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Review Article

Edible Vaccines: A Nutritional Substitute for Traditional Immunization

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ABSTRACT

Edible vaccines are created from transgenic plants and animals and contain immunostimulant. Edible vaccines, to put it simply, are medications generated from plants or animals. In underdeveloped countries, oral vaccines are less expensive and more widely available. Researchers came up with the idea of edible vaccines, in which edible plant pieces are employed as a vaccine factory. To make edible vaccinations, scientists put desired genes into plants and then force the plants to generate the proteins expressed in the genes. Transgenic plants are the result of transformation, whereas transformation is the act of converting plants. The edible vaccination promotes mucosal immunity. Dendritic cells in the gut can assist native T cells activate and differentiate into follicular T-helpers (Tfh). T and B cells will respond precisely to a reliable, digestible immunization. Potato, tomato, banana, carrots, tobacco, papaya, algae, and a variety of other plants are utilised as alternative agents for standard vaccinations. Malaria, cholera, hepatitis, rabies, measles, rotavirus, diarrhoea cancer treatments and treatment of covid-19 are among the illnesses for which plant-based vaccines have been created. It takes time and dedication to develop and sell edible vaccinations. Many edible vaccines for animal and human ailments have been developed and have gone through various levels of clinical testing. The importance of plant-based vaccinations is emphasized in this article.

Keywords: Edible vaccine, Transgenic plants, Plant-based vaccine, Infectious diseases, Vaccination.

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INTRODUCTION

Vaccines have developed as an efficient strategy against a variety of infectious diseases because they provide a direct and effective defence against communicable diseases and deaths. Vaccination cannot protect the lives of millions of people in poor and developing countries throughout the world due to factors such as high cost and storage. Approximately 20% of newborns are still unvaccinated, resulting in approximately 2 million unnecessary deaths each year, primarily in remote and impoverished areas of the world.[1] The provision of immunizations prevented the spread of infectious illnesses such as diphtheria, tetanus, polio, measles, mumps, rubella, and hepatitis.[2,3] Immunization for certain infectious diseases either does not exist, is unreliable, or is prohibitively expensive, such as immunization with DNA vaccines, which is a viable alternative but comes with some unwanted immune reactions. These vaccines are not only pricey, but they also offer a storage and transit issue, as many of them require refrigeration. As a result, there is a quest for readily administrable, storable, fail-safe, and widely approved bio friendly vaccines and delivery methods, especially in developing nations. As a result, because traditional vaccinations must be replaced, it was thought that plants could be potential agents for a more efficient vaccine production system, giving rise to the innovative notion of edible vaccines.[4] Oral vaccinations are more affordable and accessible to people in developing countries. Researchers developed the notion of edible vaccines, in which edible plant parts are used as a factory for the production of vaccines.[5] Edible vaccinations are being promoted as a viable alternative to traditional immunizations. Edible vaccines are often antigen-expressing plants, necessitating a fundamental understanding of agriculture and how to grow plants. In addition, the purification and downstream processing processes that make traditional vaccinations expensive are avoided in edible vaccines.[6-8] So, Edible vaccines are made from transgenic plants and animals, and they contain agents that stimulate an animal's immune response. To put it simply, edible vaccines are pharmaceuticals derived from plants or animals. This essay emphasises the significance of plant-based vaccines.[9] The main purpose of the review on development and current status of edible vaccine is to draw people's attention towards research on this topic.

