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Plants That Can be Used as Plant-Based Edible Vaccines; Current Situation and Recent Developments

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Abstract

Among the purposes of genetic engineering technology applications in plants, improving product quality, increasing resistance to harmful organisms and improving agronomic properties, the most important one is the production of drugs, hormones and vaccines for humans and animals (for example, the use of potatoes in cholera vaccines). Today, the use of plants as bioreactors to obtain recombinant proteins from plants has been further developed and accelerated thanks to the developments in plant genetics, molecular biology and biotechnology. Appearing as a concept about a decade ago, plant bioreactors are genetically modified plants whose genomes have been manipulated to incorporate and express gene sequences of a number of useful proteins from different biological sources. Plant-derived bioreactor systems offer significant advantages over techniques used for other biological-based protein production. Easy and inexpensive production from plant tissues, providing appropriate post-translational modifications for the production of recombinant viral and bacterial antigens, and showing similar biological activity to recombinant vaccines obtained in microorganisms are important reasons that encourage the use of plant tissues in vaccine production. Edible vaccines, which create an immune response in the body against a foreign pathogen that causes disease, have a working mechanism that serves as both a nutritive and a vaccine that we consume in our daily lives. In the development of edible vaccines, the gene responsible for the production of the part of the foreign pathogen that causes the disease, that is, the antigen, which provides the immune response in the body, is transferred to the plants. With this technique, antigen production is carried out in plants. For example, thanks to today's advancing technology, enough hepatitis B antigens to vaccinate all of the world's approximately 133 million live births each year can be grown on a field of approximately two hundred hectares. In addition to these, edible vaccine technology also makes edible vaccines an interesting concept as second-generation vaccines, as they allow several antigens to approach M (microcoat) cells at the same time, by offering multi-component vaccine proteins that are possible by crossing two plant lines.

Keywords: Plant-Based Edible Vaccines; Plant Bioreactors; Transgenic Plant; Plant Biotechnology

Abbreviations: ETEC: Enterotoxigenic E Coli; MIS: Mucosal Immune System; IBDV: İnfectious Bursal Disease Virus; PA: Protective Antigen; RPV: Rinderpest Virus; NDV: Newcastle Disease Virus; eHN: Hemagglutinin-Neuraminidase; HIV: Human Immunodeficiency; VLPs: Virus Like Particle.

Introduction

Probably one of the most serious achievements of the nineteenth century was the development of vaccines. The first vaccine developed was the smallpox vaccine developed by Edward Jenner in 1796 against the negative effects of the smallpox virus on humans. Afterwards, studies on developing vaccines to combat infectious diseases were continued by Louis Pasteur [1]. Vaccine is the name given to a biological preparation that stimulates the immune system against any disease agent and is effective in remembering the immune system with a memory factor in case of encountering this agent [2]. With the vaccine, the entire microorganism or capsule, protein, nucleic acid material, etc. structures are introduced to the body in various ways (by mouth, injection into the muscle, nose) without suffering the disease caused by the microorganism or its toxin immunity is achieved. With vaccination, the host either does not catch the disease caused by that microorganism at all, or survives mildly, as the antibodies are ready before encountering the microorganism. Although the effect of the immune response that occurs with vaccination varies according to the type of vaccine and the route of administration, it is protective for a lifetime or for a certain period of time [3].

Vaccination is the practice of introducing antigens into the body to create an immune response to prevent disease and combat infectious diseases. With the development of vaccines, the smallpox virus has been completely eliminated worldwide, especially with the vaccine programs developed as a result of the widespread vaccination of people for infectious diseases, and the incidence of other infectious diseases has been greatly reduced by successful vaccination programs [4,5]. Vaccine studies, which started with the development of smallpox vaccine by Jenner in 1789 and continued until today, have been developed with different techniques. Today, researchers are trying to develop vaccines to improve existing vaccines and combat some chronic diseases such as infections, cancers and HIV/AIDS Corona/Covid19 [6].

