surfaces, encompassing the respiratory, digestive, urinary, and reproductive systems, occupy most of the body's immunologically active tissue. The mucosal immune system (MIS) is the first line of defense against viruses that specifically target mucosal surfaces and is the best place for immunization. An edible vaccine seeks to promote humoral and mucosal defenses against diseases. During oral delivery, edible vaccines are chewed up, and the majority of plant cell death is caused by digestive or bacterial enzyme action on edible vaccines in the colon. In transgenic plants, antigens are transported by bio-encapsulation. Plant cells have a tough outer coating that shields them from stomach acids and eventually disintegrate in the intestines. M-cells that are positioned on top of Peyer's patches take up the antigens once they are released in the colon. Peyer's Patches (PP)-derived IgA-producing plasma cells have the potential to enter mucosal tissue and act as mucosal immune effector sites. They consist of 30 to 40 lymphoid nodules on the outside of the digestive tract, each of which has follicles from which germinal centers form in response to the antigenic stimulation caused by the breakdown of ingested vaccines. The antigen enters the intestinal epithelium through these follicles and gathers inside specialized lymphoid tissues. As a result of interaction, the antigen is subsequently carried by M-cells, which express class II MHC molecules beyond the mucosal barrier and activates B cells in lymphoid follicles. When it interacts with the lumen, a group of B-cells, T-cells and macrophages produces a profound invagination in the basolateral plasma membrane. In the mucosal-associated lymphoid tissue (MALT), activated B-cells travel from lymphoid follicles and mature into plasma cells that produce IgA antibodies. Epithelial cells transport IgA antibodies into lumen secretions where they interact with antigens found there (Table 1; Fig. 2). The memory cells and IgA response would quickly stop the actual infectious agent's invasion (Mishra *et al.* 2008; Esmael and Hirpa 2015).

Clinical trials

Foreign antigens factory-made in plants: Before the development of edible vaccines, several concerns had been expressed about the use of injectable vaccines, which have been the standard form of immunization since 1995. Arntzen and his associates engineered a tobacco plant that could synthesize a protein from the hepatitis B virus by introducing the gene for that protein into the plant. This is just one example of how many foreign genes were generated in diverse plants in the proper conformation. The immune system components of the mice were then activated similarly to how a virus could by administering this antigen via injection.

Plant-based diabetes vaccinations: Langridge created plant-based diabetes vaccines, such as potatoes infused with insulin or GAD linked to the harmless B fractional monetary unit of the deadly toxin *V. cholerae* (to increase the antigens' absorption by M cells). The immunizations were given to a strain of mice that developed diabetes as a result of the vaccinations. This assisted in limiting the immunological response and delaying the onset of high blood pressure (Langridge 2000).

Mouse LT-B antibody production in serum and mucosa: Numerous researches for vaccine development in plants were conducted, and it was also shown that mice may create serum and mucosal antibodies against LT-B. These antibodies were generated by feeding mice transgenic potato tubers with an altered LT-B at a level of 2 pg gg' tuber tissue. The principal source of LT inhibition in mammalian cells was therefore considered to be antibodies produced in response to potato LTB. They were effective in protecting as a result. In order to boost LT-B production in potato plants, a fake LT-B (sLT-B) encryption sequence that has been altered to include plant-preferred codons and reduce erroneous mRNA process signals was utilized. When administered to mice, the sLT-B tubers produced significant blood and mucosal antibody responses against LT-B and provided some protection against an oral LT-B challenge

