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Review Article

EDIBLE VACCINES FROM GM CROPS: CURRENT STATUS AND FUTURE SCOPE

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ABSTRACT
The idea of an edible vaccine is coming closer to reality as scientists have found a way to incorporate the protein gene with some antigen in some plants. The major hurdles in the path of an emerging vaccine technology are being overcome. In this context, genetically modified (GM) plants are being investigated for the production of vaccines, antibodies and therapeutic proteins. The development of GM crops to produce drugs and vaccines has received considerable investment and is relatively well advanced. The myth surrounding edible vaccines and 'food as pill' is the difficulty to control their intake and distribution, particularly in developing countries where education levels and literacy may be low. However, this concept suffers from the fact that the potency of this class of vaccines and drugs remains unmasked to the majority of the population, which has to be publicized and campaigned in a scientific manner, to make it realistic and useful for the common man. Creating edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. This process is known as "**transformation**," and the altered plants are called "**transgenic plants**." Like conventional subunit vaccines, edible vaccines are composed of antigenic proteins and are devoid of pathogenic genes. Thus, they have no way of establishing infection, assuring its safety, especially in immuno-compromised patients. Conventional subunit vaccines are expensive and technology-intensive, need purification, require refrigeration and produce poor mucosal response. In contrast, edible vaccines would enhance compliance, especially in children and because of oral administration, would eliminate the need for trained medical personnel. Their production is highly efficient and can be easily scaled up. If the technology is properly nurtured and given the right direction, it may usher into a new era where we will be asked to take "food" rather than "drugs" when we are ill. This review attempts to discuss the current status and future of this new preventive modality.
Keywords: Chimeric, edible vaccines, transgenic

INTRODUCTION

Vaccine is basically a substance used to stimulate the production of antibodies and provide immunity against one or several diseases. Vaccines have accomplished near miracles in the fight against infectious disease. Due to vaccine smallpox has become a history and should soon same would happen to polio. Yet these victories mask tragic gaps in delivery. The 20 percent of infants still missed by the six vaccines-against diphtheria, pertussis (whooping cough), polio, measles, tetanus and tuberculosis-account for about two million unnecessary deaths each year, especially in the most remote and impoverished parts of the globe. Upheavals in many developing nations now threaten to erode the advances of the recent past, and millions still die from infectious diseases for which immunizations are nonexistent, unreliable or too costly. This situation is worrisome not only for the places that lack health care but for the entire world. [1]

Keeping these statistics and situation in view, genomics has opened up other entirely new avenues. Plants can be engineered to produce antigens, so an edible vaccine is feasible. [2]

Edible vaccines are antigenic proteins that are genetically engineered into a consumable crop. The idea is that the crop food product contains the protein which is derived from some disease causing pathogen. As the people eat the crop, the food is digested, and some of the protein makes its way into the blood stream. Through this enough of this protein gets into the blood stream and it causes an immune response. This immune response would now neutralize the pathogen should the person ever encounter it in the future. [3]

The plant's capacity of producing different classes of proteins with pharmaceutical value and the need for new technology for the production and delivery of inexpensive vaccines has led to the use of transgenic plants. Also, large amounts of antibodies can be produced at relatively low cost, using agriculture instead of sophisticated and expensive cell culture-based expression systems. [4]

Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and socioculturally readily acceptable vaccine delivery system, especially for the poor developing countries. Introduced as a concept about a decade ago, it has become a reality today. A variety of delivery systems have been developed. Initially thought to be useful only for preventing infectious diseases, it has also found application in prevention of autoimmune diseases, birth control, cancer therapy, etc. Edible vaccines are currently being developed for a number of human and animal diseases. There is growing acceptance of transgenic crops in both industrial and developing countries. Resistance to genetically modified foods may affect the future of edible vaccines. They have passed the major hurdles in the path of an emerging vaccine technology. Various technical obstacles, regulatory and non-scientific challenges, though all seem surmountable, need to be overcome. This review attempts to discuss the current status and future of this new preventive modality. [5]