In poorer countries of the world, inadequate healthcare infrastructures, the cost of the vaccine, and the lack of cooling systems are limiting vaccine use, and this is now seen as the leading cause of death in these countries. The Child's Vaccine Initiative, which was established in 1992 with the cooperation of the World Health Organization and some voluntary organizations in order to find a solution to this problem, has undertaken the discovery of cheaper vaccine production technologies that do not have transportation problems and the creation of alternative immunization programs at the global level. Easy and inexpensive production from plant tissues, providing appropriate post-translational modifications for the production of recombinant viral and bacterial antigens, and showing similar biological activity with recombinant vaccines obtained in microorganisms are important reasons that encourage the use of plant tissues in vaccine production [7]. The concept of edible vaccine is the use of edible tissues of transgenic plants. It has been designed as a production system for subunit vaccines and has been shown to safely induce an immune response in humans. Edible vaccines have eliminated the necessity of cold chain conditions. In addition, edible vaccines attracted attention due to the increase in the cost of access to vaccines and the delay in vaccination in underdeveloped countries [8].

Obtaining Plant-Derived Edible Vaccines Using Plants as Bioreactors

Genetic engineering technology applications in plants are carried out in order to improve product quality, improve resistance against harmful organisms and improve agronomic characteristics [9]. Among the objectives of applying genetic engineering technology in plants, the most important one is the production of substances such as drugs, hormones and vaccines for humans and animals (for example, the use of potatoes in cholera vaccines) [10,11]. Plants have the potential to act as bioreactors in the execution of many biological processes such as VLPs (Virus Like Particle) or vaccines. By manipulating the plants using foreign genes and then obtaining and using drugs, vaccines and antibodies against different human pathogens of these transformed plants, a safe production method can be developed without the need for storage. Many studies have shown that herbal vaccines are effective and safe. Monoclonal antibodies against cholera, hepatitis B virus, dengue virus, HIV and Ebola and glucocerebrosidase used for the treatment of Gaucher disease are among the most important examples of plant-derived vaccines and therapeutics [12].

Plant-based vaccines are called third-generation vaccines. This vaccine production technique is based on the production of antigenic or protective protein in the plant by cloning the vaccine in the plant expression system. In this way, vaccines; plants are produced by using them as bioreactor, a large number of productions can be carried out at the same time and continuous production can be ensured [13]. Edible vaccine; consumption of transgenic plants that produce antigens of pathogens leads to the formation of immune proteins against various infectious diseases in humans and animals are vaccines. Edible vaccines are sub-unit vaccines in which a selected gene is transferred to the plant and the protein (antigen) encoded by this gene is produced [14].

Today, the use of plants as bioreactors to obtain recombinant proteins from plants has been further

developed and accelerated thanks to the developments in plant genetics, molecular biology and biotechnology. Appearing as a concept about a decade ago, plant bioreactors are genetically modified plants whose genomes have been manipulated to incorporate and express gene sequences of a number of useful proteins from different biological sources. Plant-derived bioreactor systems offer significant advantages over techniques used for other biological-based protein production. Plant-derived bioreactor systems are grown in land and controlled closed greenhouses called green houses, using more economical inputs such as light, water and minerals. Plant bioreactor systems offer more practical advantages by being easily adapted for the production of large-scale biologically derived proteins. For example, thanks to today's advancing technology, enough hepatitis B antigens to vaccinate all of the world's approximately 133 million live births each year can be grown on a field of approximately two hundred hectares [15]. In addition to these, edible vaccine technology also makes edible vaccines an interesting concept as second-generation vaccines, as they allow several antigens to approach M (microcoat) cells at the same time, by offering multi-component vaccine proteins that are possible by crossing two plant lines. It has been reported that a multicomponent edible vaccine could be of great value in that it can be designed to simultaneously protect against multiple diseases such as enterotoxigenic E. coli (ETEC), cholera and totavirus [16].

