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PRODUCTION OF EDIBLE VACCINES FOR ORAL IMMUNIZATION IN TRANSGENIC PLANTS, CURRENT AND FUTURE PROSPECTIVES

RAVINDRA B. MALABADI*
Division of Plant Biotechnology, Department of Botany, Karnatak University, Pavate Nagar, Dharwad-580003, Karnataka state, India
*Present address: Forest Biotechnology Lab (6.06), Instituto de Tecnologia Quimica e Biologica (ITQB), Universidade Nova de Lisboa, Av. Da Republica, Apartado 127, 2781-901, Oeiras, Portugal

This review highlights the recent advances made in the use of transgenic plants as biofactories for the production of edible vaccines and its long-term potential with demonstrated utility to the medical community. The use of transgenic plants to express orally immunogenic protein antigen is an emerging strategy for vaccine production. Foods under study include legumes, potatoes, banana, tobacco, rice, wheat, soybean and corn. This concept has particular suitability for developing countries. Although, the first human clinical trials for edible vaccine have been performed recently, many challenges including maximization of expression levels, stabilization during post-harvest storage, remain to be met. Public acceptance of edible vaccines is highly variable on the global scale, and similar issues of social acceptance will influence the commercial feasibility of a plant-made vaccine. Edible vaccines can be improved for their oral immunogenecity by the use of appropriate adjuvant which could be used either as a fusion to the candidate gene or as an independent gene. Concern about immune tolerance and allergy to edible vaccines has been expressed and needs to be addressed suitably. The production of antigenic proteins in genetically engineered plants provide an inexpensive source of edible vaccines, in turn, increases the value of plants as novel sources of medicinal drugs. Therefore, one would perhaps expect edible vaccines from transgenic plants to be safer than their counterparts derived from animal-based sources, which have the potential for contamination with human pathogens.

Keywords : Edible vaccine; Immunization; Oral vaccine; Risk, Transgenic plants.

Introduction

The expression of vaccines in plants is an exciting application of biotechnology. Vaccination is a great asset for eradication of infectious diseases in humans and animals[1]. In the recent several years, a novel approach for developing subunit vaccines has emerged as a result of the genetic engineering technology: the use of plants as hosts-biological bioreactors[2-4]. Therefore, plants have been considered as an alternative production systems for subunit vaccines as they are likely to contribute to all of these critical features of effective vaccines. Biotechnology is one such domain advancing at a rapid rate with new applications arising in many areas for the benefit of society. A vaccine is primarily defined as an antigenic substance(s) from a disease-causing organism administered into a host cells against the same pathogen causing disease. Vaccination involves the stimulation of the immune system to prepare it for the event of an invasion from a particular pathogen for which the immune system has been primed[5]. For most part, vaccines have relied upon serum responses, although there are good examples of oral vaccines *(i.e.*, vaccine against polio virus).

The terms vaccination and vaccine were derived from the work of Edward Jenner who, over 200 years ago, showed that inoculating people with material from skin lesions caused by cowpox (*L. vaccines*, of cows) protected them from the highly contagious and frequently fatal disease smallpox. The idea for transgenic plant-derived vaccines originated in the early 1990s. At that time, Charles Arntzen and his colleagues envisaged a cost-effective vaccine production system through the use of plants specifically engineered to deliver safe subunit preparations of candidate antigens for major diseases affecting developing and developed nations[3, 6, 7]. Vaccination is also called active immunization because the immune system is stimulated to develop its own immunity against the pathogen. Passive immunity, in contrast, results from the injection of antibodies formed by another animal (*e. g.* horse, monkey, human, pig) which provide immediate, but temporary, protection for the recipient. A subunit vaccine composed of one or more subunits of an antigenic protein from a disease-causing organism also can be immunogenically protective. Because of their relative ease of genetic manipulation and rapid growth, genetically

engineered mammalian and yeast cells are the most widely used large-scale production systems for recombinant proteins or subunit vaccines[1]. With the tools of genetic engineering and molecular biology, genes encoding immunogenic proteins of an infectious agent are transferred into the nuclear genome of a plant system *via* genetic transformation protocols, and these transgenic plants are then capable of producing the desired immunogenic protein subunit vaccines.