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HISTORY OF EDIBLE VACCINE

Researchers backed by the National Institute of Allergy and Infectious Diseases (NIAID) demonstrated for the first time in 1998 that an edible vaccine may safely induce large immune responses in people, ushering in a new era in vaccine delivery. The study was published in the May issue of Nature Medicine by researchers from Tulane University in New Orleans, the Boyce Thompson Institute for Plant Research in Ithaca, N.Y., and the University of Maryland in Baltimore said "Edible vaccines offer great possibilities for dramatically decreasing the burden of diseases like hepatitis and diarrhoea, particularly in the developing world, where storing and administering vaccinations is often a huge difficulty," said the then-Director of NIAID.[10] Hiatt and colleagues proposed a proposal to develop a plant-based vaccination in 1989.[11] In 1990, Dr. Arntzen was the first to employ transgenic plants to produce and administer monomer vaccines. Arntzen's concept demonstrated that an edible vaccine can eliminate the limitations in the production of standard vaccinations. They achieved a breakthrough in edible vaccine manufacturing by expressing a surface antigen from hepatitis B in tobacco plants (Streptococcus mutants).[12] They started producing hepatitis B and heat-labile toxin B in potato and potato plants at the same time that they started producing edible vaccine in tobacco.[11]

CONCEPT OF EDIBLE VACCINE

Edible vaccines are made by inserting desired genes into plants and then forcing the plants to produce the proteins expressed in the genes. The transformed plants are known as transgenic plants, and the conversion process is known as transformation. Edible vaccines are made up of antigenic proteins and lack harmful genes, just like traditional subunit vaccinations. As a result, especially in immunocompromised individuals, they have no way of establishing infection or ensuring its safety. Conventional subunit vaccinations are costly and high-tech, requires processing and refrigeration, and have a poor mucosal response. Edible vaccinations, on the other hand, would improve compliance, particularly among children, and would reduce the need for trained medical workers because they are administered orally. Their manufacturing process is highly efficient and scalable.[13] Oral administration of edible vaccinations to moms might help immunise the foetus in gestation via transplacental transfer of maternal antibodies or the child through breast-feeding. Edible vaccinations allow for seroconversion in the presence of maternal antibodies, potentially protecting infants from infections such as group B Streptococcus, respiratory syncytial virus (RSV), and others. For numerous human and animal illnesses, edible vaccinations are currently being developed (cholera, measles, foot and mouth disease and hepatitis B, C and E). They can also be used to prevent rare illnesses such as hookworm, rabies, and dengue fever by combining them with other immunisation programmes to deliver numerous antigens. Tomato, rice, banana, lettuce, potato, and a variety of other foods are being studied for use in edible vaccines.[14] As edible vaccines come into touch with the digestive tract lining, they stimulate both mucosal and systemic immunity. This dual effect would offer first-line protection against pathogens that enter the body through the mucosa, such as Mycobacterium tuberculosis and agents that cause diarrhoea, pneumonia, STDs, HIV, and other diseases. The diarrheal agents Norwalk virus, vibrio cholerae, rota virus, and enterotoxigenic *E. coli* (ETEC) are responsible for roughly three million newborn fatalities every year, mostly in underdeveloped nations, according to scientists.[15]