Plant-derived vaccines; in addition to its nutritional properties, it is rich in vitamins and protein and acts as a vaccine. It includes all vaccines produced in edible form, such as a part of a plant, its fruit or byproducts derived from that plant. The most important feature is that it is taken orally (by mouth). Therefore, research and application of genetically modified plant vaccines has become an important issue in recent years [17]. Antigens in transgenic plants are given orally in the form of bio-capsules. Through the mucosal immune system (MIS), the antigens of the plant cells are protected from gastric secretions and delivered to the hard outer wall of the stomach of the disintegrating parts of the intestinal cells. Here, antigens are released, taken up by M cells in the intestinal lining lining the lymphoid tissue (GALT) in the digestive tract. It is transferred to other antigen-presenting cells, especially macrophages. Site-specific lymphocyte cells; the attack of real infectious agents destroys serum IgG and IgE responses, local IgA responses, and transforms memory cells to give the same response when faced with these cells again [18].

Techniques Used to Develop an Edible Vaccine

- The first step is to engineer the selected plant to produce peptides and proteins suitable for the purpose. After this step, the recombinant virus is integrated into the appropriate plant, which succeeds in producing many plants from which the chimeric virions are isolated and refined, and the resulting edible plant vaccine can then be used for immunological applications.
- In another method, the desired gene suitable for the purpose is combined with the plant vector by transformation technique. Combination of the desired gene with the plant vector is generally performed by three techniques: Agrobacterium-mediated gene transfer, plasmid/vector mediated, gene gun or biolistic method, and electroporation methods [19].

The edible vaccine concept was originally developed for study purposes only, and later in this case, the vaccine molecule was placed in non-food plants such as tobacco. It has been observed that tobacco is the best of the ideal plants for the production of such vaccines. Edible vaccines have been developed using the same transformation techniques as transgenic plants. The first step in edible vaccine development involves the appropriate identification of antigenic compounds of pathogens. The antigenic protein should provide satisfactory levels of immunogenic stimulation. The next step involves transferring the antigen sequence to a suitable vector. The transformation process is usually accomplished using several traditional technologies. The most appropriate method is the use of plant viral vectors [18]. When viral vectors are used for transformation, it involves modifying the viral vector to express the vaccine molecule. The modified virus is then transferred to the desired plants [20]. This method is very promising because most of the soluble vaccine molecules can integrate into the viral capsid, thereby effectively replicating and reinfecting surrounding tissues [5,18].

Another edible vaccine conversion process is non-viral methods. They include classical methods such as Agrobacterium-mediated methods and biolistic methods. For the Agrobacterium-mediated method, the Ti (tumor inducer) plasmid is selected as a vector. The plasmid is then deactivated by removing its t-DNA and oncogenes. The left margin and right margin are conserved as well as the origin of replication and virulence genes. Relevant gene and marker genes (antibiotic resistance) are inserted in place of t-DNA. The modified Ti plasmid can then introduce the graft sequence into the plant cell. Plants are co-cultured with the vector and transformed plants are then selected using antibiotic markers. Other edible vaccine development methods are adding antigenic genes to the plant; techniques such as direct and indirect gene transfer technique, electroporation and transgenic plant screening are trying to develop edible vaccines [5].

Plant and Therapeutics That Can be Used to Develop Plant-Based Edible Vaccines

Banana (Musa Sapientum)

Other agents that will serve as bioreactors and vehicles for edible vaccines are the delicious fruits that color our daily lives. Banana, one of these fruits, gives promising results in edible vaccine development studies [21]. Bananas are available in abundance in countries where the vaccine is most needed [22]. Banana is still considered an ideal source of vaccines as it is widely grown in both the tropics and subtropics [17,23]. Bananas are loved by both adults and children, and they are eaten raw, they do not need to be cooked, so the protein they contain is not denatured, which makes them an ideal candidate for an edible vaccine. As a result of studies on bananas, it has been reported that banana plants express HBsAg and especially leaves contain antigens [1]. In a different study on banana, it was observed that it is a strong edible vaccine candidate against diarrhea [19].