First encounter[6] - Edible vaccines were first tested on humans in 1997, when scientists asked volunteers to eat anti-diarrheal transgenic potatoes. . After consuming the potatoes, almost all the volunteers produced antigens in their bodies

just as if they had received a traditional anti-diarrheal vaccination. And they experienced no adverse side effects. Volunteers are also testing raw potatoes engineered to produce a Hepatitis B antigen. The first final human trial that confirmed the feasibility of edible vaccine was done in 1998. Volunteers ate bite-sized pieces of raw potato that had been genetically engineered to produce part of the toxin secreted by the Escherichia coli bacterium, which causes diarrhea. Encouraged by the results of this study; NIAID-supported scientists explored the use of this technique for administering other antigens.

Current development - Oral vaccines, whether living or nonliving, must be protected during passage through the hostile environment of the stomach and intestine to the sites where immune stimulation occurs. In recent years, a variety of delivery systems have been developed for presenting nonliving antigens to mucosal surfaces, which will allow these antigens to persist and survive in the hostile gastric and enteric environments. These include polylactide/polyglycolide, microspheres, liposomes, proteosomes, cochleates, virus-like particles, and immune-stimulating complexes. [4]

There is research going on to make up digestive losses of the antigen and to elicit a significant immune response. Less immunogenic proteins would require even larger doses to be effective. Even with more palatable alternatives to potatoes (e.g. bananas), these accumulation levels may 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. These include optimization of the coding sequence of bacterial or viral genes for expression as plant nuclear genes, and defining the subcellular compartment in which to accumulate the product for optimal quantity and quality. Several laboratories are also developing alternative expression systems to improve accumulation.

The second approach is to enhance the immunogenicity of the orally delivered antigens by using mucosal adjuvants. One such approach is making use of bacterial entertoxins such as CT or LT, mammalian and viral immunomodulators as well as plant-derived secondary metabolites. [6]

From the graph given below we can conclude that there has been a significant increase in the amount of research done on the edible vaccine in past one decade.