MECHANISM OF EDIBLE VACCINE

Mucosal immunity is primarily stimulated by the edible vaccine. Both the innate and adaptive arms of the immune system are represented in this design (T and B cells). These mucosa associated lymphoid tissues (MALT) have a well-structured makeup. SIgA also protects mucosal surfaces against microbe and toxin adherence. The key to improving vaccination effectiveness is the development of novel platforms for the delivery of pathogens or toxin-specific SIgA and systemic IgG.[16,17] One of the most significant antigen capture mechanisms in the gut is microfold(M) cells. They are some kind of follicular-associated enterocyte (FAE) that is mostly found in the gastrointestinal tract. From small intestinal canals to antigen submucosal cells (APCs) on Peyer's patches, these cells may successfully gather a wide spectrum of macromolecules.[18] Dendritic (DC) cells appear to be the most potent antigenic cells for priming naïve T cells to mount an adaptive immune response.[19] DC is seen in a stable form in the immediate phase, with high endocytic activity and a poor capacity for primary naïve T cells. Inflammatory circumstances, on the other hand, cause DCs to develop, increase co-stimulatory chemicals, and move to T-cell regions in lymph nodes. To assist convert naive antigen-specific T cells into effector cells and move to a particular inflammatory location, antigens and the release of cytokines are used.[20] DCs in the intestine can boost naïve T-cell activation and follicular T-helper differentiation (Tfh) by either directly promoting Tfh differentiation or indirectly promoting Tfh development in later converted T-17 cells.[21,22] Active B cells exit the follicle and migrate to the lymphoid MALT, where plasma cells release antibodies against immunoglobulin A (IgA).[23] The same IgA antibodies are secreted past epithelial cells into the lumen to bind with antibodies.[22] DCs can also acquire luminous antigens via the epithelial cell layer and subsequently into the lumen through dendritic projection.[24] The goblet cell, a type of cell involved in the synthesis of mucins, was a new strategy for trapping antigen in the small intestine.[25] A dependable, edible vaccination will elicit precise responses from T and B cells, as well as long-lasting memory cells for later infection gathers. Although the phrase "oral tolerance" refers to the T-cell-mediated contradiction of a reduction in a particular immune response to previously met antigen when administered orally, it was one of the challenges with oral vaccination administration.[26,27] Antigens are created in the intestinal immune system because there is minimal inflammation, and juvenile dendritic cells introduce T cells, culminating in resistance.[28] When regulatory T cells obstruct the growth and development of dendritic cells to change their tolerogenic mechanism, they secrete cytokines like IL-10 and make intimate cell-to-cell contact.[29]

HOW TO PRODUCE EDIBLE VACCINE

Antigens that are delivered into the body are divided into two categories: Proteins and peptides. The antigen is either the full-length protein or a peptide fragment of the protein. The decision to utilise a protein or peptide antigen is case-specific and is influenced by a variety of circumstances.[30] Both plant viruses were utilised to establish the two major techniques for expressing the immunogenic protein or peptide in the host plant. Epitope presentation systems and polypeptide expression systems are the first and second, respectively. "Short antigenic peptides fused to the coat protein (CP) that are presented on the surface of formed viral particles" are employed in epitope presentation systems.[31] "The complete unfused recombinant protein that accumulates within the plant" is expressed by polypeptide expression systems.[31]

HOW TO MAKE EDIBLE VACCINE FROM POTATOES

The bacterium Agrobacterium tumefaciens is used to transfer the genetic blueprints for viral or bacterial "antigens"—proteins that provoke a targeted immune response in the recipient—into plant cells, resulting in edible vaccines. The manufacture of vaccine potatoes is depicted in Figure 1.

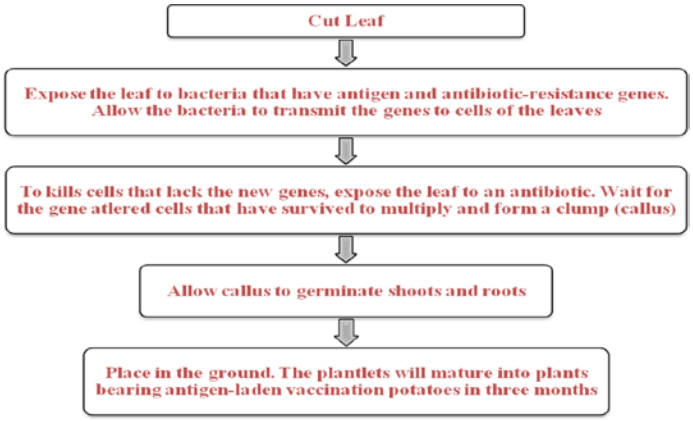

Figure 1: Production of edible vaccine from potatoes.

METHODS FOR TRANSFORMATION OF DNA/ GENE INTO PLANTS

The approaches listed below have been reported in the production of Edible Vaccines. The Agrobacterium tumifaciens method, the biolistic method, chimeric viruse's method and electroporation are all ways for transforming desired DNA/gene into plants.