Rice (Oryza sativa)

Cereals such as rice are rich in soluble proteins and can be easily separated from the plant, thereby increasing antigen concentrations and therefore rice is a promising vaccine candidate. Because it can be used to express certain target proteins at high levels using structural and endosperm-specific promoters [21]. Vaccines created from rice will have a major impact on overall health where rice is the major food source. Its benefits over different plants are generally used in children's foods and high antigen articulation. In 2007, a study of genetically modified rice called *Oryza sativa* found enormous amounts of antibodies to *Escherichia coli*. In addition, the expression of hepatitis B surface antigen in rice seeds was confirmed in 2008 [24]. Transgenic rice is self-pollinated to prevent loss of vaccine molecules. The development of Japanese cedar allergy vaccines using the rice, Cry j 1 and Cry j 2 allergens is currently being studied. When the research results were evaluated, the vaccine successfully induced an immune response. A combined vaccine using roundworm antigen fused with cholera toxin is also being developed in rice [25]. An edible vaccine is also being developed against *E. coli* in rice using the B-subunit epitope. The vaccine has been observed to effectively induce an immune response. Rice is another ideal option for edible vaccines, especially since it is consumed frequently in daily life and is widely cultivated in tropical regions. As a result of different studies, developments for the zoonotic pathogen *Chlamydophila psittaci* antigen are carried out using rice. Preclinical vaccination in mice has been proven to produce both IgG and IgA antibodies [17,26]. In addition, according to recent studies, the genome of a rice plant was transferred from the microbe responsible for producing cholera toxin, and it was reported that these plants produce toxins and provide immunity against the bacteria that cause diarrhea when rice grains are given to mice [27].

In other studies on rice, Helicobacter pylori against [28], Anti-Amyloid β antibodies against Alzheimer's disease [29], infectious bursal disease virus (IBDV) against [30], rice-based cholera vaccine in Japan [31], reported that rice is a potent edible vaccine candidate in their study.

Lettuce (Lactuca sativa)

Lettuce is one of the indispensable vegetables for salads that we consume abundantly in our daily life, it is targeted as a host and carrier for edible vaccines. Trials are underway to develop an HBV vaccine in lettuce [22]. One of the most important developments is the expression of measles virus MVH in lettuce. The vaccine has proven to be immunogenic in preclinical trials [25]. In addition, the heat-stable B-subunit of cholera toxin was expressed in lettuce. It has been observed that the expressed protein constitutes 2% of the total proteins of the plant [18,32].

Potatoes (Solanum tuberosum)

Potato, which ranks fourth in the world as a food item after corn, wheat and rice, is still the most common food item in many countries, although it is responsible for only about 2% of the energy supplied from food [33,34]. Potatoes, which are generally seen only as a carbohydrate source, are an excellent source of high-quality protein and also an excellent source of lysine amino acids [35]. It is also a vegetable containing antioxidant compounds such as polyphenols, carotenoids and vitamins [33]. It is also used in gluten-free food formulations for celiac patients with its gluten-free feature. Although potato cannot compete with citrus fruits in terms of vitamin C, it meets 30% of the daily vitamin C requirement of an adult person and is rich in vitamin B6 [36] and folic acid [37].

Potatoes are considered not only fast food that we consume in restaurants, but also an ideal model to serve as a vehicle for an edible vaccine. Potatoes are used for many edible vaccines that have been successfully applied as a result of clinical studies. In addition, it has been proven that enteric pathogens such as ETEC LT-B and Norwalk virus capsid proteins can be successfully expressed in potato tubers. Studies have proven that a low dose vaccine in raw potato tubers can produce significant amounts of antibodies, both systemic and mucosal HBV vaccine is also being developed in potatoes [22]. Apart from this, vaccines are also being developed for enteric pathogens such as cholera, *E.coli* and rotavirus in potatoes. It has been observed that human papillomavirus surface antigens can also be expressed

in potato tubers [25]. In addition potato, HBV against [38], diarrhea against [32], Norwalk virus and hepatitis B against [18,39], a strong reported that it is an edible vaccine candidate.