Table 1: Edible vaccines produced in transgenic plants

Disease	Antigen	Plant Host	Effect of vaccine	Reference
Cholera	*E. coli* heat labile enterotoxin (LT-B)	Potato	Protection against LT-B in mice was induced	(Mason *et al.* 1998)
Newcastle	Fusion (F) and haemagglutinin neuraminidase (HN) epitopes	Tobacco	The expression of the heterologous gene and production of the recombinant protein was confirmed by PCR, dot-blot and ELISA assays.	(Shahriari *et al.* 2015)
Measles	Measles virus hemagglutinin (MVH) antigen	Tobacco	The serum antibodies were induced which were able to neutralize wild type MV. The animals immunized with MV-H had IgA antibodies in the fecal samples.	(Ishiwada *et al.* 2001)
Rabies	A chimeric peptide comprising antigenic determinants from glycoprotein and nucleoprotein of rabies virus	Spinach	The mice showed immunity against challenge infection	(Yusibov *et al.* 2002)
Rabies	A full-length nucleoprotein gene of the rabies virus	Tomato and *N. benthamiana*	1–5 % of total soluble protein was expressed in tomato and 45% in *N. benthamiana*	(Perea *et al.* 2008)
Hepatitis B	A recombinant hepatitis B surface antigen (HBsAg)	Potato	Mice fed transgenic HBsAg potato tubers showed a primary immune response	(Richter *et al.* 2000)

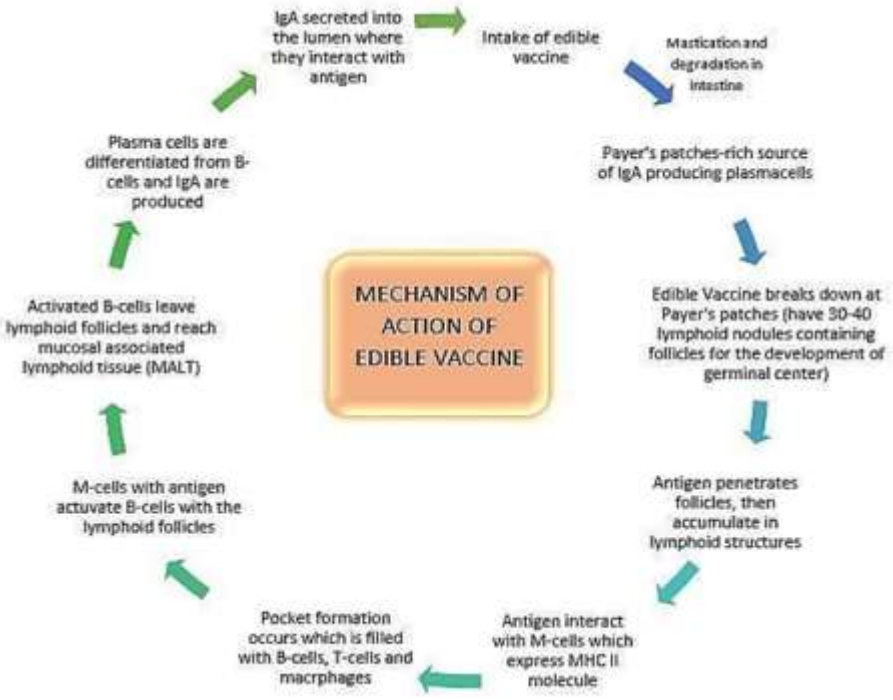

Fig. 2: Representation of immunological mechanisms of action of plant-based edible vaccines

(Mason *et al.* 1998). LT B was also expressed in corn by S.J Streatfield and also its immunogenicity was described when fed to mice.

Immune response aggravated by transgenic potatoes: When HBsAg was expressed in transgenic tomato leaves; VLPs with a diameter of 22nm were produced. Afterward, it was employed in mice for duct creation, and VLPs induced a B and T cell immune response, which is equal to yeast derive vaccination (Richter *et al.* 2000).

