

Current Status of Plants as Vaccine Production Platforms

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Abstract

Plant-derived biopharmaceuticals offer enormous potential as a cost-effective, rapid and safe means to produce vaccine and therapeutic proteins. Plant-made vaccines can be administered orally to elicit a mucosal immune response, and represent a method by which vaccine coverage can be improved for many who reside in developing countries. Vaccines such as these offer great promise on many levels, from providing relief to those who have little access to modern medicine, to producing large-scale stockpiles of vaccines available to offset global pandemics, and even to playing an active role in the battle against cancer. Plant-derived vaccines can both deliver an antigen to the mucosal immune system in the form of a food product, as well as prevent the antigen from degradation as it passes through the gastrointestinal tract. Both transgenic plants and plant virus expression vectors are routinely used to express biopharmaceutical proteins. The following review details recent advances concerning the production of vaccines against Hepatitis B virus, Human papilloma virus, Influenza virus and Non Hodgkins Lymphoma using plant expression platforms.

Introduction

It is an unfortunate reality that the morbidity and mortality rate caused by preventable infectious diseases remains high among the world's poor. Factors such as high cost, ineffective healthcare infrastructure and poor access to refrigeration and transport severely constrains the accessibility of much needed vaccines for developing countries. Often the pathogen enters the host through mucosal surfaces located in the gastrointestinal, respiratory and reproductive tracts. In the early 1990's, the Children's Vaccine Initiative, comprised of an assembly of philanthropic groups and in conjunction with the World Health Organization, was established to move forward new technologies that aim to provide accessible vaccines and improved immunization programs on a global scale [1,2]. Ideally, such vaccines would be safe, efficacious, stable at room temperature, easy to store and transport and inexpensive to produce. The Initiative strived to generate vaccines for diseases that were difficult to manage or were poorly funded. It was amidst this background that the preliminary concept of using plants as a delivery platform for vaccine proteins first came into being [3,4,5]. Plants have been demonstrated to have the capacity to produce recombinant antigens that undergo the appropriate post-translational modifications to retain the same structural integrity and biological activity as their mammalian-derived counterparts. Importantly, plant-derived vaccines offer the dual advantage of providing an oral route of administration through the consumption of edible plant tissue, as well as providing a measure of protection for vaccine antigens as they pass through the harsh environment of the gut [5,6].

Transgenic, or genetically modified (GM) plants are produced over much of the globe today. GM plants have been available commercially for the past 15 years and continue to offer enormous potential for crop improvement. Transgenic plants will provide a valuable tool for attaining future food security, particularly with the combination of a rapidly increasing world population and the advance of climate change. Transgenic plants also present a novel cost-effective and safe expression platform for the large-scale production of proteins for industrial, pharmaceutical, veterinary and agricultural uses [7].

Mucosally acquired infections can best be controlled via mucosal vaccination, which can elicit both IgG and secretory IgA responses. Since different segments of the mucosal immune system throughout the body appear to be linked, delivery of an antigen to any mucosal

surface can potentially induce immunity at others. Thus, the type of immune response can be determined by a combination of the nature of the antigen, the route of administration and the delivery system utilized.

The focus of the field of plant-made pharmaceuticals has been largely on the generation of vaccines and other therapeutic proteins in plants that are biologically active and can be produced inexpensively and in amounts substantial enough to elicit an immune response. Specifically, plant-derived vaccines which prioritize diseases affecting the intestinal tract and are major causes of mortality in developing countries have been the principal targets. Orally administered, plant-derived vaccines therefore have the potential to enhance vaccine coverage in children and infants against a number of gastrointestinal diseases, particularly in resource-poor regions. As a result, this has been the focus of a number of Phase 1 clinical trials [8].

One obstacle for the delivery of antigens to the intestinal immune system stems from the fact that many are prone to become rapidly degraded within the harsh environment of the digestive tract. A select advantage of plant-made vaccines is the fact that plant cells provide protection and prevent degradation of the vaccine antigen as it passes through the gut [9]. Another obstacle is that many antigens do not serve well as immunogens as they are poorly recognized by the immune system. The use of immunogen such as Cholera toxin B subunit (CT-B), which largely affect the immunogenic context in which an antigen is encountered, can help to overcome this problem. CT-B can not only modify the cellular environment in order to present the antigen in a

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highly efficient manner, but can also act as an efficient transmucosal carrier molecule and delivery system for plant-derived subunit vaccines. For example, a protein which is only weakly immunogenic can be coupled to CT-B as part of a fusion protein and then expressed in plant tissue. Proteins presented as part of a fusion protein with CT-B have been shown to exhibit an enhanced antigenicity within the gut [10,11].