Tomatoes (Solanum lycopersicum)

Bringing a new perspective to edible vaccines, the tomato-based vaccine, which is a must in our daily diet, has been a new development and hepatitis, HIV and rabies antigens have been successfully developed in tomatoes [21]. The tomato plant was first used in the development of an edible rabies vaccine. Tomatoes have also been used to express the epitope for respiratory syncytial virus along with Hepatitis E virus, Yersinia, DPT endotoxin and synthetic HBV and HIV antigen. In addition, tomatoes were developed for the Norwalk virus vaccine, which is known to provide better protection than the potato plant, and tomatoes were also used in research for the development of Cholera vaccines. Another important vaccine development using tomatoes has been for Alzheimer's syndrome [40]. In recent studies, recombinant structures of tomato plants (Lycopersicon esculentum Mill var. UC82b) were designed and engineered to express a gene for a glycoprotein (G-protein) that coats the outer surface of the rabies virus to develop an edible vaccine. (CaMV) and reported that they contain the G-protein gene from the ERA strain of rabies virus, including the signal peptide, under the control of the 35S promoter [27].

Neem (Azadirachta Indicia)

First of all, it should be emphasized that Neem *Azadirachta indicia* is not an edible plant; however, it is often explored as a model plant in biotechnology and as a proof-of-concept model type for edible vaccine studies. Neem *Azadirachta indicia* is one of the most widely used medicinal plants in the cosmetic and herbal industries. The raw extract of the neem leaf is an Ayurvedic medicinal herb widely used to treat normal fever and malaria fever [41-43]. It has been observed that it is effective against the clinical symptoms of Dengue fever in rats ingesting the aqueous extract of both neem leaves. Neem leaf extract has been observed to have inhibitory potential on Dengue virus type-2 replication both in vivo and in vitro [41,44]. Neem leaves are also used as a traditional practice in different parts of India to treat gastrointestinal ailments such as diarrhea [45].

In addition, it has been reported that plant extracts obtained from neem leaves, flowers or root bark have strong antioxidant potential as a result of different studies [46]. Neem bark extract has been observed to have antioxidant potential by scavenging hydroxyl radical and abolishing hydroxyl radical-mediated oxidative damage in a mouse model [47,48]. Neem leaf extract was observed to induce a cell-mediated and humoral immune response in an albino mouse model [49]. In addition, neem leaf glycoprotein has been observed to induce dendritic cell maturation [50] and macrophage-mediated antigen presentation in a mouse model [41,51].

Corn (Zea mays L.)

Corn is used as a serious candidate especially for edible vaccine production due to its refining, grinding and processing procedures and high yields [25]. Corn is currently used as a source of transgenic protein in many biotechnology companies for testing, research and production of some vaccine candidates. As a result of research on the maize plant, it has been proven that both humans and animals have satisfactory levels of rabies antigen and are expressed in maize and produce antibodies. The vaccine content is 2.7% of total phytoproteins and is stable to post-harvest processing. It has been observed that the vaccine stimulates both IgG and IgA antibodies [18]. Corn kernels have also proven to provide the benefits of expressing significantly higher levels of vaccine molecules in their grains [5,17].

Pea (Pisum sativum)

Peas are a delicious plant rich in protein, especially loved by children and highly nutritious for adults. It is another important model plant due to its short life cycle and high protein content. As a result of the researches, it has been reported that pea plants will be used in the expression of a protective antigen (PA) against rinderpest virus (RPV) and hemagglutinin protein (H). Peas need to be cooked before being consumed as food, which is seen as a disadvantage as it may reduce immunogenicity [38,52].

Tobacco (Nicotiana tabacum)

First, it should be emphasized that tobacco is not an edible plant; however, tobacco is generally used as a model plant in biotechnology and as a proof-of-concept model strain for edible vaccine studies [1]. It has been reported that tobacco is a very suitable plant especially for its easy transformation, short growing period and recombinant protein production [53]. Tobacco, which is widely used for the production of recombinant proteins in the laboratory; contain a large number of seeds, high yield and fast scaling, proteins stored in the leaves are the primary benefits and these proteins are very delicate and must be extracted without spoiling. Tobacco tissues may also contain phenols and toxic alkaloids, which must also be removed by other processes to make the products safe [54].