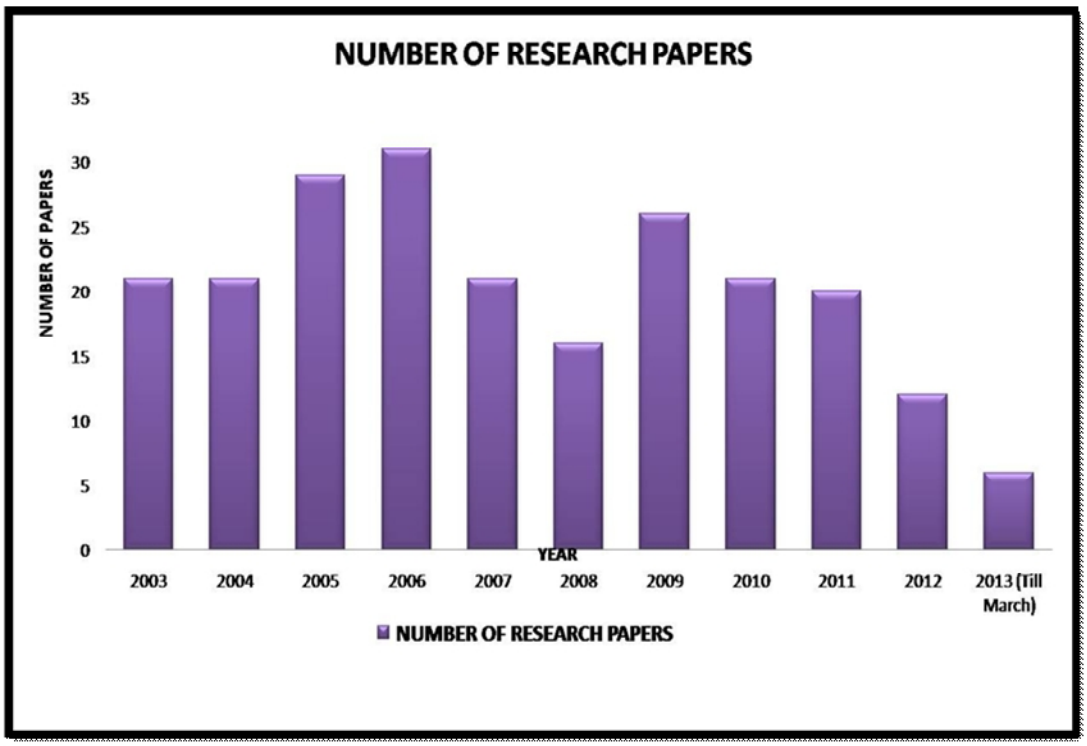

Fig. 1: Number of research papers published in PubMed.com in last 10 years

Advantages

Factors in favor of plant systems as sources of animal derived proteins, compared with other conventional methods, include: [4]

- The potential for large-scale, low-cost biomass production using agriculture
- Low risk of product contamination by mammalian viruses, blood-borne pathogens, oncogenes and bacterial toxins
- The capacity of plant cells to correctly fold and assemble, not only antibody fragments and single chain peptides, but also full-length multimeric proteins
- Low downstream processing requirements for proteins administered orally
- Elimination of the purification requirement when the plant containing the recombinant proteins is edible, such as potatoes.
- Reduced anaphylactic side effects.
- The ability to introduce new or multiple transgenes by sexual crossing of plants
- The avoidance of ethical problems associated with transgenic animals
- Production size is flexible and easily adjustable to the needs of changing markets

Disadvantages

Along with its numerous advantages it is accompanied by a few disadvantages-

- Some proteins used in experiments may be extraordinarily potent inducers of immune responses. Whereas, other immunizing proteins may not work as well when taken orally. In fact, they can have the opposite effect as many proteins in a diet induce tolerance, making the immune system less able to mount a response against them.
- There may be allergic reactions to plant protein glycans and other plant antigens
- Plant and product maybe contamination by mycotoxins, pesticides, herbicides and endogenous metabolites
- Some other compounds in plants may compromise the ability of the vaccine protein to induce immunity.
- The food containing it must be palatable
- Some foods need to be heated before ingesting, which could possibly cause the vaccine protein to denature, reducing or eliminating its ability to elicit immunity. [4]
- To date researchers have had difficulty achieving high levels of chloroplast gene expression in the edible parts of the plant. [7]
- Each type of plant poses its own challenges. Dosage of vaccine will be variable
- It is not convenient for infants. [8]
- The vehicles like tomato or banana don't come in standard sizes due to which people may consume too much vaccine which will be toxic or too little which would lead to outbreak of disease among population which is believed to be immune. [9]

Mechanism

Regardless of how vaccines for infectious diseases are delivered, they all have the same aim: priming the immune system to swiftly destroy specific disease-causing agents, or pathogens, before the agents can multiply enough to cause symptoms. Classically, this priming has been achieved by presenting the immune system with whole viruses or bacteria that have been killed or made too weak to proliferate much. On detecting the presence of a foreign organism in a vaccine, the immune system behaves as if the body were under attack by a fully potent antagonist. It mobilizes its various forces to root out and destroy the apparent invader-targeting the campaign to specific antigens (proteins recognized as foreign). The acute response soon abates, but it leaves behind sentries, known as "memory" cells, that remain on alert, ready to unleash whole armies of defenders if the real pathogen ever finds its way into the body. Some vaccines provide lifelong protection; others (such as those for cholera and tetanus) must be re-administered periodically. [1]

The antigens in transgenic plants are delivered through bio-encapsulation, i.e. the tough outer wall of plant cells. This protects them from gastric secretions and finally breaks it in the intestines. These antigens released are taken up by M cells in the intestinal lining that overlie peyer's patches and gut-associated lymphoid tissue (GALT), passed on to macrophages and other antigen-presenting cells; and local lymphocyte populations, generating serum IgG, IgE responses, local IgA response and memory cells, which would help promptly neutralize the attack by the real infectious agent. [5]