Mechanisms Used to Express Vaccine Epitopes and Proteins in Plant Tissue

A number of techniques are available by which to express vaccine and therapeutic proteins in plants. For example, transgenic plants have long been used to express the protein of interest in a stable fashion. In order for a plant cell to become transgenic, it must undergo a transformation event; each transformed cell can then be regenerated into an entire plant. Alternatively, the transient expression of a protein using Agroinfection or a plant viral expression vector.

Plant transformation involves the stable integration of the gene of interest into a plant genome, and was originally performed with the bacterial strain responsible for crown-gall disease, *Agrobacterium tumefaciens* [12]. Biolistic delivery is another commonly used mechanism and involves the use of a device known as a 'gene gun' [13]. In both instances, cells which are successfully transformed are then selected on media containing the appropriate antibiotic, and generated into plantlets on tissue culture media. When large enough, the plantlets are transferred to soil and grown into mature plants.

Several disadvantages can be found for stable plant transformation as a means to express vaccine proteins, including the length of time taken for transgenic plants to be generated, which can be months or even years, depending on the plant species used. In addition to this, containment issues such as the escape of transgenes into the environment through outcrossing with weedy relatives have been a sizeable concern [12]. As a result, alternative methods by which to express proteins in plants, such as the use of plant cell culture bioreactors or greenhouses rather than plants grown in outdoor fields has become popular. The use of plant virus expression systems for the production of biopharmaceutical proteins is another viable option. The utilization of virus expression vectors as a technology enables the protein of interest to be produced both in large quantities and within a relatively short time period. Plant viruses-based expression systems can be divided into two main branches; epitope presentation systems (short antigenic peptides fused to the coat protein [CP] that are displayed on the surface of assembled viral particles) and polypeptide expression systems (these systems express the whole unfused recombinant protein that accumulates within the plant). Problems such as insert size limitations and host range restrictions have been known to provide major stumbling blocks for plant virus expression vectors to be universally used for any plant species [3,4,8]. The means by which a vaccine or therapeutic protein is expressed therefore becomes a matter of selecting the appropriate plant species, and determining whether the protein is expressed in a whole plant or cell culture, or whether stable transformation or transient expression works best for the therapeutic protein under investigation.

Although mostly similar in structure, there are a few significant differences between plant-derived and traditional vaccines synthesized in mammalian cell cultures. Many human therapeutic proteins are in fact glycoproteins, and some of the N-glycoproteins which are synthesized in plants differ in their glycosylation patterns from

those derived from their mammalian counterparts. These differences may result in increased allergenicity or a reduced ability to elicit the appropriate immune response in mammals who are administered plant-derived glycoproteins. Plant-derived therapeutic proteins can be further humanized by altering various glycosylation pathways unique to plants [14,15]. Protein retention within the ER and engineering of plant strains which lack enzymes necessary to produce plant-specific glycan structures or which acquire mammalian-specific glycosylation machinery are actively being pursued [16].

The Mucosal Immune Response to Plant-derived Biopharmaceuticals

Mucosal surfaces are an essential part of the mammalian immunological repertoire and represent the portal of entry for most pathogens [10]. Epithelial layers line the mucosal surfaces of the gut, respiratory and urogenital tracts. The mucosal surface separates the internal and external environments, and is further protected by both innate and adaptive immune pathways. The epithelial mucosa encompasses lymphoid follicles and consists of mucin-producing glandular cells, lymphocytes, plasma cells, dendritic cells, macrophages, cytokines and chemokines. The uptake, processing and presentation of antigens to elicit a mucosal response also takes place within the epithelial mucosa [17,18].