In the early 1990s, research on plant-based vaccine development as vaccine production platforms has just begun.

Antigen production for plant-based vaccines has emerged as a safer and cost-effective alternative to traditional vaccine development techniques. In a study investigating edible vaccines, an effective *Nicotiana tabacu*m L. cv. For Newcastle disease virus (NDV), a transgenic tobacco plant expressing the immunogenic eHN protein was developed by constructing a BY-2 cell system expressing the ectodomain of the hemagglutinin-neuraminidase (eHN) protein from the AF2240 strain. It has been reported that all mice receiving purified eHN protein from transgenic tobacco plant cells expressing the established BY-2 system immunogenic eHN protein produced antibodies and produced a transgenic tobacco plant immune response that expressed the eHN protein. These results determined that the tobacco plant could be a candidate vaccine against NDV [55].

In addition, Type I diabetes or insulin-dependent diabetes is a disease caused by autoimmune destruction of insulin-secreting beta cells in the pancreas, and transgenic plant tobacco with the gene encoding GAD67 has been developed as an edible vaccine mechanism. As a result of clinical trials, it has been observed that when diabetic mice are fed with these vaccines, transgenic plant tobacco with the gene encoding GAD67 can help suppress the autoimmune attack and delay the rise in blood sugar levels [56]. In addition, tobacco was tested in a study on mice in diphtheria, tetanus and pertussis diseases, and it was found that strong antibody response was induced. In addition, tobacco is used in plant-derived oral vaccines against infectious diseases of gaucher, malaria, ebola, acetylcholinesterase, human immunodeficiency (HIV), bluetongue, rabies, dengue fever, norwalk, avian flu H5N1 influenza, *Taenia solium*, *Toxoplasma gondii* and has a serious potential as an edible vaccine [1,19]. In addition, it has been proven by these studies that the tobacco plant is not only used for cigarettes, pipes, cigars and hookahs.

They also reported that tobacco is a potent edible vaccine candidate against Cottontail rabbit papillomavirus [57], human papillomavirus [58], and Norwalk virus [59].

Aloe vera (Aloe barbadensis)

Aloe vera is not an edible plant, but oral administration of aloe vera gel has been found to exhibit immunostimulatory activity in mice, and the main polysaccharide Acemannan (ACM) in its gel has immunomodulatory effects [60,61]. The pharmacological properties and phytochemistry of aloe vera, one of the most researched medicinal plants worldwide, have been documented [62]. Viruidal and cytotoxic properties of Aloe vera, which is considered nutraceutical, including antiviral activities, have also been detected in studies [63]. In addition, it has been observed that the extracts obtained from the Aloe vera plant show active activities against RNA and DNA viruses, especially in terms of toxicity, the harmlessness of Aloe vera extracts has been experimentally proven both in vitro and in vivo. Aloe vera alone or SARSCov-2, such as some antiviral drugs (Lopinavir, ritonavir). It contains viruidal secondary metabolites such as anthraquinones, which may be in synergy with pharmacological targets such as the protease 3CLPro. In addition to its intrinsic antiviral activities, Aloe vera also has anti-inflammatory and immunomodulatory effects [63-66].

Carrot (Daucus carota)

Carrot is a very tasty and nutritious food for both children and adults and it has been reported that carrots are being worked on in the development of an edible vaccine against ETEC (enterotoxigenic E. coli). Considered the carrot as an option as the vegetable is usually eaten raw, it has been proven to be rich in Vitamin-A and Vitamin-A precursors, which are powerful white blood cell boosters. They also contain lutein and cellulose fibers. Carrots have the potential to enhance IgG and IgA [67]. The genetic engineering of the carrot is highly advanced as the carrot was one of the first GM crops. Satisfactory levels of vaccine molecules were expressed in carrot cells [21]. Carrot has been targeted as a candidate for edible vaccines for enteric pathogens. The genes of *E.coli* and *Helicobacter pylori* antigens are expressed in carrots. Carrot is also a target vaccine candidate for HIV and has shown satisfactory results in rodents [18].

