Chapter 13
Plant-Based Vaccines as a Global Vaccination Approach: Current Perspectives

Dania O. Govea-Alonso, Edward Rybicki and Sergio Rosales-Mendoza

Introduction

Plant-made vaccines were popularized in the 1990s as an innovative source of “edible vaccines.” A number of edible crops such as potato, tomato, lettuce, bananas, corn, and rice were used to express antigens from various pathogens, including *Vibrio Cholerae*, enterotoxigenic *Escherichia coli* (ETEC), *Norwalk virus*, hepatitis B virus, and human and animal rotavirus, among others (Walmsley and Arntzen 2000, 2003; Rybicki 2010). However, although the initial prospect was highly attractive, subsequent research identified problems that would limit the advance in the use of plants as a robust platform for the production of convenient vaccines. Perhaps, the main modification on the focus was the obvious need for introducing some processing in the vaccine formulation to ensure plant biomass stability and proper dosage of the vaccine antigen. Among the technologies that have addressed the identified limitations are the use of bioreactors for biomass production, which allows full containment of the production process; freeze drying of partially purified material; the use of seeds as expression vehicles in order to yield an edible and stable biomass for dosage; and refining of expression systems for higher yields to allow for better purification and formulation of parenteral vaccines (Yusibov and Rabindran et al. 2008). These developments have resulted in sophisticated approaches that are currently making the adoption of plant-based vaccines by the pharma industry a lot more likely than in previous years (Table 13.1). The following section presents some examples of plant-derived biopharmaceuticals adopted by the industry, which illustrate the positive impact of these advances in the adoption of the technology.
| Aspect | Limitation | Developed alternatives |
|--------|------------|------------------------|
| Low yields | Initial expression strategies resulted in poor protein expression, hampering successful immunization | New expression strategies, such as transplastomic technologies, and transient expression by means of viral vectors, have led to yield improvements in several orders of magnitude, and have allowed for successful immunization of test animals |
| Poor stability and lack of accurate dosage | Unprocessed plant tissues used in early attempts are labile, which difficulties proper dosage | Stability and dosage are currently controlled by means of using freeze-dried biomass |
| Poor immunogenicity/induction of oral tolerance | Oral tolerance is frequently induced when antigens are orally administered, and thus vaccine fails on the induction of immune responses | Adjuvants able to conveniently polarize the immune response have been used |
| Establishment of production under good manufacturing practices (GMPs) | Conventional regulations for biopharmaceutical-favored products produced under GMPs Initial focuses of plant-based vaccines did not contemplate this aspect | Procedures under GMPs have been implemented with the modalities of culturing plant cells in bioreactors as well as transient expression systems |
| Biosafety concerns | Undesired gene flow is associated with the risk of contaminating food chain with antigenic proteins | Transplastomic approaches minimize this risk, since plastome typically shows maternal inheritance Culture of plant cells in bioreactors as well as transient expression system allows for the production under full containment |
Successful Cases of Plant-Made Biopharmaceuticals

**Influenza Vaccine**

One of the most advanced plant-based human vaccines is under development by Medicago Inc. (USA and Canada). Candidate influenza vaccines have been developed by means of expressing the hemagglutinin (HA) protein of H5N1 influenza (A/Indonesia/5/05), as well as of seasonal and H1N1pdm viruses, so as to obtain virus-like particles (VLPs) in a transient expression system in *Nicotiana benthamiana* plants. The candidate H5N1 vaccine is safe and immunogenic when intramuscularly administered to humans, and is currently under evaluation in phase II clinical trial (Landry et al. 2010; Penney et al. 2011). This platform is proposed as an ideal approach for producing vaccines quickly, which is critical for new pandemic influenza strains and viruses such as SARS-CoV and MERS-CoV. It is estimated that plant-based vaccines produced in a transient expression system can be generated within 3 weeks from the release of sequence information.

**Anti-HIV Antibody**

Although not a vaccine, the EU FP7 Pharma-Planta consortium successfully produced a topically applied anti-HIV monoclonal antibody-based microbicide named P2G12: This was expressed in transgenic tobacco plants, and subjected to a phase I trial carried out at the University of Surrey Clinical Research Centre, UK with the participation of 11 healthy volunteers. Previous studies reported that this antibody is capable of recognizing a cluster of high-mannose-type N-glycans on the HIV envelope protein gp120, leading to a high neutralizing activity both in vitro and in vivo, since it prevents transmission by both parenteral and mucosal routes (Mascola 2002; Veazey et al. 2003). Tobacco plants (*N. tabacum*) producing P2G12 were grown in adequate containment greenhouses at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in Germany (Fraunhofer 2011). At present, no results from the clinical trial are available, but investigators envisage that P2G12 may be used in combination with other plant-produced antibodies for the formulation of a broadly protective vaginal microbicide (Pharma-Planta 2011).