Human Trials

In the first human experiment of an edible plant vaccine designed to boost active immunity, 14 adult volunteers were randomly assigned to receive either 100 g of transgenic potatoes, 50 g of transgenic potatoes, or 50 g of wild-type potatoes (Tacket *et al.* 1998). According to the promoter's tissue specificity, there were different amounts of LT-B per gram of potato, which may indicate that LT-B was expressed in different potato tissues to varying degrees. Whereas the potatoes were ingested raw in this study, following research has demonstrated that only about 50% of the CTB pentameric GM1-binding form may be lost by cooking transgenic potatoes encoding the cholera toxin B component for 3 min until the tissue softens (Wang *et al.* 2004). Serologic reactions also appeared after immunization. When transgenic potatoes were ingested by 11 participants, 10 (91%) of them developed IgG anti-LT antibodies, with half of them responding after the first dosage. In six (55%) of the 11 patients, serum IgA anti-LT

levels multiplied (Tacket *et al.* 1998). The National Institute of Allergy and Infectious Diseases claims that transgenic plant immunization can effectively stimulate people's immune systems (NIAID). Little, raw potato pieces that had been genetically altered to generate a tiny quantity of the diarrheal toxin produced by *E. coli* were given to participants (Ball *et al.* 1999). Transgenic potatoes producing this toxin component induced potent immunological responses in mice, according to in vitro and preclinical research previously funded by NIAID. The creation and development of transgenic potatoes use scientific methods. The genetically modified potatoes were given to 11 of 14 healthy individuals at random, while the remaining three received conventional potatoes. The potential of the vaccination to trigger intestinal and systemic immune responses was assessed using routine blood and stool samples from patients. After vaccination, six out of the eleven volunteers (55%) experienced a fourfold rise in intestine antibodies, and ten out of the eleven (91%) experienced a fourfold increase in blood antibodies at some time after consuming the transgenic potatoes. The participants tolerated the potatoes well and none of them reported any serious side effects. The NIAID-supported researchers were inspired to look into using this method to deliver other antigens by the study's encouraging results. To combat the Norwalk virus, diarrhea, and hepatitis B, potatoes, tomatoes and bananas are being grown. Research is also being done on edible vaccines for other intestinal illnesses (Pyrski *et al*. 2019).

Conclusion

Being able to fold and assemble proteins correctly, plants and fruits are increasingly used as expression sites for the development of ingestible vaccines. Transgenic plant vaccines provide a number of advantages over conventional vaccinations, including safety, cost-effectiveness, consistency, and competence. Orally given edible plant vaccinations can stop the spread of illness. Transgenic plant vaccines will, however, take a long time to produce in large quantities and become commercially available. Yet, a lot of vaccination research is still in the testing phase. The marketing of a transgenic plant vaccine that prevented Newcastle disease was authorized by the United States Department of Agriculture (USDA) in 2006. This illustration will encourage the development and manufacture of further transgenic plant vaccines. Plants may overtake other molecular agricultural production methods in the near future because to their benefits in terms of safety and economics. It follows that the application of inexpensive edible vaccinations against a variety of illnesses is anticipated in the future.

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Author Contributions

All authors contributed equally.

Conflict of Interest

All authors declare no conflict of interest.

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References

Arakawa T, J Yu, DK Chong, J Hough, PC Engen, WH Langridge (2007). A plant-based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes. *Nat Biotechnol* 16:934–938

Ball JM, DY Graham, AR Opekun, MA Gilger, RA Guerrero, MK Estes (1999). Recombinant Norwalk virus–like particles given orally to volunteers: phase I study. *Gastroenterology* 117:40–48

Chan HT, H Daniell (2015). Plant-made oral vaccines against human infectious diseases – Are we there yet? *Plant Biotechnol J* 13:1056–1070

Cortina C, FA Culiáñez-Macià (2004). Tomato transformation and transgenic plant production. *Plant Cell Tiss Org Cult* 76:269–275

Diane EW, MC Thomas, RA Strugnell, IB Dry, SL Wesselingh (2002). Appetizing solutions: An edible vaccine for measles, *Med J Aust* 176:434–437

Dorokhov YL, AA Sheveleva, OY Frolova, TV Komarova, AS Zvereva, PA Ivanov, JG Atabekov (2007). Superexpression of tuberculosis antigens in plant leaves. *Tuberculosis* 87:218–224

Doshi V, H Rawal, S Mukherjee (2013). Edible vaccines from GM crops: current status and future scope. *J Pharm Sci Innov* 2:1–6