In the intestinal tract, the gut-associated lymphoid tissue (GALT), represents approximately 70% of the body's entire immune system. Peyer's Patches, comprised of clusters of lymphoid follicles and distributed throughout the length of the small intestine, contain various, highly specialized cells known as M (minifold) cells which deliver antigen from the lumen to antigen-presenting cells. This in turn is followed by the activation of T cells, B cells and dendritic cells [19-21]. In the respiratory tract, the mucosal surface of the respiratory system is highly specialized. Antigens are taken up into alveolar spaces by antigen presenting cells, and translocated to regional lymph nodes, the site of the primary immune response. B cells generated as part of this response then return to the lung where they differentiate into either antibody-secreting plasma cells or memory cells [22]. Thus, an antibody response of the respiratory tract can occur either quickly if there has been prior exposure to the pathogen, through activation of resident memory B cells or, more slowly through the induction of both systemic and local mucosal immunity, if the host is naive to the pathogen. Both IgG and IgA are involved in pathogen clearance, with the entry site playing a major role in determining the nature of the antibody that is produced. For pathogens of the respiratory tract, mucosal vaccination is required to effectively stimulate a rapid local and systemic IgA and IgG response. This latter point underscores the great importance of developing vaccines which can be delivered as an aerosol by inhalation [22,23]. The major antibody isotype in mucosal secretions, IgA, can not only block the entry of antigens into the epithelium, it can neutralize virus production, and additionally trigger the release of inflammatory mediators.

Clinical Trials and Plant-Derived Vaccines

The first plant-made vaccine that underwent clinical trials took place over 20 years ago, and concerned *Streptococcus mutans* surface protein A expressed in transgenic tobacco plants. Mice immunized with this transgenic plant material were demonstrated to elicit a response to intact *S. mutans* [16]. Later, plants which expressed *E. coli* enterotoxin B subunit (LT-B) were developed and exhibited successful induction of both mucosal and sera antibody responses [24,25]. Many

other plant-made vaccines have been proven to be effective by animal antigenicity and challenge trials, and more currently, a number of human clinical trials (Table 1). Many of these clinical trials using plant-derived vaccines have focused predominantly on diseases which are major causes of infant mortality in developing countries. The next section of this review describes some of the results of studies involving the production of plant derived vaccines against Hepatitis B Virus, Human Papilloma Virus, Influenza Virus and an anti-cancer vaccine for Non Hodgkins Lymphoma.

Hepatitis B Virus (HBV)

Despite the introduction of a commercially available recombinant vaccine in 1981, Hepatitis B Virus (HBV) continues to be a major global health concern. Approximately two billion people are infected with the virus worldwide, 400 million of whom have developed chronic infection [21]. An estimated 75-100 million chronic carriers die of HBV-induced liver cirrhosis and/or hepatocellular carcinoma each year [26]. Skilled administration and cost continue to be principal prohibitive factors in the distribution of current HBV vaccines in developing nations. Plant-based HBV surface antigen expression systems have played an important role in advancing the viability of oral plant-based delivery as a safe, practical, and cost-effective alternative for inducing immunogenicity.

Recombinant subunit vaccines currently licensed for human immunization contain Hepatitis B surface antigen (HBsAg) derived from yeast [27]. HBsAg has thus served as the standard in transgenic plant research on HBV vaccine development, with research groups examining its expression and immunogenicity in the leaf, fruit, and grain tissue of various plant systems. Proof-of-concept was first established in tobacco and revealed the viability of HBsAg transgenic plant production, however antigen expression levels have been too low (< 0.01% of total soluble protein) to be practical for oral vaccination [28-30]. Unfortunately, expression levels in other leafy plants, such as lettuce as well as fruit transformation, including tomato and banana, has yielded similarly low antigen expression levels [31-33].

Animal trials using transgenic potatoes expressing HBsAg have provided more successful results, with levels of major surface protein in tubers nearing 0.002% fresh weight [34]. Moreover, mice fed HBsAg transgenic potatoes with a mucosal adjuvant over a three-week period elicited a primary response of up to 100 mlU/ml, and the ingestion of HBsAg was found to serve as an effective boost in mice primed with a subimmunogenic parenteral dose of yeast-derived recombinant HBsAg [34,35]. Human immunogenicity to plant-derived HBsAg was evaluated in a recent clinical trial, in which volunteers previously immunized with a commercial HBV vaccine consumed two or three doses of uncooked HBsAg transgenic potatoes (at 850 µg HBsAg/dose) [31]. Oral administration of the antigen was found to boost anti-HBsAg blood serum antibody titers up to 33 times after two doses and 56 times after three doses. The results of this study provide compelling evidence that oral delivery of an antigen derived from HBV, a nonenteric pathogen, can generate a systemic immune response in humans.