The use of plants and other botanicals as a source of medicines exists of the earliest stages of civilization. The main goal of oral vaccine is the induction of a mucosal immune response and a subsequent systematic immune response. Edible vaccines are the sub-unit vaccines that introduce selected genes into the plants and facilitate the production of the encoded protein. Recently, and through modern biotechnology, there has been a revival of interest in obtaining new pharmaceuticals from botanical sources. The release of vaccine is practiced so that T and B cells specific for the pathogen vaccinated against, or specific for part of it, will be ready to proliferate and differentiate a lot faster in the event of a natural challenge by a pathogen. Vaccination has become an important and effective public-health measure for safeguarding against devastating outcomes of infectious diseases. Current vaccines rely on the use of either attenuated (weakened) or killed strains of pathogens *e. g.* against diphtheria, tetanus, measles and mumps. For some vaccines, such as the one against human smallpox, a strain from a different species (cowpox) is used instead. Some of these vaccines (especially parenteral-vaccines) contain toxic preservatives such as formaldehyde, thiomersal (a mercury-based compound), and aluminum phosphate[8, 9]. In recent years there has been a move towards developing subunit vaccines, linear immunogenic epitopes of the pathogen that elicit production of antibodies. This alleviates concerns over risk of reversion of attenuated strains to aggressive forms in pathogen-based-vaccines[8]. Scale up production of current vaccines takes place either in specific pathogen free eggs or mammalian cells grown in large fermentors or bioreactors. Therefore, these vaccines require purification, before they are available for use. Moreover, most are delivered *via* intramuscular injection, and, therefore, require the use of sterile hypodermic needles. As the products of genetically modified plants make their way from concept to commercialization, the associated risks and acceptance by the public has been become a focal point. In this review, I summarize the recent advances made in the use of transgenic plants and plant cell cultures as biological factories for the production of vaccines. This review also updates and highlights the importance of plant-based vaccines verses existing vaccine system and problems of social acceptance of the oral vaccine concept.

Edible vaccines - The important features of any effective vaccine include safety, protective immunity that is sustained for long periods of time (preferably a life time), ease of administration, low cost and few side-effects. In recent years, plants have emerged as alternative production systems for subunit vaccines as they are likely to contribute to all of these critical features of effective vaccines. Plants that have been engineered with genes encoding antigenic proteins of various pathogenic viral and bacterial organisms have been shown to correctly express the proteins that elicit production of antibodies in animal and human hosts. Plant systems do not harbor human or animal pathogens (such as virions or prions) and, therefore, they do not transmit such pathogens along with the target subunit vaccine. Pathogens that infect plants do not infect humans, whereas mammalian pathogens can infect human and other animal populations[1]. Moreover, they cost less to produce than *via* fermentation or bioreactors; plants can be grown in the field or in a greenhouse relatively inexpensively[10]. When produced in edible parts of the plant, such as grain, fruit or even leaves, subunit vaccines may not require purification. Also, any required processing of an edible vaccine in the form of juice, powder or sauce, would be less complicated and easier than purification. Maintaining the antigenic protein within plant cells that are edible may also contribute to stability and reduce degradation. Another advantage of producing subunit vaccines in edible parts of a plant is the potential to deliver them orally rather than intramuscularly[9], providing a simple and easy means of administration to humans and animals. Moreover, oral delivery stimulates mucosal immunity (the first line of defense) in the tissues lining the mouth, nose and esophagus (among others) that provide the first target of opportunity for pathogens to enter and infect the human or animal body. Mucosal immunity is the term for the production of antibodies in those regions of the body that are exposed to the environment such as the mouth, nose, stomach and intestines[4]. In addition, production in plants reduces the overall cost of vaccinations, which is often prohibitive in developing countries; for example, sterile hypodermic syringes are not required. Plants can readily and properly handle the downstream processing of foreign proteins, including expression, folding, assembly, and glycosylation, all contributing to the fidelity of antigenic proteins[11]. As a result, these proteins maintain their activity and efficacy, thus contributing to their viability as subunit-vaccine candidates. Plants can produce not only single, simple foreign proteins, but also complex multimeres, such as secretary proteins and antibodies. All these capabilities render plants as targets of opportunity for marketing of high-value protein products[11]. The advantages from producing subunit vaccines in plants or edible vaccines or plant-based-vaccines may be summarized as follows;

1) Adjuvant for immune response is not necessary.