Esmael H, E Hirpa (2015). Review on Edible Vaccine. *Acad J Nutr* 4:40–49

Famulare M, H Hu (2015). Extracting transmission networks from phylogeographic data for epidemic and endemic diseases: Ebola virus in Sierra Leone, 2009 H1N1 pandemic influenza and polio in Nigeria. *Intl Health* 7:130–138

Guan ZJ, B Guo, YL Huo, ZP Guan, JK Dai, YH Wei (2013). Recent advances and safety issues of transgenic plant-derived vaccines. *Appl Microbiol Biotechnol* 97:2817–2840

Habibi-Pirkoohi M, A Mohkami (2015). Recombinant vaccine production in green plants: State of art. *J Cell Mol Res* 7:59–67

Haq TA, HS Mason, JD Clements, CJ Arntzen (1995). Oral immunization with a recombinant bacterial antigen produced in transgenic plants. *Science* 268:714–716

Hefferon K (2013). Plant derived pharmaceuticals for the developing world. *Biotechnol J* 8:1193–1202

Huang Z, I Dry, D Webster (2001). Plant derived measles virus hemagglutinin protein induces neutralizing antibodies in mice. *Vaccine* 19:2163–2171

Ishiwada N, MM Addae, JK Tetteh (2001). Vaccine modified measles in previously immunized children in Accra, Ghana: clinical, virological and serological parameters. *Trop Med Intl Health* 6:694–698

Karaman S, J Cunnick, K Wang (2006). Analysis of immune response in young and aged mice vaccinated with corn-derived antigen against Escherichia coli heat-labile enterotoxin. *Mol Biotechnol* 32:31–42

Karasev AV, S Foulke, C Wellens, A Rich, KJ Shon, I Zwierzynski, D Hone, H Koprowski, M Reitz (2005). Plant-based HIV-1 vaccine candidate: Tat protein produced in spinach. *Vaccine* 23:1875–1880

Kim YS, MY Kim, TG Kim, MS Yang (2009). Expression and assembly of cholera toxic B subunit (CTB) in transgenic carrot (*Daucus carota* L.). *Mol Biotechnol* 41:8–14

Kumar GS, TR Ganapathi, CJ Revathi, L Srinivas, VA Bapat (2005). Expression of hepatitis B surface antigen in transgenic banana plants. *Planta* 222:484–493

Langridge WH (2000). Edible vaccines. *Sci Amer* 283:66–71

Li HY, S Ramalingam, ML Chye (2006). Accumulation of recombinant SARS-CoV spike protein in plant cytosol and chloroplasts indicate potential for development of plant derived oral vaccines. *Exp Biol Med* 231:1346–1352

Lou XM, Z Zhang, HQ Yao, AS Xiong, HK Wang, RH Peng, X Li (2005). Expression of human hepatitis B virus large surface antigen gene PRS-S1S2S in transgenic apples. *J Fruit Sci* 22:601–605

Maliga P (2002). Engineering the plastid genome of higher plants. *Curr Opin Plant Biol* 5:164–172

Maliga P (2001). Plastid engineering bears fruit. *Nat Biotechnol* 19:826–827

Malik A, VK Vashishta, R Rizwan, S Sharma, J Singh (2011). Edible vaccine-vegetables as alternative to needles. *Intl J Curr Res* 3:18–26

Marquet-Blouin E, FB Bouche, A Steinmetz, CP Muller (2003). Neutralizing immunogenicity of transgenic carrot (*Daucus Carota* L.) derived measles virus hemagglutinin. *Plant Mol Biol* 51:458–469

Mason HS, H Warzecha, T Mor, CJ Arntzen (2002). Edible plant vaccines: applications for prophylactic and therapeutic molecular medicine. *Trends Mol Med* 8:324–329

Mason HS, TA Haq, JD Clements, CJ Arntzen (1998). Edible vaccine protects mice against Escherichia coli heat-labile enterotoxin (LT): potatoes expressing a synthetic LT-B gene. *Vaccine* 16:1336–1343