Another promising plant expression platform for the expression of HBsAg is rice, a staple crop that can be easily maintained and processed. It further offers the advantage of natural bioencapsulation of the antigen in the plant wall, thus providing enhanced protection from intestinal degradation [37]. Levels as great as 31.5 ng/g dry weight of HBsAg have been expressed in rice seeds, forming VLPs (virus-like particles) of proper size and morphology. Additionally, mice immunized with the recombinant antigen developed immunological responses that imply the potential of rice-derived HBsAg to serve as an effective oral alternative for HBV vaccination.

Human Papilloma Virus

Cervical cancer is the second most prevalent cancer in women worldwide, with the Human papillomavirus (HPV) being the causative agent of approximately half a million new cervical cancer cases and almost 250,000 deaths each year [38]. There are over 130 genotypes of HPV; of these, 16 are considered high-risk types in the development of malignant tumors [39]. In particular, HPV-16 and HPV-18 are the major established etiological agents of cervical cancer and are

Vaccine Produced	Host Plant/Expression System Used	Number of Human Subjects	Details, Immune Response Obtained	Reference
Norwalk Virus nucleocapsid	Transgenic Potato	24 volunteers	Oral consumption; IgA, IgM and IgG responses detected	24, 34
LT-B from enterotoxigenic E. coli	Transgenic Corn	13 volunteers	Oral consumption; IgA, IgG response detected	24, 34
LT-B from enterotoxigenic E.coli	Transgenic Potato	14 volunteers	Oral Consumption; IgA, IgG response detected	24, 34
HBsAg from Hepatitis B virus	Transgenic potato	16 volunteers	Oral consumption; 10 volunteers exhibited IgG serum response	36
HBsAg from Hepatitis B virus	Transgenic Spinach	3 volunteers	Oral consumption; 2 exhibited serum IgG response	31
Glycoprotein from Rabies virus	Spinach infected with virus vector	9 volunteers	Oral consumption; 5 demonstrated serologic IgG response	63
HA protein of H5N1 Influenza virus	<i>N. benthamiana</i> infiltrated with vector	Phase I completed successfully with 48 volunteers, Phase II in progress	Virus-like particles, delivered by injection, IgG response as determined by hemagglutinin inhibition and neutralization assays	57
Non-Hodgkin Lymphoma anti-idiotype scFv	<i>N. benthamiana</i> infiltrated with vector	Phase I in progress with 16 patients suffering from NHL	Delivered by injection, 11 had cellular immune response (T cell proliferation) and of these, 7 had a humoral response	58

Table 1: Human Clinical Trials using Vaccines derived from Plants.

associated with two-thirds of all cases [39]. As the development of attenuated or dead vaccine particles is hindered by HPV's inability to be propagated in culture, a significant amount of biotechnological research has focused on the development of non-infectious VLPs of HPV, particularly those composed of the HPV major capsid protein L1 [40]. L1 can self-assemble into highly immunogenic VLPs, and such a bivalent HPV vaccine has given excellent results in human phase 2 clinical vaccine trials, showing to be 91.6% efficacious in the prevention of incident and persistent cervical infections with HPV-16 and HPV-18 [41]. Two L1-based vaccines currently on the market, Gardasil and Cervarix, both contain HPV-16 and HPV-18 high-risk types, with the addition of HPV-6 and HPV-11 in Gardasil [42].

The expression of L1 VLPs in plants has been the subject of extensive study. Nuclear expression of HPV L1 capsid protein in a variety of hosts including tomato, potato, and tobacco have been tested, however expression levels in plant systems destined for direct oral administration have been problematic. Expression levels that would be acceptable for economic production (ie. >50 mg/kg for antibodies) have been difficult to achieve. HPV L1 protein production in transgenic *Arabidopsis* plants has thus far been detected at yields of up to 12 µg/g, while transient expression with a tobacco mosaic virus (TMV) – derived vector has yielded up to 37 µg/kg L1 protein product [3,43-44]. Of all transformation methods, however, chloroplast transformation has resulted in one of the most successful expression systems developed to date. Chloroplast expression allows significantly greater protein accumulation than cytoplasmic expression due to high plastid gene copy number, with the highest expression level yet achieved being 3 mg/g fresh weight for HPV-16 L1 protein, or 24% total soluble protein [45]. There is also some indication that a human codon-optimised gene design results in improved expression levels, while plant codon-optimised genes work least well [46].