2) Elimination of risk of contamination with infectious agents.

3) Convenience and safety in storing and transporting vaccines.
4) Cost-effective in larger quantities.
5) Edible plants are very effective as a delivery vehicle for inducing oral immunization.
6) Reduced need for medical personnel and sterile injection conditions.
7) Easy for mass production system by plants compared to an animal system.
8) Storage near the site of use.
9) Antigen protection through bioencapsulation.
10) Subunit vaccine (not attenuated pathogens) means improved safety.
11) Seroconversion in the presence of maternal antibodies.
12) Generation of systematic and mucosal immunity- first line of defensive mechanism.
13) Delivery of multiple antigens.
14) Integration with other vaccine approaches.
15) Improved patient compliance (especially in children).
16) Longer shelf-life.
17) Help in attaining eventual independence foreign supply.
18) Stimulation of humoral immunity.

The development of plant-based vaccines directed at human and animal diseases has opened up an innovative and exciting opportunity for adding new high value to food crops, thus increasing the uses and profitability of these value crops. The production of antigens in genetically engineered plants provides an inexpensive source of edible vaccines and, in turn, increases the value of plants as novel sources of medicinal drugs[1]. This new field of biological science, referred to as molecular biopharming has received much attention in the past decade and promises to become more important in the next decade. Oral vaccines are more affordable and accessible to the inhabitants of developing countries, who needlessly die, in the thousands, from diseases, which can easily be prevented by vaccination. Food vaccines are like subunit preparations in that they are engineered to contain antigens but bear no genes that would enable whole pathogens to form. These vaccines basically work in the same way as the injected DNA vaccines, since a peptide sequence similar to an infectious part of a pathogen is synthesized, by itself, and is used to prime T and B cells in the body[12]. The major difference in this case is that the protein sequences are encoded in a plant to form the desired protein. This protein is then ingested, as the plant or its fruit is eaten. One becomes immune against the ingested protein, as T and B cells become stimulated to proliferate and differentiate[12]. Thus, food crops can play a significant new role in promoting human health by serving as vehicles for both production and delivery of vaccines.

Background- There are many examples of successful expressions of antigens in plants was achieved for Cholera toxin B subunit (CT-B) in lettuce (*Lactuca sativa* plants)[13], potato[14] and tomato plants[15], *E-coli* heat-labile enterotoxin B subunit (LT-B) in tobacco and potatos[16], Norwalk virus capsid protein in tobacco and potato[17], Hepatitis B surface antigen in tobacco and potato [18-23] and in banana plants[24], human milk protein β-casein in potato plants[25], antimicrobial human lactoferrin in potato plants[26] and Rabies virus G-protein in tomato[27]. Food vaccines are also used to suppress autoimmune disorders like type-1 diabetes, multiple sclerosis, rheumatoid arthritis etc[28]. Foods under study include potatoes, banana, lettuce, rice, wheat, soybean, corn and legumes. Bananas is a good candidate for edible vaccines since they were eaten raw, appealing to children, inexpensive to produce, native to many developing countries[29]. A measles vaccine that can be directly consumed would significantly increase the availability in places where maintenance of a cold-chain during storage and transport is difficult. Tacket[30] conducted the first human clinical study where they demonstrated that humans given a plant-derived oral vaccine (fed raw transgenic potato tubers carrying the recombinant LT-B antigen) produced both serum IgG- and mucosal IgA-specific antibodies in humans. The capsid protein of the Norwalk virus was also expressed in potato tubers and found to be immunogenic in test mice as well[17]. Very recently, a human clinical trial was conducted by feeding 24 healthy adult volunteers two or three doses of these potato tubers and found that 19 of 20 volunteers fed the transgenic potato (carrying the capsid protein of the Norwalk virus) developed an immune response, although the level of serum antibody increases was reported to be modest[30]. The potato was also used for the production and delivery of the human insulin antigen[31, 32]. In another effort, constructs carrying the gene encoding the binding subunit of *Escherichia coli* heat labile enterotoxin (LT-B) were introduced into tobacco and potato plants[33]. Heat labile enterotoxin is produced by enterotoxigenic *E. coli* (ETEC), the causal agent of an enteric disease, and also immunogenically interacts with the cholera toxin of *Vibrio cholerae*. Thus, LT-B is a candidate vaccine against both EETC and cholera[1].