When vaccines are taken orally the intestinal immune response to these food-antigens is called tolerance. Oral tolerance is an active immunologic response, which prevents the development of responses to the many antigens ingested by a host. The M cells which take up the antigen present them to specific suppressor T cells, which secrete cytokines that allow for the propagation of specific antibodies. Tolerance is dose- schedule-, and antigen-specific. Therefore, an antigen that is orally administered and is tolerant will show no response, because the expression of that antigen has been suppressed. Thus when synthesizing a protein from a transgenic plant, oral tolerance must be taken into consideration. [4]

Manufacturing

The various steps involved in transferring the genes from pathogen into a plant vector as an edible vaccine are-

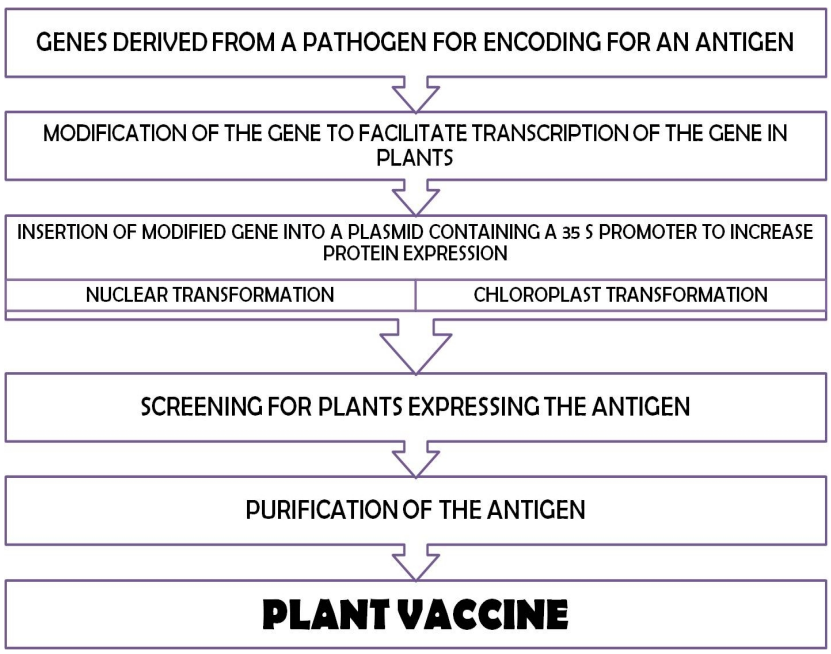

Fig. 2: Steps involved in transferring genes from pathogen into plant vector

The above steps are primary for transferring genes into the vector. These steps are used to manufacture and administer the vaccine into a human. Thus the processes undertaken before administration of this vaccine are-

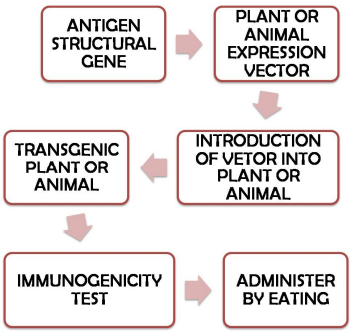

Fig 3: Steps are used to manufacture and administer the vaccine

Transgenic plants used for Delivery of Edible vaccine

Recent progress in the area of transgenic plants has attracted attention of the scientists, and plants are being looked upon as potential bio-reactors or bio-factories for the production of immunotherapeutic molecules. Transgenic plants capable of producing several different products can be created at any given time by crossing plants producing different products. [9] The choice of plant system was initially driven by convenience and the need to evaluate genetic constructs quickly. For this reason, tobacco plants were generally used, however, potatoes and bananas were considered for the purpose of acquiring a delivery system of vaccines for humans. [4]

Table 1: Some very important Plants which are used as Edible vaccines [8]