Plasmid/vector carrier system: Agrobacterium tumifaciens method

In this approach, the micro-organism species is responsible for delivering the genetic blueprints for an infectious agent or microbe "antigens" proteins into plant cells, which induce a targeted immune response in the recipient.[32] *A. tumifaciens* is a bacterium found in soil that is used to turn a small amount of DNA into plant ordination. A single plant cell can regenerate the entire plant. When genes that have been successfully expressed in experimental model plants are administered orally to animals, serum antibodies are produced. Agrobacterium tumefaciens and Agrobacterium rhizogenes, two vegetable pathogens, have the ability to integrate transfer DNA (T-DNA) into the nuclear genome of the infected cell. The research of exogenous genes' stable integration in the plant's genome and synthesis of a transgenic protein that serves as an EV resulted from the introduction of exogenous genes into the appropriately modified T-DNA of Agrobacterium cells and subsequent infection of a vegetable tissue.[33-36]

Micro projectile bombardment (biolistics)/gene gun method

The biolistic approach, often known as the gene gun or microprojectile bombardment method,[37] is a vector-independent method. If Agrobacterium-mediated transformation is not possible, this is an alternate way of gene transfer for nuclear transformation.[12,23,38] It includes coating DNA with gold or tungsten as a microcarrier.[12,39] The coated DNA will then be placed on top of the macrocarrier, injected into the gene gun, and exposed to helium gas under high pressure.[12,39] The coated DNA will travel at a high speed within a vacuum, penetrating into the cells of the targeted plant due to the high pressure.[40] This approach has the benefit of forming a stable integration of the transgene into the plant genome and may be used to transfer foreign DNA into a range of plant host species and cell types.[41] This approach does not require a vector, and it can help with cotransformation.[41] However, it necessitates the purchase of a high-priced particle gun, is labor-intensive, and can result in serious plant tissue damage.[42,39]

Chimeric Viruses method

Viruses like CPMV (cowpea mosaic virus), alfalfa mosaic virus, TMV (tobacco mosaic virus), CaMV (cauliflower mosaic virus), potato virus X, and tomato bushy stunt virus can be modified to express snippets of antigenic proteins on their surface. Overcoat and epicoat techniques are used in this project.[43] Overcoat technology allows the plant to generate the whole protein, whereas epicoat technology only allows the foreign proteins to be expressed. A plant-derived mink enteritis virus (MEV) injectable vaccine based on chimeric CPMV has been shown to protect minks from the clinical illness. In mice, fragments of the HIV virus's gp41 surface protein were able to elicit a significant neutralising antibody response.[44] On the surface of the plant virus, it may even be feasible to provide a cocktail of specific HIV epitopes. Genetically and thermally, CPMV is a stable virus. For one hour, it can withstand acidity (pH 1). In CPMV, a wide range of epitopes have been expressed. HIV-1 (gp41), (gp120), human rhinoviruses, foot and mouth disease virus, canine parvovirus, mammalian epitopes from hormones or colon cancer cells, and fungal and protozoan epitopes from Plasmodium falciparum are among them. Parenteral and nasal (purified particles), oral (formulated leaf extracts), entire and homogenised leaves, fruits, or vegetable tissues are all options.[43] In all of these cases, plant viruses are genetically modified to carry the desired genes and then utilised to infect natural hosts like edible plants, where the cloned genes are expressed to differing degrees in various areas of the plant, including the edible parts.