There are several reports on the development of transgenic plants that express antigenic proteins of pathogenic human and animal organisms[34-36]. Tomatoes were used to produce the first plant-derived rabies vaccine[27], and have proven more palatable than potatoes while offering other advantages such as high biomass yields and the increased contaminant that is offered by growth in greenhouses. Mor[37] used tomato to express the human acetylcholinesterase (AChE) that provides protection against organophosphate poisoning. Soybean was used for production of the glycoprotein B antibody of the herpes simplex virus 2(HSV-2) [38], while corn was used for the production of an LT-B subunit vaccine[39]. Recently, Stoger[40] expressed the single-chain Fv (ScFv) antibody of the human

carcinoembryonic antigen (CEA), a marker antigen to diagnose tumor onset, in both rice and wheat grains. Both lupine and lettuce were used to express a hepatitis B surface antigen (HbsAg) either in the pods or leaves, respectively, and these tissues were found to be useful systems for production and delivery of this antigen vaccine[41]. A plant-based oral vaccine was also developed in tomatoes for the respiratory syncytial virus (RSV), a serious pathogen that causes bronchiolitis and pneumonia-type diseases in all human-age groups[1]. RSV infects virtually all children worldwide and can cause symptomatic infections throughout life. An oral vaccine is desirable for its ease of use[42, 76].

Hepatitis B- Hepatitis B virus (HBV) infection is an important global health problem, and vaccination is a proven strategy to control HBV infection. Hepatitis means inflammation of the liver, which in turn causes damage to individual liver cells. The hepatitis B virus is estimated to have infected 420 million people throughout the globe, making it one of the most common human pathogens. Hepatitis B is a serious liver-cancer disease that may result in long-term complications. These chronically infected persons are at high risk of death from cirrhosis of the liver cancer. This is the most common cause of infection with viruses called hepatitis A, B, C, D and E. HBV is much more contagious than AIDS virus. HBV is commonly called as the liver cancer. Many patients with acute hepatitis B have no symptoms, or symptoms are mild and mistaken for flu. Their bodies are able to fight the virus off quickly. Some however become quite sick while their bodies are fighting off the virus. Hepatitis B virus causes acute diseases lasting several weeks including loss of appetite, nausea, vomiting, fever; aching muscles, joint pain; yellowing of skin and eyes (Jaundice); dark urine and putty-like or white stool.

Diagnosis of the disease is made by a blood test. It is called hepatitis B surface antigen test (HBsAg). No specific treatment is available or usually necessary for acute hepatitis infection. The physician may recommend supportive measures to help the patient maintain strength and avoid taxing the liver while body's natural defenses are fighting the virus. Hepatitis may be either acute or chronic. Acute hepatitis B patients recover completely within six months and develop antibodies that give them a life-long immunity. Chronic hepatitis can develop over a number of years without the patient ever having acute hepatitis or even feeling sick. As the liver repairs itself, fibrous tissue develops, much like a scar forms after a cut or injury to the skin heals. Advanced scarring of the liver is called cirrhosis. Over time, cirrhosis irreversibly damages the liver, eventually ending in liver failure. Liver transplantation is the only successful form of therapy for the people with the chronic hepatitis B with a damaged liver.