**Therapeutic Enzyme for Gaucher’s Disease**

This example constitutes the first plant-derived biopharmaceutical approved for use in humans, illustrating their potential to reach the marketplace. Protalix Biotherapeutics (Carmel, Israel) established the production of a human recombinant glucocerebrosidase in suspension cultures of transgenic carrot cells (*Daucus carota* L.). This is a therapeutic enzyme for Gaucher’s disease, a lysosomal storage disorder
caused by mutations in the human glucocerebrosidase gene. Unlike the Chinese hamster ovary (CHO) cell-derived product, the carrot-derived enzyme has terminal mannose residues on its glycans, allowing for efficient uptake via macrophage mannose receptors. Phase III clinical trial was completed in 2009, and orphan drug designation has been granted from the Food and Drug Administration (FDA). The product is currently approved for human use by health agencies from a number of countries (Protalix 2011).

What Is Next in the Field of Plant-Based Vaccines?

The cases mentioned above exemplify how plant-based platforms have shown a sufficient degree of maturity in development as well as robustness, leading to modalities of production that are finding their niche in the biopharmaceuticals industry.

It is clear that production of biopharmaceuticals such as antibodies or enzymes are prone to less complex evaluations than the case of vaccines, where complex parameters such as achieving long-lasting immune responses capable of supporting immunoprotection should be addressed. Therefore, it is envisioned that vaccines, whose target antigens, immunogenic properties, and conventional large-scale production and production processes are well established, will be the first plant-based vaccines to be produced in transient systems, as this implies a standardized and approved path for the development of biosimilars. An influenza virus vaccine is the case that highlights this potential. However, the reluctance of Big Pharma to move from conventional production platforms to those of the new generation will still be a hurdle to overcome.

The road to reaching the market with plant-based vaccines and deriving a substantial exploitation of this technology is still a long one. The following section summarizes perspectives that are identified at both the basic-research and the regulatory industrial levels for pursuing this goal through technical innovations that meet the regulatory requirements, promoting social acceptance in parallel, and the politics of exploiting the technology in poor countries.

**Perspectives in the Context of Basic Research**

**Advancing on the Development of Oral Formulations**

A major challenge in the field of plant-based vaccine development has been in the modality of oral immunization with plant biomass. The high potential is attributed to this immunization approach, such as simple needle-free delivery, very low costs because minimally processed plant biomass is administered as the vaccine, and the possibility of inducing mucosal responses which can protect against orally or sexually transmitted diseases. However, several factors need to be addressed before the successful elicitation of safe and robust immune responses through oral immunization is assured.
One challenge consists of eliciting robust immune responses by the oral route, which is hampered by both antigen degradation and tolerogenic nature of the system (Fujkuyama et al. 2012; Mestecky et al. 2008). Several factors may impact the immunogenicity of oral vaccines. Among these, formulation is a critical aspect (Gebril et al. 2012). In the case of plant-based vaccines, it is proposed that a protective effect is exerted by the plant cell wall, which is termed as a “bioencapsulation” effect. Interestingly, a number of studies have revealed higher immunogenicity when the antigen is administered in the form of a plant-derived preparation rather than as a purified soluble antigen (Rosales-Mendoza et al. 2011; Hayden et al. 2012; Pniewski et al. 2011), suggesting a matrix effect that may be associated with (1) a delayed degradation rate or (2) the adjuvant effects exerted by plant compounds (Otsuki et al. 2010).

However, the reality is that no systematic studies to characterize this effect have been conducted, with only a few reports that have focused on studying the bioavailability of oral plant-based vaccines and the effect of the plant tissue used for expression and immunization (e.g., Pelosi et al. 2011, 2012). Thus, future evaluations of the effect of vaccine formulation will provide new insights on the bioencapsulation effect and optimization of the immunogenic potential of this kind of vaccine. Among the parameters to be studied are particle size, pill/capsule composition, and the type of plant tissue to be administered.