Plant/Fruit	Main Features	Advantage	Disadvantage
Potato	It has been used as a vehicle for diabetes related proteins, vaccine against a strain of E.Coli, cholera vaccine, and vaccine against Norwalk virus.	• Dominated clinical trials • Easily manipulated/transformed • Easily propagated from its "eyes" • Stored for long periods without refrigeration • Cooking of the potatoes does not always destroy the full complement of an antigen.	Needs cooking which can denature antigen and decrease immunogenicity
Banana	Bananas are sterile so the genes don't pass from one banana to another which is the main reason why bananas are a good choice for edible vaccine.	• Does not need cooking • Proteins not destroyed even if cooked • Inexpensive • Grown widely in developing countries • They grow quickly. • They have high content of vitamin A which may boost immune response.	• Trees take 2-3 years to mature • Transformed trees take about 12 months to bear fruit • Spoils rapidly after ripening • Contains very little protein, so unlikely to produce large amounts of recombinant proteins
Tomato	A possible edible vaccine against HIV/AIDS, hepatitis B, rabies, norovirus, Alzheimer's, SARS, anthrax and respiratory syncytial virus. It was the first time when foreign gene had been introduced into the plastids (chloroplasts).	• Grow quickly • Cultivated broadly • High content of vitamin A may boost immune response • Overcome the spoilage problem by freeze-drying technology • Heat-stable, antigen-containing powders, made into capsules • Different batches blended to give uniform doses of antigen • Cuts down the likelihood of passing of infections. • Donot need special facilities for storage and transportation. • They taste good.	Spoils easily

Table 2: Some other plants used for Vaccine include [8]

Rice	Can be used for the treatment of cholera, flu, botulism, hay fever.	• Commonly used in baby food because of low allergenic potential • High expression of proteins/ antigens • Easy storage/transportation • Expressed protein is heat-stable. • Vaccine does not dissolve when exposed to stomach acids. • Less risk of contaminating than normal crop and has a broader utility.	• Grows slowly • Requires specialized glasshouse conditions
Tobacco	Tobacco plant was made to produce Interleukin 10 to treat Crohn's disease. They carry immunologically active cost effective vaccine against HPV which are the causative gents for cervical cancer.	• Good model for evaluating recombinant proteins. • Low cost preserving system • Easy purification of antibodies stored in the seeds • Large harvests, number of times/year	Produces high level of toxic alkaloids.
Lettuce		Fast-growing	Spoils readily
Soybean		• Direct consumption • Large harvests, number of times/year	Spoils readily

Application of Edible Vaccine

Vaccines and antibodies play a major role in healthcare. However, the cost of production and maintaining vaccine distribution has so far hampered realizing their full potential. Expression of antigens as vaccines and of antibodies against antigens of pathogens in transgenic plants is a convenient and inexpensive source for these immunotherapeutic molecules. Various antigens and antibodies have already been expressed successfully in plants and have been shown to retain their native functional forms.

Few of the antigen and antibodies successfully expressed in plants include-

- Maize plants produce a protein used to make the hepatitis B virus vaccine. Maize is immunogenic and protective when administered orally for Enterotoxigenic E. Coli in humans. [9]
- An edible vaccine for viruses such as Severe Acute Respiratory Syndrome (SARS) had found a breakthrough in milk. [10]
- A vaccine for measles have been made which can be grown in fruit, like tobacco leaves, and eaten instead of being injected.[11]
- HIV gene has been inserted in Agrobacterium and then transferred into a plant like tomato and spinach.
- Edible vaccines were probed as tools for quashing autoimmunity. Some of the evidence that ingesting autoantigens, or "self-antigens," might suppress autoimmunity comes from studies of type I diabetes, which results from autoimmune destruction of the insulin-producing cells (beta cells) of the pancreas. To that end plant-based diabetes vaccines have been developed, such as potatoes containing insulin or GAD linked to the innocuous B subunit of the V. cholerae toxin (to enhance uptake of the antigens by M cells). [1]

Table 3: Some common plants undergoing usage as vehicles for protein[8]