Current vaccines use yeast-derived recombinant hepatitis B surface antigen (rHBsAg) delivered by intramuscular injection, requiring trained medical practitioners and refrigerated storage, and are thus expensive to use. In many areas of the developing world the expense of immunization programs prohibits the use of the currently available vaccines for large segments of the population. The plant based production of vaccine for hepatitis B in the edible fruits may be an economical alternative. Hepatitis B surface antigen (HBsAg) expression has been reported in transgenic tobacco plants[18, 23], lettuce and lupin[41], carrot[43], potato[20, 44] and banana plants[24, 45]. The plant derived HBsAg, self assembles with respect to the following; size, density sedimentation, antibody binding, in eliciting HBsAg specific antibodies in mice, primes T cells *in vivo*. This can be stimulated *in vitro* by tobacco derived rHBsAg, yeast derived rHBsAg, and by a synthetic peptide that represents an epitope of the HBsAg[19]. HBsAg is a transmembrane protein with uncleaved internal signal sequences that facilitate cotranslational translocation and integration of HBsAg into the endoplasmic reticulum (ER) membrane[22]. Plant derived HBsAg assembles into virus-like particles as in human and yeast cells[22]. Following initial clinical trials, Kapusta[41] reported that oral delivery of the HBsAg stimulated development of anti-HBsAg IgG in humans. In subsequent clinical trials performed at Roswell Park Cancer Institute (Buffalo, NY), patients who had previously been vaccinated with yeast recombinant injectable HBsAg and were fed raw potatoes expressing the HBsAg showed stimulation of antibody titers. Based on these promising preliminary results, trials continue to more forward[46]. Further studies are underway to increase the level of the HBsAg by using different promoters such as patatin promoter, and different transcription regulating elements.

Viral diarrhea -Norwalk virus capsid protein (NVCP) from the diarrhea causing Norwalk virus was also expressed in transgenic tobacco and potato. This protein in the host cells stimulated serum IgG and gut IgA specific for NVCP when fed to mice cells[17]. The clinical trial was conducted at the Center for Vaccine Development with NVCP potatoes[47]. Twenty adults ingested either two or three doses each of 150 g raw potato containing 310-700μg NVCP. Nineteen of twenty adults showed significant increases in the numbers of specific anti-NVCP-antibody-secreating cells of the IgA subtype. This study proved that orally delivered plant-expressed VLPs could stimulate immune responses and further that GM1 binding activities not required for oral immunization[48].

Measles- Measles is a highly contagious viral disease caused by the *Paramyxo* virus spread by air. Each year, almost one million children die from the measles and many of the survivors are weakened by pneumonia or encephalitis or become deaf. The symptoms of the disease are high fever, skin rash and spots and it can lead to many different

complications[12]. Recent studies of expression of the *Paramyxo* virus surface antigen protein hemagglutinin in banana, tobacco, potato, rice and lettuce were recorded[49]. Serum samples from healthy experimental animals, fed with transgenic banana, were analyzed for the presence of anti-hemagglutinin-specific antibodies. The results are highly significant and demonstrate that banana plant can produce the antigenic hemagglutinin protein of the measles virus and elicit immune responses in the experimental animals[49].

Cholera- Cholera is a severe diarrheal disease caused by the bacterium *Vibrio cholerae*. The secretes cholera toxin that is responsible for the profuse watery diarrhea. The holotoxin comprises of one A and B five subunits[50]. The pentameric B moiety is a strong immunological adjuvant. The ideal vaccine for cholera would be one that provided antitoxin and anticolonizing immunity. Such vaccines are currently being tested[15]. Since parenteral cholera vaccines are not considered to be very effective, both killed and live oral vaccines have been investigated. An oral vaccine composed of CTB mixed with inactivated *V. cholerae* cells gives protection against cholera[51]. However, the cost of production of CTB is too high for developing countries to use it as a vaccine component.

Oral administration of *Vibrio cholerae* enterotoxin (CTX) and the nontoxic cholera toxin B subunit (CTB) induce both systemic and mucosal antibody production in animals and man[52]. Cholera toxin B subunit has been expressed in transgenic tobacco[53,54], potato[14] and lettuce[13] plants. The CTB protein, purified from transgenic tobacco plants was found to be antigenically similar to authentic protein[54]. The production of immuno-modulatory transmucosal carrier molecules, such as CTB, in food plants may greatly improve the efficacy of edible plant based-vaccines[17,19,33], and may also provide novel oral toleration agents for prevention of such autoimmune diseases as type I diabetes[55], rheumatoid arthritis[56] and multiple sclerosis[57]. In the human trial (Phase I Proof of-concept trial) performed with 14 healthy adults, 11 were chosen at random and three received pieces of ordinary potatoes. The investigators analyzed blood and stool samples from the volunteers and evaluated the vaccine's ability to stimulate both systematic and intestinal immune responses. Ten out of eleven volunteers (91%) who ingested the transgenic potatoes had a four-fold increase in serum antibodies at some point after immunization and six of eleven volunteers (57%) developed a four fold increase in intestinal antibodies. The potatoes were well tolerated and no one experienced serious adverse side effects[46, 48].