It is well known that plants synthesize a complex population of metabolites that sometimes exert profound biological effects. Some of the plant compounds that are recognized as immunomodulators are the following: flavonoids, terpenoids, and saponins (Potterat and Hamburger 2008; Castro-Díaz et al. 2012; Schepetkin and Quinn 2006). Some plant compounds can also act as mucoadhesive agents, accounting for more efficient antigen uptake (Garg et al. 2010). Thus, a relevant research objective in this field would be the characterization of specific plant compounds responsible for enhancing the immunogenicity of the plant-derived formulations: this is of particular relevance, as a significant challenge in the field of oral vaccination consists of providing appropriate adjuvants, which is in general accomplished by high-cost formulations.

Another concern around the immunological events associated with oral vaccines is the possibility of breaking tolerance towards food proteins present in the plant delivery vector. One of the few studies focused on this issue was published by Nojima et al. (2011). They observed that humoral responses against rice proteins were elicited when rice expressing a chimeric protein comprising cholera toxin B (CTB) and an Alzheimer’s disease-related antigen were orally administered to mice. However, the authors hypothesized that immunological tolerance against those non-target rice proteins could be induced by means of breast-feeding. Therefore, immunized mice were subsequently fed by lactating mothers who had consumed rice proteins. This led to a successful induction of tolerance, suggesting that the design of specific immunization schemes has a potential for modulating undesired immune responses. However, this field of investigation is still in its infancy, which highlights the need for expanding not only the evaluation target immune responses and challenge experiments in animal models but also addressing questions regarding safety in terms of the elicitation of responses against non-target proteins. Systematic research in
this direction must be conducted to better understand how immune responses elicited by plant-based vaccines could be modulated in order to avoid undesired immune responses that may eventually mediate allergic events and other undesirable side effects.

Expanding the Modalities of Expression of Immunogens

Although current expression strategies are considered robust in terms of yields suitable for industrial applications, certain aspects of the expression systems may allow for innovative and improved formulations. The induction of broader immune responses is a relevant goal, especially in the case of hypervariable pathogens (e.g., influenza or HIV viruses). One approach to address this is the simultaneous expression of several antigens from a single transformation or transient event. When a transplastomic technology is used, the use of polycistronic vectors can allow simultaneous expression of proteins (see Modalities for Expression of Antigens in Plants: Plastid-Based Expression Strategies). In addition, some virus-derived vectors may allow this in nuclear expression approaches. One such strategy involves the use of internal ribosome entry site sequences (IRES), which allow for the translation of several open reading frames (ORFs) via CAP-independent translation (Ha et al. 2010; Gouiaa et al. 2012). Another alternative is the use of the picornaviral 2A endopeptidase sequence, which mediates a translational skip mechanism, allowing the production of different polypeptides from a single ORF where target antigens are linked by a 2A sequence (Halpin et al. 1999; Ha et al. 2010). These approaches remain essentially unexplored in the field and are considered to have great potential to aid in the improvement of plant-based vaccine production.

Diversifying Full-Contained Production Platforms

Among the options for implementing processes under full containment and good manufacturing practices (GMP) are the use of cell suspensions and organ cultures such as hairy roots systems. These approaches offer tightly controlled bioprocesses where environment concerns related to undesired gene flow are eliminated (Franchini et al. 2010; Michoux et al. 2011; Skarjinskaia et al. 2013). In addition, some platforms implemented in the biopharmaceutical field remain to be explored for vaccine production. In particular, moss (Physcomitrella patens) has been proposed by Rosales-Mendoza et al. (2013) as a robust platform for the production of recombinant vaccines. This non-vascular plant can be propagated in a filamentous development stage, named protonema, which can be grown in liquid media using bioreactors. This approach has several singular advantages, such as a low cost and well-established production system a platform for the production of plant-based vaccines, full containment of production, efficient secretion of the antigen to the media (facilitating purification), and the possibility of producing specific glycoforms by the use of strains genetically engineered for alternative glycosylation.
machinery. Exploring emerging plant production platforms may lead to innovative developments, for example, the production of immunogens with specific glycosylation patterns with improved immunogenic properties, and new processes performed under full containment.

**Targeting a Broader Number of Diseases**

A number of highly relevant pathologies considered as vaccine preventable still cause significant epidemiologic impact. As costs are in general the main obstacle to widespread vaccination, the adoption of the plant-based technologies for the development and production of vaccines for an expanded list of vaccine preventable diseases represents an important field of opportunity for medical biotechnology. The following section describes some diseases for which new vaccines are urgently needed, and which constitute logical targets for the plant-based vaccine production technologies.