Electroporation method

DNA is introduced into cells by exposing them to a high-voltage electrical pulse for a short period of time, which is thought to cause transitory holes inside the plasmalemma (a thin layer of tissue that covers surface). DNA is effectively blocked by the cell wall. As a result, enzymatic treatment must be used to weaken it, allowing DNA to enter the cell.[45,46]

MAJOR PLANT SPECIES USED AS VACCINE

Potato

Potato is a good model for developing vaccines for hepatitis B, diphtheria, tetanus, and Norwalk virus. In humans, potato may serve as an oral strengthening agent for hepatitis B vaccines.[47] The fundamental advantage of making edible vaccines from potatoes is the simplicity with which they may be transformed and propagated. Refrigerators are not required for storage, and one of the main disadvantages is that heating causes antigens to denature.[7]

Tomato

An effective vaccine against acute respiratory illness, SARS was first developed in tomato. It has a better antiviral effect against the Norwalk virus.[48] Tomatoes were used to create vaccinations for septicemia, pneumonia, and the bubonic plague. It grows swiftly and may be cultivated in a wide range of environments. Tomatoes include a lot of Vitamin A, which may help your immune system. It, on the other hand, quickly spoils.[49,50]

Carrots

Carrots are not only nutritious and tasty, but they may also be used to make edible vaccinations. When created in transgenic carrots, vaccines against *E. coli*, Helicobacter pylori, and HIV indicate potential effects. Consumption of this sort of antigen-containing carrot consumable vaccine benefits those with weakened immune systems.[51-53]

Banana

In the manufacture of edible vaccines, banana is the most usually used plant species. There is no need to prepare it. Even after cooking, proteins were not degraded. When compared to other plants, it is inexpensive. HBsAg is produced by banana plants. Antigen is present in the leaf. The biggest drawback is that it takes 2–3 years to mature and spoils quickly once ripe.[54]

Rice

Rice is the other plant species that has been employed in the creation of edible vaccinations. Benefits over other plants included being regularly used in infant food and having a high antigen expression level. However, it develops slowly and necessitates the use of a glasshouse. Vaccines made from rice plants will have a huge effect on the health in areas where rice is an important food source.[7,55]

Tobacco

Tobacco is not a plant that can be eaten. It's being utilised to produce edible vaccinations as a model. In 1996, a vaccine for the Norwalk virus, which causes gastroenteritis, was created in tobacco. VP1 protein is expressed in transgenic tobacco to protect chickens from infectious anaemia. Tobacco has the ability to produce a hepatitis B-related polypeptide. It's also being used to create a coccidiosis vaccine.[56-58]

Lettuce

Lactuca sativa expresses the B-subunit of *E. coli's* thermolabile protein, which causes both human and animal enteric disease, indicating that this vegetable could be used as an edible vaccine. In 2005, lettuce expressed the glycoprotein E2 of the normal swine fear hog pest virus.[59]

Soybean

The B-subunit of thermolabile toxin from *E. coli* bacteria was expressed in the endoplasmic reticulum (ER) of soybean (Glycine max) in this work, yielding a total antigen level of up to 2.4 percent of the total soybean seed protein without any problems after drying for further processing. Furthermore, giving this protein to rats orally leads to rise in systemic IgG and IgA.[60,12]

Algae

Chlamydomonas reinhardtii (green algae) has been exploited to produce a huge number of animal and human-specific proteins for medicinal purposes.[60,12] Because algae has such a fast rate of growth, the entire system can be used as a raw material for the production of edible vaccines. In addition, bioreactors can be used to cultivate algae that are already quickly growing. One chloroplast exists in *C. reinhardtii*, which aids in the maintenance of the desired antigens in the algal line. Notably, the efficacy of algal vaccines after lyophilization is unaffected, suggesting that global delivery of edible algae vaccine could be facilitated.[47]

Papaya

By producing synthetic peptides in 19 transgenic papaya clones, a papaya (*Carica papaya*) vaccine was produced in 2007 to combat cysticercosis caused by *Taenia solium*. The vaccine was evaluated in rats, with a 90 percent immunogenic response in vaccinated animals. These edible vaccinations may provide effective alleviation in both people and pigs, the disease's two main carriers.[61-63]