Mishra N, PN Gupta, K Khatri, AK Goyal, SP Vyas (2008). Edible vaccines: A new approach to oral immunization. *Ind J Biotechnol* 7:283–294

Perea AI, EL Rubio, ER Anaya, TO Flores, LGDL Vara, MA Gómez Lim (2008). Expression of the rabies virus nucleoprotein in plants at high-levels and evaluation of immune responses in mice. *Plant Cell Rep* 27:677–685

Pyrski M, AA Mieloch, A Plewiński, A Basińska-Barczak, A Gryciuk, P Bociąg, M Murias, JD Rybka, T Pniewski (2019). Parenteral–oral immunization with plant-derived HBcAg as a potential therapeutic vaccine against chronic hepatitis B. *Vaccines* 7:211–220

Ramshaw IA, AJ Ramsay (2000). The prime-boost strategy: exciting prospects for improved vaccination. *Immunol Today* 21:163–165

Richter LJ, Y Thanavala, CJ Arntzen, HS Mason (2000). Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nat Biotechnol* 18:1167–1171

Rossi L, S Reggi, M Zaninelli, F Saccone, D Gottardo, A Crotti, A Baldi (2015). Evaluation of antigens stability of tobacco seeds as edible vaccine against VTEC strains. *Intl J Health Anim Sci Food Saf* 2:2283–3927

Roy S, A Tyagi, S Tiwari, A Singh, SV Sawant, PK Singh, R Tuli (2010) Rabies glycoprotein fused with B subunit of cholera toxin expressed in tobacco plants folds into biologically active pentameric protein. *Protein Exp Purif* 70:184–190

Sala F, MM Rigano, A Barbante, B Basso, AM Walmsley, S Castiglione (2003). Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. *Vaccine* 21:803–808

Singh B (2002). *Biotechnology*, Vol. 1, p:323. Kalyani publishers, New Delhi, India

Shahriari AG, A Bagheri, MR Bassami, SM Shafaroudi, AR Afsharifar (2015). Cloning and expression of Fusion (F) and Haemagglutinin-neuraminidase (HN) Epitopes in hairy roots of tobacco (*Nicotiana tabacum* L.) as a step toward developing a candidate recombinant vaccine against newcastle disease. *J Cell Mol Res* 7:11–18

Sharma M, B Sood (2011). A banana or a syringe: journey to edible vaccines. *World J Microbiol Biotechnol* 27:471–477

Stander J, S Mbewana, AE Meyers (2022). Plant-derived human vaccines: Recent developments. *Biol Drugs* 36:573–589

Streatfield SJ, JM Jilka, EE Hood, DD Turner, MR Bailey, JM Mayor, IR Tizard (2001). Plant based vaccines: unique advantages. *Vaccine* 19:2742–2748

Tacket CO, HS Mason (1999). A review of oral vaccination with transgenic vegetables. *Microb Infect* 1:777–783

Tacket CO, HS Maso, G Losonsky, JD Clements, MM Levine, CJ Arntzen (1998). Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. *Nat Med* 4:607–609

Tregoning J, P Maliga, G Dougan, PJ Nixon (2004). New advances in the production of edible plant vaccines: chloroplast expression of a tetanus vaccine antigen, Tet C. *Phytochemistry* 65:989–994

Walmsley AM, CJ Arntzen (2000). Plants for delivery of edible vaccines. *Curr Opin Biotechnol* 11:126–129

Wang L, MW Goschnick, RL Coppel (2004). Oral immunization with a combination of Plasmodium yoelii merozoite surface proteins 1 and 4/5 enhances protection against lethal malaria challenge. *Infect Immun* 72:6172–6175

Wang YQ, T Li (2008). Transformation of HBsAg gene into tomato and production of transgenic tomato plants. *JSW Univ (Nat Sci Edn)* 30:78–83

Yusibov V, DC Hooper, SV Spitsin, N Fleysh, RB Kean, T Mikheeva, D Deka, A Karasev, S Cox, J Randall, H Koprowski (2002). Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine* 20:3155–3164