Respiratory syncytial virus (RSV)- A plant-based oral vaccine was also developed in tomatoes for the respiratory syncytial virus (RSV), a serious pathogen that causes bronchiolitis and pneumonia-type diseases in all human-age groups[1]. Tomato is one of the most important vegetable crops of commercial importance in world. RSV is a leading cause of viral lower respiratory tract illness in infants and children worldwide and can lead to infant mortality. RSV infects virtually all children worldwide and can cause symptomatic infections throughout life. An oral vaccine is desirable for its ease of use[42]. The United Nations 1992 Children's Vaccine Institute calls for the development of a oral vaccine against RSV as no vaccine is available so far. RSV infects virtually all children worldwide and can cause symptomatic infections throughout life. Risk factors for severe RSV disease include congenital heart diseases, bronchopulmonary anomalies, immunodeficiency, prematurity, and age of less than six weeks[58]. Approximately, 25 to 40% of infected infants, elderly people and adults with immuno-compromised systems develop symptoms of bronchiolitis or pneumonia. RSV disease occurs throughout the world and is more severe in underdeveloped countries where it usually takes the form of a "common cold" but can be more severe. The virus enters human body mainly through the nose and eyes, but also through the mouth[1]. In the northern hemisphere, the yearly peak seasons for RSV infections occur from December through March and, in urban areas, the virus is detected from the Fall through the Spring. A major difficulty in developing a RSV vaccine that works *via* the serum immune system has been the fact that natural infection confers, at most, only temporary protection against reinfection[1, 42, 59]. There is no consistently effective treatment available for RSV infections, and these infections can occur repeatedly in the same individual[1]. Another problem is that the mechanism by which even partial immunity to RSV develops is not well understood. Upon analysis of the tomato fruit for the localization of the antigenic RSV-F protein, it was found that the majority of the antigenic protein is localized in the seed, while the pulp contained only marginal levels of the antigen. This suggested that in order to deliver a high amount level of the RSV-F antigen vaccine, the whole tomato fruit (seed and pulp) must be homogenized and used for the delivery of the vaccine to insure presence of high enough levels of the antigen[1].

Novel vaccine targets- Edible vaccine development for the prevention or treatment of cancer is difficult since tumor antigens are also auto-antigens[60]. Auto-antigens are nothing but the body's own proteins recognized as foreign by the immune system. Autoimmune diseases include arthritis, myasthenia gravis, multiple sclerosis and type I diabetes. A scFv antibody fragment of the immunoglobulin from a mouse B-cell lymphoma in tobacco with a viral vectory and showed that mice injected with this vaccine were protected from challenge by a lethal dose of tumor. Another scFv fused to the potato virus X coat protein generated protection against lymphoma and myeloma[61]. Recently, a poly-epitope isolated from human melanoma tumor was integrated into the nuclear and chloroplast DNA of tobacco in an attempt to develop a plant-derived melanoma vaccine[62]. Ma and Jevnikar[63]

expressed glutamic acid dehydrogenase in potatoes and fed them to non-obese diabetic mice, in which the reduced pancreatic islet inflammation suggested immuno-tolerization of cytotoxic T-cell-mediated autoimmune disease. As usual an appropriate oral dose of a plant derived auto antigen will inhibit development of the autoimmune disease[48,62,64].

Human lactoferrin- Lactoferrin is an iron-binding glycoprotein found in high concentration in mammalian milks and to a lesser extent, in exocrine fluids such as bile and tears[26]. Lactoferrin plays a significant protective role in human milk. Based on its iron-chelating properties, lactoferrin impedes bacterial iron utilization causing bacteriostasis[65]. Human lactoferrin (hLF) protein also contains a specific antimicrobial domain consisting of a loop of 18 amino acid residues. This peptide region significantly inhibits growth of *E. coli* and is distinct from the iron-binding region. Lactoferrin is also important in the regulation of myelopoiesis, the modulation of inflammatory responses, as an essential growth factor for lymphocytes, in DNA binding and RNase cleavage[26]. A cDNA fragment encoding human lactoferrin (hLF) linked to a plant microsomal retention signal peptide was stably integrated into potato plants[26]. Antimicrobial activity against four different human pathogenic bacterial strains was detected in the extracts of lactoferrin-containing potato tuber tissues. This is the first report of synthesis of full length, biologically active hLF in edible plants.