Studies on the assembly and immunogenicity of plant-derived L1 VLPs are also encouraging. VLP assembly that is structurally analogous to natural HPV viral particles has been demonstrated via electron microscopy studies and the elicitation of neutralising antibodies in orally- or intraperitoneally-treated mice has been consistently successful [40,47]. Moreover, recent work expanding on immunization to include immunotherapy has led to the design of chimeric HPV VLPs expressed in tomato that include T-cell epitopes from E6 and E7 early nonstructural viral proteins [48]. Similarly, Massa et al. [49] showed that transgenic tobacco plants expressing oncoprotein E7 induced E7-specific IgG and cytotoxic T-cell responses in mice protected against challenge with E7-expressing tumor cells and prevented tumor development.

Influenza Virus

Influenza, commonly referred to as the *flu*, is an RNA virus which causes high mortality and morbidity in human populations worldwide. It is thought that influenza is responsible for 300,000 to 500,000 deaths globally every year, and between 3 and 5 million hospitalizations [50]. Control of influenza is a costly global endeavour: the World Bank alone reported \$4.3 billion in pledges from bilateral and multilateral donors from 2005 - 2009 for the WHO for the purpose of influenza response [50,51]. The push for global vaccine coverage necessarily compels the refinement of biotechnical vaccine development to reduce both cost and response time as yearly influenza strains evolve and threaten human populations, and plant-based approaches to the production of influenza vaccines are gaining popularity and acceptance.

The virally encoded haemagglutinin (HA) and neuraminidase (NA) surface glycoproteins are the most immunogenic in human and animal hosts and are therefore responsible for most of the variation arising between strains. The segmented nature of the influenza genome allows for these two proteins to be rapidly reassorted over the course of host co-infection, giving rise to new and harmful strains almost on a yearly basis [51].

The factor limiting global pandemic influenza vaccine coverage is the availability of sufficient quantities of influenza antigen for large-scale vaccine preparation [52]. Most widely-used influenza vaccines are produced in embryonated chicken eggs using a live virus attenuation strategy with directed chromosomal reassortment to induce cross-protection against a variety of strains, determined on a seasonal basis [52]. This method has been in use for over 60 years, and is thought to lack sufficient potential for scalability beyond production of hundreds of millions of doses per year.

Recently, a number of plant-based technologies have evolved showing promise for the large-scale production of influenza antigen. One such technology produces influenza VLPs for prophylactic host inoculation by transient expression of recombinant antigen in *Nicotiana benthamiana* [53]. VLPs containing lipid-anchored recombinant HA budded from the inner plasma membrane of *N. benthamiana* cells were shown to induce a fully protective immune response against lethal viral challenge in mice when inoculated in doses as low as 0.5 µg. The scaling and automation of this technology by Medicago Inc. is reported to have the potential for a surge capacity of 10,000 doses/month at a single plant costing \$35 million to build, far outstripping current pace of vaccine production in the event of an influenza pandemic at a significantly lowered cost [54-57]. Their system is also capable of producing a candidate VLP vaccine within 3 weeks of the release of influenza strain sequence information. Other biotech firms, including Icon Genetics (a subsidiary of Bayer Pharmaceuticals) have developed similar large-scale facilities for plant-based production of pharmaceutically valuable recombinant proteins.

It is clear that plant-based methods for influenza vaccine production present a significant opportunity for improving global responses to both pandemic and endemic influenza by reducing both cost and response times to the emergence of new strains. The wide applicability of these methods makes them a compelling target for future research and investment in current technologies.

Non-Hodgkins Lymphoma

A 'deconstructed' version of the Tobacco mosaic virus (TMV) expression system, in which the genome and gene of interest is expressed in the form of different modules, has been used recently as a novel personalized medicine strategy to produce a cancer vaccine. This technique is being used to undergo clinical trials for the treatment of Non-Hodgkin's Lymphoma (NHL) [58]. NHL is the fifth highest cause of death in North America, and is a tumor disease involving the proliferation of B-cells, which then accumulate in the lymph nodes, bone marrow and other tissues of the body. Each individual NHL patient expresses a unique idiootype from these degenerate B cells, and the idiootype can be used as a tumor marker. Nucleotide sequences corresponding to these idiotypes can be subcloned, then rapidly and inexpensively expressed in plants using a TMV-based expression vector which is agroinfiltrated onto tobacco leaves. Large amounts of vaccine protein containing an epitope to the idiootype can be generated from these plants within a few days time, then purified and injected

back into the patient, enabling them to generate an immune response against their own cancer. Plant-made vaccines against cancers such as NHL could provide a powerful short-term therapy which could be administered immediately after diagnosis to block tumor progression [58].