Target species for vaccines	Plant used for expression	Route of administration
Enterotoxigenic *E.coli*	TOBACCO	Oral
Enterotoxigenic *E.coli*	POTATO	Oral
Enterotoxigenic *E.coli*	MAIZE	Oral
Vibrio cholerae	POTATO	Oral
Hepatitis-B virus	POTATO	Oral
Hepatitis-B virus	LUPIN	Oral
Hepatitis-B virus	LETTUCE	Oral
Norwalkvirus	TOBACCO	Oral
Norwalkvirus	POTATO	Oral
Rabies virus	TOMATO	--
Human cytomegalovirus	TOBACCO	--

Table 4: Besides that some of the main vaccines and its vehicles, for humans and animals, explored till date[6]

Vaccine for-	Vehicle
Hepatitis B (humans)	Tomato, Banana, Maize, Tobacco, Lettuce
Rabies Virus (humans)	Tobacco, Tomato,
Enterotoxigenic E.Coli (humans)	Tobacco, Potato, Maize
Vibrio Cholerae [Cholera] (humans)	Potato
Norwalk Virus (humans)	Tobacco, Potato, Tomato
Stomach Virus (humans)	Potato
HIV (humans)	Tomato, Arabidopsis, Spinach
Cervical Cancer (humans)	Tobacco
Crohn's Disease (humans)	Tobacco
Alzheimer's Disease (humans)	Tomato
Insulin	Arabidopsis, Potato
SAARS (humans)	Milk, Tomato
Measles (humans)	Tobacco
Human cytomegalovirus (humans)	Tobacco
Rabbit hemorrhagic disease virus (rabbits)	Potato
Foot-Mouth disease (agricultural domestic animals)	Arabidopsis, Alfalfa
Transmissible gastroenteritis coronavirus (pigs)	Arabidopsis, Tobacco, Maize

At least 350 genetically engineered pharmaceutical products are currently in clinical development in the United States and Canada. Scientists believe that potent drugs and vaccines will soon be harvested just like wheat and corn.

Future Prospects

Several issues will still have to be resolved before this intriguing idea can become a reality. The studies completed so far in animals and people have provided a proof of principle; they indicate that the strategy is feasible. Yet many issues must still be addressed.

Researchers are also grappling with the reality that plants sometimes grow poorly when they start producing large amounts of a foreign protein. One solution would be to equip plants with regulatory elements that cause antigen genes to turn on--that is, give rise to the encoded antigens-only at selected times (such as after a plant is nearly fully grown or is exposed to some outside activator molecule) or only in its edible regions. This work is progressing.

Among the other foods under consideration are lettuce, carrots, peanuts, rice, wheat, corn and soybeans.

In terms of acceptance there is a hurdle which is yet to be crossed. In many countries in the world, plants engineered to produce vaccines fall under the very restrictive rules set up to control GM-crop plants. The present concern, especially in Europe, over the use of biotechnology for the genetic improvement of crop plants also negatively affects the acceptance of GM-plants for medicinal use. As a consequence, while the demonstration that plant-derived vaccines are effective on populations at risk is expected to arrive within 1-2 years, a further quarantine of 2-3 years will be required in order to fulfill the requirements for registration and marketing. It is hoped that simpler rules will be set up for GM-plants producing vaccines and that they are seen as clearly and legally distinct from GM-plants grown for nutrition purposes.

Vaccines have been one of the most far-reaching and important public health initiatives of the 20th century. Advancing technology, such as oral DNA vaccines, intranasal

delivery and edible plant derived vaccines, may lead to a future of safer and more effective immunization. Edible vaccines might overcome some of the difficulties of production, distribution and delivery associated with traditional vaccines. Significant challenges are yet to be overcome before vaccine crops can widely be used. However, while access to essential healthcare remains limited in much of the world and the scientific community is struggling with complex diseases such as HIV and malaria, plant derived vaccines represent an appetizing prospect.[9]

CONCLUSION

In contrast, edible vaccines would enhance compliance, especially in children and because of oral administration, would eliminate the need for trained medical personnel. Their production is highly efficient and can be easily scaled up. If the technology is properly nurtured and given the right direction, it may usher into a new era where we will be asked to take "food" rather than "drugs" when we are ill.

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