Social acceptance of plant-made vaccines- The expression of vaccines in plants has shown a fundamental ability to induce systematic and mucosal immune responses, and phase I initial clinical studies have demonstrated that plant-made vaccines(PMV's) to be safe and functional[7,66]. Public acceptance of GM food is highly variable on a global scale. Similar issues of social acceptance will influence the commercial feasibility of a plant-made vaccine. Despite advances in agricultural biotechnology that have resulted in the approved release of several GM food crops, the extension of plant engineering technologies for human vaccines has been comparatively low. Underlying this suggestion is the assumption that social acceptance will not prevent introduction of an effective plant-made vaccines to the market[67]. Perhaps more importantly, that the potential for nonacceptance would not prevent commercial parties from exploring and developing this new technology[68]. If social acceptance is of sufficient importance, then gauging public attitudes toward any new technology becomes an important step in market assessment. This will lead to the justification of financial investment to conduct research and development. People express their preferences directly in the market place. Public perceptions of biotechnology are extremely complex and can not be generalized easily. There are numerous opinion studies about genetically modified foods, but few address the use of biotechnology to produce pharmaceuticals[69]. Consumer preference for plant made vaccines could be crudely estimated by evaluating the acceptance of genetically modified foods. However, the risks and benefits of vaccines are significantly different than those of food commodities. In one of the recent survey, public opinion is that plant made vaccines were advantageous for use in developing countries. They offer significant cost benefits, and are more appropriate than other transgenic plant technologies due to the preventative medical application. The interpretation of delivering edible vaccines to developing countries is not dissimilar to much of the literature on this topic[70].

In one of the survey, it was found that males were slightly more accepting of genetically modified vaccines (72%) than females (64%), which may be grounded in the same trends shown by Fischhoff and Fischhoff[67]. Other studies have also shown this gender gap regarding perception of genetic technology. Women perceive lower benefits and are less accepting of genetic technology than men. But more empirical studies need to be done to specifically address this problem[71]. Studies have shown that even if people associate technology with relatively high risks and unknown consequences, they still might not reject the technology[71,72]. Oversight by regulatory agencies (throughout the world) may give confidence to the general public and facilitate acceptance of new technologies, despite negative perceptions with regard to specific risks. More empirical research on public perception of agricultural biotechnology specific to producing novel vaccines is needed before substantive generalizations can be made. Given that oral vaccines are preferred, that people believe that most vaccines are genetically modified, and the public has expressed a high acceptance of plant-made-vaccines. Further development of this technology by commercial parties is favorable, if paralleled with appropriate demand for specific products. Investment in clear communication by scientists and regulators will further enhance the public trust, optimism, and ultimate acceptance for plant made vaccines[68,73].

Risk analyses - New technology brings risk and benefit, both of which have some degree of uncertainty before introduction to society and environment. To protect the interests of the greater population, assessment of risk is necessary before release of new technologies[68]. Although plant-based technology has presented significant perceived advantages for cost and utility of vaccine production, it is yet to be demonstrated in commercial practice[74]. Commercial potential of this technology is dependent on showing broad protective immunity in humans, demonstrating a viable manufacturing process, and forecasting accurate cost of production. Kirk and Webb[73] have recently reviewed the strength and weaknesses of the plant-made vaccine platform. Many of the uncertainties associated with this technology

can not be either validated or disproved until a first product emerges. Two major milestones in moving this technology forward are the successful development of a model product and demonstration of protection in humans. The achievement of these milestones will stimulate maturation of the regulatory framework in which risk assessment, management, and communication standards can be defined.