CURRENT STATUS AND APPLICATIONS

The evolution and marketing of edible vaccines takes time and patience. Many edible vaccines have been produced for animal and human illnesses, and have progressed through various stages of clinical testing. Numerous clinical trials were carried out to validate the vaccines' opportunities for human ingestion. Several communicable diseases in humans and animals, including hepatitis B, measles, and cholera, have been studied for edible vaccines.[64,65] The discovery of effective and economical medicinal chemicals in transgenic plants sparked significant developments in medical research and plant biology. Since 1986, many pharmaceutical therapeutic proteins, antigens, antibodies, monomers, enzymes, hormones, and growth regulators have been produced in various plants such as tobacco, banana, tomato, carrots, rice, maize, lettuce, Alfalfa, potatoes, peanut, spinach, apple, papaya, bean (Viciafaba), Arabidopsis, soybeans, and clover as edible vaccines against various diseases with various purposes.[66,67] Which are given below:

Malaria

Malaria is one of the leading causes of morbidity and mortality in the globe, with 300 to 500 million new infected individuals each year, resulting in 1.5 to 2.7 million fatalities. Merozoite surface protein (MSP) 4 and MSP 5 from Plasmodium falciparum, as well as MSP 4/5 from *P. yoelli*, are now being studied for the creation of a plant-based malaria vaccine.[68]

Cholera

Transgenic potatoes containing the CT-B gene of Vibrio cholerae have been demonstrated to be effective in mice. It was claimed that eating one potato every week for a month, along with occasional boosters, would offer immunity. The co-utterance of mutant cholera toxin subunit A (mCT-A) and LT-B in agricultural seed has been proven to be successful and feasible via nasal delivery.[69,70]

Hepatitis

Hepatitis According to WHO projections, two billion individuals worldwide have evidence of previous or ongoing HBV infections. Over 360 million people are chronically infected with HBV, and over 600,000 people die from HBV-related illnesses such as liver cirrhosis or hepatocellular carcinoma. HBsAg, or hepatitis B surface antigen, is employed in the manufacturing of edible hepatitis B vaccination. Potatoes are the plant of choice for the creation of an edible hepatitis vaccine. The expression of HBsAg is more prevalent in the roots than in other regions of the plant.[13,14,71,72]

Measles

Every year, measles kills 800,000 people worldwide. The measles live-attenuated vaccine (LAV) has no oral effectiveness and is destroyed if a cold chain of refrigeration is not maintained. The presence of maternal antibodies in the LAV decreases its efficacy. There are two surface proteins, hemagglutinin (H) and fusion proteins, with H protein contaminated with wild-type measles virus. The results showed that IgA antibodies were present in the faeces of animals vaccinated with MV-H. According to research, transgenic carrot plants are the wisest option for measles immunizations.[74,75] Mice fed tobacco that produced MV-H (Edmonston strain measles virus haemagglutinin) had antibody titers five times higher than what is considered effective and preventing for humans, as well as secretory IgA in their excrement.[74]

Anthrax

The possibility of using Bacillus anthracis as a bioweapon has increased the urgency of establishing a vaccination against it. Tobacco leaves inundated with the pag gene (anthrax protection antigen - PA) using a gene gun might produce a protein that is physically equivalent to the main protein found in the current vaccine. Anthrax antigen might be

manufactured in billions of units. Furthermore, this vaccination lacked the edoema and fatal factors that were accountable for the toxic side effects. Tomato plants are now being inoculated with the same anthrax antigen. Scientists are also attempting to convert spinach by transfecting it with TMV-expressing PA, in the hope that spinach will be a safer vaccine.[76,77]

Rabies

Antibodies against rabies might be induced in mice by tomato plants producing rabies antigens. TMV can also be used as an alternative. CaMV-transformed tomato plants bearing the rabies virus (ERA strain) glycoprotein (G-protein) gene were shown to be biologically active in mammals.[73]