Some food plants that humans and animals consume frequently in daily life have also been identified as good sources of edible vaccines. Some of these are legumes, maize and rice, in addition to these, green and leafy crops such as alfalfa, spinach, legumes and lettuce are potentially preferred for edible vaccines. The hepatitis B antigen has been enhanced in both lupine and lettuce. They have proven to be immunogenic in both human and animal test models [25]. Alfalfa is a good source as it is high in protein and low in secondary metabolites. In addition, it has been reported that researches have been carried out on arabidopsis, celery, cabbage and cauliflower plants as edible vaccines [21].

Conclusion

Plants have both cost-effective and technical advantages over traditional expression systems for the production of pharmaceutical or non-pharmaceutical products; It has unique features that enable it to reach higher quality production and more diverse product targets in a short time with various advanced technologies such as chloroplast expression and viral transfection applications [68]. Plants have the potential to act as bioreactors in the execution of many biological processes such as VLPs (Virus Like Particle) or vaccines. By manipulating plants using foreign genes and then obtaining and using drugs, vaccines and antibodies

against different human pathogens from these transformed plants, a safe production method can be developed without the need for storage [12]. The concept of edible vaccine has been accepted as a research topic by showing its versatile use against many health hazards with many studies today. Advances in plant biotechnology have succeeded in introducing pathogenic antigens into plant vectors, allowing antigens to be produced in plant parts. In addition, plant-based edible vaccination provides a very important advantage over other vaccines. The vaccine can be consumed as food, as well as where the World lacks adequate facilities to provide general vaccine coverage, edible vaccination has been reported to be a promising candidate [5,26,40].

Edible vaccines are free of therapeutic protein and other pathogens and toxins. Since they are heat resistant, they eliminate the need for storage and cooling near the usage area. Edible vaccine producing plants can be grown in third world countries. Plants are used regularly in pharmaceuticals and established purification protocols exist. Among the advantages of edible vaccines, it has been observed that the vaccines can be easily separated from plant materials and obtained in a short time, and especially that they are very easy to purify, and that pathogenic contamination from animal cells can be effectively prevented [69,70]. The leading plants for edible vaccine research are living organisms that can change, so the continuity of vaccine production may not be guaranteed. Edible vaccines can be mixed with regular fruit and consumed in larger quantities than regular fruit, so the dose of vaccines may vary. For example, since edible bananas of different sizes contain different amounts of vaccine, the consumer should be informed about this by experts who develop edible vaccines. Also, some foods are not eaten raw (for example, potatoes or peas) and must be cooked before these foods can be consumed, so cooking can denature or weaken the protein they contain [70].

The articles reviewed in this review are from selected studies to demonstrate the feasibility of transgenic plant expression systems for proteins of microbial and viral pathogens. The rationale for edible vaccine mechanisms is that essential immunogenic proteins of major pathogens can be obtained from plant tissues and then developed to deliver a palatable edible vaccine to humans or animals [71]. The concept of edible vaccine has become a subject of interest in the scientific community for the last few decades. Advances in plant biotechnology have made it possible to insert pathogenic antigens into plant vectors in such a way that antigens are produced in plant parts. Where there are insufficient facilities to provide the world's general vaccine coverage, edible vaccination is seen as a promising candidate and the concept of edible vaccine has recently been recognized as the most promising strain [5,72,73]. In addition, the plants from which edible vaccines are obtained are food products that are grown in very large areas and are still very important for trade. The edible vaccine model has become a prominent practice to protect both humans and animals from infectious diseases. For this reason, it is thought that the concept of edible vaccine can be given a new direction, especially in agriculture. It is foreseen that in the coming days, perhaps our farmers will be able to grow edible vaccines that can combat infectious diseases in their existing fields.

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