There are several risks during production and delivery stages of this technology, with potential impact on the environment and on human health. Risk to the environment includes gene transfer and exposure to antigens or selectable marker proteins. Risks to human health include oral tolerance, allergenicity, inconsistent dosage, worker exposure and unintended exposure to antigens or selectable marker proteins in the food chain. These risks are controllable through appropriate regulatory measures at all the stages of production and distribution of a potential plant-made vaccine. Risk can be defined as the probability that a substance or situation will produce harm under specified conditions. Risk is a function of the probability that an adverse event will occur, and the consequences of that adverse event. Risk is important to all persons who either individually or collectively may be influenced by a specific activity. Risk occurs on a variety of scales from individual risk, through community risk, to global or biosphere significance[75]. Therefore, there is some degree of risk in taking an action, and in not taking action. We must accept that there will always be risk as a consequence of decision-making[68]. The process of formal risk analyses requires the integration of science-based framework with the social, cultural, and economical impacts that may result through implementation of that technology. The current process of drug approval is largely confined to the manufacturer and the regulatory agencies. It should be expected however, that public interest groups might have persuasion with regulators if sufficient support is generated within the general public. Most of the risks described are low in severity and are increasingly monitored by a range of stakeholders during the development of the technology[68].

Safety concerns- The two main concerns over edible vaccines are the contamination of food crops through cross pollination and of the vaccine itself in plant debris spreading as dust and as pollutants in surface and ground water. The vaccine antigen may affect browsing animals and humans living in the area drinking vaccine-polluted water or breathing vaccine-polluted dust. It is imperative that the cultivation and production of pharmaceutical crops should be limited to controlled production facilities such as greenhouses, or in plant tissue culture, that prevent the environmental reasea of the biopharmaceuticals. The main safety concern is that the oral vaccine preparations will induce 'immune tolerance', thereby making the individual susceptible to, fore example, the hepatitis B virus.

Limitations - At the present time, the prospect of developing effective edible vaccines for oral immunization is not without limitations. Expression levels obtained thus far in transgenic plants are below optimum and need to be enhanced. In addition, not all vaccine candidate proteins are highly immunogenic in plant tissues and secondary metabolites found in plants may compromise the ability of the vaccine candidate protein to induce immunity. For example one could develop immunotolerance to the vaccine peptide or protein. Little research has been done on this topic[62]. Most of the examples discussed above commonly showed that plants accumulate foreign proteins to relatively low levels. Less immunogenic proteins would require even larger doses to be effective. Even with more palatable alternatives to potatoes (e.g., banana), these accumulation levels limit the practicality of edible vaccines. Two solutions to overcome this limitation are being explored. First, techniques to enhance antigen accumulation in plant tissues are being explored. A number of factors during gene expression affect the trasgene expression and ultimately vaccine epitope accumulation in plants. Optimization of coding sequences of bacteria or viral genes for transient expression for product accumulation to obtain optimal quality and quantity is also being considered. Clearly, additional experimentation in this area is needed[48]. Another limitation is storage of edible vaccines. Potatoes containing vaccines proteins seem to store well at 40°C but tomatoes will not last very long. Using potatoes or bananas may require some processing such as smashing and a liquoting as in baby food jars. Other concerns are about transgene escape and identification of 'vaccine' fruit verses a normal fruit. Fruit vaccines should be easily identifiable to avoid the misadministration of the vaccine, which may lead to complications such as immunotolerance.

Conclusions- Producing vaccines in plants offers numerous advantages over current vaccine methodologies. Among them, safety, ease of production and low cost of production provide strong justification for developing this novel technology. As the technology to produce vaccines in plants goes through the regulatory pathway and demonstrates its economic feasibility, it may also overcome public-perception concerns that seem to have been dodged by the pharmaceutical industry. Nevertheless, edible vaccines are an exciting and novel strategy for the development of oral vaccine. Edible vaccine is a milestone on the road to creating inexpensive vaccines that might be particularly useful in immunizing people in developing countries. There is potential for major impacts on global health, particularly in developing countries. Plants might one day surpass other production systems because of the economic and safety benefits, and ultimately, it should be possible to make edible vaccines available to everyone who needs them, at a lower cost that everyone can afford. This leads to a new area of agriculture, now referred to as

"bio-pharming" where plants are used as factories for the production of edible vaccines and /or other antimicrobial agents. The use of foods as vehicles for production and delivery of human vaccines is an exciting and novel field of biotechnology and should pay dividends for both human health and the agricultural sector in the near future.

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