Allergies and Oral Tolerance to Plant-Derived Biopharmaceuticals

The vast majority of compounds which reach the gastrointestinal tract through the oral consumption of food are not in fact immunogenic, and this lack of response prevents the onset of unnecessary and damaging inflammatory responses to benign substances, leading to conditions such as inflammatory bowel syndrome and food allergies. Oral tolerance, which results in a diminished response to challenge with a particular immunogen, is also a concern for oral routes of vaccination. Takagi et al. [59] examined the ability of plant-derived vaccines to elicit oral tolerance by developing transgenic rice plants expressing mouse T cell epitope peptides specific for pollen allergens of *Cryptomeria japonica* (Japanese Cedar). Mice fed transgenic rice expressing these common allergens were later challenged by feeding with total protein extracts of pollen, and displayed allergen-induced oral tolerance, demonstrating that the plant-derived vaccine strategy for oral tolerance has also been demonstrated to successfully suppress asthma-based allergies. Similarly, Sunflower Seed Albumin (SSA), a common allergen, has been expressed in transgenic narrow leaf lupin (*Lupinus angustifolius L.*) [60]. Oral consumption of plants expressing SSA prevented a delayed-type hypersensitivity response, including asthmatic symptoms. The data from these two studies demonstrates that plant-based vaccines may have potential applications in the protection against allergic diseases such as asthma.

The Future of Plant-Derived Biopharmaceuticals

A principal motivation for generating plant-derived vaccines has been to block the spread of preventable infectious diseases in remote rural areas of developing countries. Plant-derived vaccines would also be helpful against 'orphan' diseases which are poorly financed, such as dengue fever, hookworm and rabies. Since plant-derived vaccines are inexpensive to produce and easy to administer, they could provide relief to many in resource poor regions [61].

Plant-derived biopharmaceuticals can be produced in both food and nonfood crops, in the open field, greenhouse and in cell culture. Cell suspension cultures enable plant tissues to be grown in precisely controlled environments or even continuously, making it easier for the product to follow the Good Manufacturing Practices essential for commercialization. Protein purification from plant tissues is in general a fraction of the cost of mammalian and bacterial protein purification. Moreover, some forms of plant-derived therapeutic proteins require only partial purification. Recently, the first plant derived biopharmaceutical, a veterinary vaccine for Newcastle Disease in poultry, has been approved by the FDA and licensed [62]. Many others are in the process of completing clinical trials and will soon approach market release.

Conclusions

Originally, plant-derived vaccines were introduced in the literature as 'edible vaccines'. In fact, the first clinical trial performed within the US required each volunteer to consume 100-150 g of raw transgenic potato expressing Hepatitis B surface antigen [36]. At the time, researchers speculated that plant-made biopharmaceuticals could be

produced and consumed as a routine/local food source from their own fields and gardens. However, in order to keep quantities of vaccine protein consistent, additional preparation steps were required, such as grinding transgenic corn kernels into a cornmeal or lyophilizing transgenic tomatoes into a powder, which could later be resuspended into a juice or paste [7]. It is unlikely that in the future one will be able to be immunized for a particular disease simply by reaching for and consuming a piece of fruit or vegetable. Even so, the results of clinical trials of plant-derived vaccines and therapeutic proteins described here provide ample evidence for the potential of plants to become oral delivery vehicles for biopharmaceuticals. It is clear that people who ingest plant tissue expressing vaccine proteins or take up the vaccine intranasally in the form of an aerosol or spray are capable of exhibiting a greater immune response, and recover more rapidly from infection than those who ingest control plants which express no vaccine. Plant-derived vaccines therefore offer concrete hope to those who need it most for more immunogenic, more effective and less expensive vaccination strategies against respiratory as well as intestinal mucosal pathogens. The examples described in this review will pave the way to future success in the control of some of the world's worst infectious diseases.

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