Diarrhoea

Diarrhoea is the third biggest cause of death among Indian children. The most common originator of diarrhoea is GIT infection. The pathogens that caused the illness included bacteria, viruses, and parasitic organisms. Although several oral vaccinations have been produced for the prevention of diarrhoea, only a few mucosal active vaccines targeting pathogens have been licenced. To be effective, oral vaccination must travel through the hostile environment of the stomach and intestine. This can be accomplished by designing edible vaccines against enterotoxigenic Escherichia coli (ETEC), cholera, and norovirus. It used Agrobacterium tumifaciens to transfer gene-encoding LT B to tobacco and potato leaves, which were then given to mice. Mice fed these potatoes and tobacco leaves generated blood IgG and mucosal IgA anti-LT-B antibodies.[23]

Cancer therapy

Certain plants have been efficiently designed to produce monoclonal antibodies, which have been shown to be useful cancer therapeutic agents. In the case of soyabeans, for example, Monoclonal body (BR-96) is a powerful antidote for the drug doxorubicin, which develops ovarian cancer, breast cancer, lung cancer, and colon tumours.[44]

Role in autoimmune disease

In terms of autoimmune diseases, research into boosting self-antigen production in plants is still in its early phases. Among the illnesses being studied include multiple sclerosis, rheumatoid arthritis, lupus, and transplant rejection. Diabetic mice were fed potatoes capable of generating insulin as well as a protein called GAD (glutamic acid decarboxylase), which was linked to the CT-B monomer in one clinical investigation. The protein was revealed to be efficient in lowering immunological assaults and delaying the onset of high blood sugar levels.[78]

Rotavirus

Rotavirus is responsible for 25% of diarrhea-related fatalities in underdeveloped nations. *A. tumafaciens* converted the vector to potato by fusing the rotavirus VP7 glycoprotein to the endoplasmic reticulum transporter SEKDEL gene. Mice were given potato tubers, which evoked blood IgG and mucosal IgA responses against the virus.[79,80]

Treatment of Covid-19

Corona viruses (COVs) are a diverse category of positivesense implanted RNA viruses with genomes ranging from 27 to 32 kb.[81] Around 20 days after the SARS-CoV-2 genetic sequence, Medicago, a Canadian biopharmaceutical company, succeeded in producing virus-like particles (VLPs) of the coronavirus. Despite using egg-based vaccine production methods, this methodology involves inserting an encoded genetic sequence of COVID 19 spike protein into Agrobacterium, a common soil bacterium that is then ingested by plants.[82] The plants that arise produce a virus-like particle made up of plant lipid membrane and COVID-19 spike protein. *Nicotiana benthamiana*, a plant that belongs to the same family as tobacco plants, is being used by Medicago to create SARS CoV2 virus VLPs.[83] There is currently no licenced vaccination or therapy that has been shown to be effective against the recently developed CoV COVID-19. Patients acquire acute respiratory symptoms as a result of the CoVs infection, which produces severe respiratory illness with clinical signs. The Spike (S) protein may be utilised to create a CoV vaccine that can be cloned into a transgenic plant like a tomato, cucumber, or lettuce. The transgenic plants can then be eaten as salad and used to immunise humans against the newly emerging virus.[84]

LIMITATIONS AND CHALLENGES

While the concept of edible immunizations is enticing, putting them into action can be difficult. Many difficulties must be addressed in order to produce a plant-based vaccination, including selection of antigen, dose, quality control, selection of plant, conveyance, efficacy, safety, public perception, and licencing.[85] Antigen selection raises the question of whether chosen antigens are suitable enough with the plant type to be expressed safely. The weight, age, and size of the fruit or plant, as well as the ripeness of the fruit or plant, all influence the dosage.[86] Because no two potatoes or bananas are the same size, considerable variances in protein content may occur. This could lead to the risk of underdosing, which would result in lower antibody production, or overdose, which would result in tolerance. As a result, dose consistency from any fruit to fruit, plant to plant, and generation to generation is a concern.[87] Plant crops must have a long shelf life. Because these fruits are employed as vaccine vectors, they must be maintained correctly to minimise infection or sickness due to spoiling.[88] Another issue to consider is transgene escape and identifying the "vaccine" fruit from a regular fruit to avoid vaccination misadministration.[87] Excess mRNA may be introduced into the plant genome as a result of methods used to increase the antigenic protein concentration in transgenic plants by stunting plant growth and reducing fruit production.[85] Furthermore, plant-based vaccinations may cause an allergic reaction or other side effects such as cytokine-induced illness, central nervous system damage, or autoimmune diseases. The issue is to make the procedures easier to follow without sacrificing quality, which is a requirement for producing plant-based edible vaccines.[89]

FUTURE PROSPECTIVE

Although edible vaccinations are not yet available, researchers in fields as diverse as agricultural and biotechnology make it feasible to imagine that the a toddler being vaccinated while eating a tomato,[90] is not far-fetched. In concept, it is now feasible to transfer an organism's gene into any plant and have that gene express a new product in any part of the plant, be it the seed, leaf, root, or tuber. Food is increasingly being seen not just as a fundamental source of nutrition, but also as a product with distinct medical benefits.[91] Many factors influence the future of edible vaccines. It must be well-accepted by the general public, thus society must be educated on the use and benefits of edible vaccinations. The stability of genetically engineered plants is the next key benchmark to assess, and proper plant isolation is required.[92,93] Future research and development on edible vaccines will evaluate if these vaccines can meet WHO quality standards, such as safety, potency, efficacy, and purity.[94] Most diseases would be able to be vaccinated worldwide if these vaccinations become a reality.

CONCLUSION

Vaccines have a critical role in infection prevention. One of the biggest advances in the field of biotechnology is the development of an edible vaccination. Edible vaccines, unlike regular vaccinations, do not require

expensive equipment and equipment's to manufacture. They are less dangerous and do not need sterile injection conditions or storage. Conventional vaccinations are mostly delivered by parenteral route, need refrigeration, excessively costly, and only cause minimal mucosal reactions. On the other hand, edible immunizations are affordable, do not require refrigeration, and stimulate both systemic and mucosal responses. Edible vaccines can synthesise complicated multimeric proteins that a microbial system cannot make. When cultivating plants for the manufacturing of edible vaccines, it is necessary to keep a close eye on them. In molecular farming, there is a risk of cross-contamination between genetically engineered and non-genetically engineered plants. Because the advantages of edible vaccinations outweigh the risks, further research and development is needed in this field, which might usher in a new era of improved control over infectious illnesses.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Tfh: Follicular T-helper differentiation; **DNA:** Deoxyribonucleic acid; **NIAID:** National Institute of Allergy and Infectious diseases; **RSV:** Respiratory Syncytial Virus; **STDs:** Sexually Transmitted Diseases; **HIV:** Human Immunodeficiency Virus; **ETEC:** Enterotoxigenic *E. coli*; **MALT:** Mucosa associated Lymphoid Tissue; **SIgA:** Secretory Immunoglobulin A; **FAE:** Follicular associated enterocyte; **DC:** Dendritic cells; **CP:** Coat Protein; **T-DNA:** Transfer DNA; **EV:** Edible vaccine; **CPMV:** Cowpea mosaic virus; **TMV:** Tobacco mosaic virus; **CaMV:** Cauliflower mosaic virus; **MEV:** Mink enteritis virus; **SARS:** Severe acute respiratory syndrome; **HBsAg:** Hepatitis B surface antigen; **ER:** Endoplasmic reticulum; **MSP:** Merozoite surface protein; **HBV:** Hepatitis B virus; **LAV:** Live attenuated vaccine; **GAD:** Glutamic acid decarboxylase; **VLPs:** Virus-like particles; **COVs:** Corona viruses; **mRNA:** messenger RNA; **WHO:** World Health Organization.

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