*Tropical neglected diseases.* This group of diseases affects the lives of 1 billion people worldwide, but no vaccines are available for them (WHO 2010). Vaccines against neglected tropical diseases (NTDs) should be of low cost and preferably needle free, in order to reduce the logistic cost of their administration. Although a number of efforts on developing vaccines to fight rabies, cysticercosis, dengue fever, and helminthiasis have been reported, there is still a need for developing plant-based vaccination models for more of this group. Development of plant-based vaccines against tropical neglected diseases is identified as a key priority for exploiting this technology, as low-cost formulations may be produced to fight these diseases that mainly impact low-income populations ((Rosales-Mendoza et al. 2012a).

*Non-communicable diseases.* These pathologies killed tens of millions of people in 2008, and a high fraction of these deaths occurred in people under the age of 60 years, comprising the most productive human cohort. The incidence of these diseases continues to rise, especially in low- and middle-income countries. Of particular interest in combating these diseases are vaccines against cancer, hypertension, diabetes, and atherosclerosis, which play a major role in the mortality rates at the global level (WHO 2011). According to the World Health Organization (WHO), the leading global risks for mortality are high blood pressure (responsible for 13% of deaths globally), tobacco use (9%), high blood glucose (6%), physical inactivity (6%), and overweight and obesity (5%). These risks are responsible for raising the risk of chronic diseases such as heart disease, diabetes, and cancers (WHO 2009).

Type I diabetes is an autoimmune disease that has been targeted by a number of groups using plant-based vaccine models. The proposed therapy consists of eliciting tolerance against the glutamic acid decarboxylase, which is a self-antigen associated with the development of immune responses responsible for diabetes development. This goal is pursued by administering autoantigens by the mucosal route, which is “tolerogenic” by nature. This kind of approach has been successfully evaluated in animal models with promising findings in terms of therapeutic effects, suggesting a considerable potential for clinical trials (Alvarez et al. 2013; Langridge et al. 2010).
**Hypertension and atherosclerosis.** Immunotherapies for the treatment of these pathologies are well documented. In particular, vaccination models using conventional formulations have been based on the induction of humoral responses against physiological proteins whose elevated levels favor the development of the pathology (Bachmann and Jennings 2011). However, plant-based vaccines against hypertension and atherosclerosis have been recently suggested (Rosales-Mendoza 2012b; Salazar-González and Rosales-Mendoza 2013).

**Cancer.** Interestingly, plants have served as a source of recombinant antibodies for use in the treatment of non-Hodgkin lymphoma. Recombinant idiotype-specific personalized vaccines have been developed by the expression of tumor-derived single-chain Fv (scFv) antibodies in *N. benthamiana* plants by means of a TMV-based expression vector (McCormick et al. 1999). Autologous full-idiotype IgG-based vaccines have been produced by *N. benthamiana* plants using the transient TMV-based magnICON platform (Marillonnet et al. 2004). Mice subcutaneously immunized with this vaccine were protected against lethal tumor challenge. Patients with follicular lymphoma have been enrolled for a phase 1 clinical trial of the full-idiotype vaccine (sponsored by Bayer Innovation GmbH.http://www.clinicaltrials.gov; NCT01022255 ; NCT01022255).

However, active immunization therapies have essentially not been developed for most other human cancers. This represents a field of opportunity, as conventional chemotherapy and radiotherapy lack specificity and show significant toxicity. Vaccines are proposed as complementary treatment. For example, the human carcinoembryonic antigen (CEA), which is over-expressed by a large number of epithelial neoplasias, including colorectal carcinoma (CRC), gastric, pancreatic, breast, lung, and ovarian carcinomas (Hammarstrom 1999), is considered a tumor-associated antigen that can be targeted in therapeutic cancer vaccines (Berinstein 2002). This has been shown to be safe, and induction of antigen-specific immune responses has been achieved (Mosolits et al. 2005; Samanci et al. 1998). It is thus expected that focusing on this kind of target will be of significant relevance in the field of plant-based vaccines.

**The Regulatory Framework**

In spite of the many candidate vaccine antigens expressed in plants, and those proven to be effective thus far, most candidates have not progressed beyond the preclinical phase. Factors that limit their progress to the market will be further elaborated on in this chapter.

Interestingly, a regulatory framework has already been established to limit the growth of crop plants expressing pharmaceuticals, including vaccines. This development limits the use of, for example, maize, rice, potatoes, tomatoes, and other foods for commercial vaccine production. Commercialization also requires facilities for manufacturing vaccines on medium and large scale under current GMPs (Streatfield 2005). These GMPs were established by the WHO, and constitute an
associated group of norms and activities to guarantee that every product meets and retains the characteristics of design required for its use. The GMPs minimize unanticipated risks that may occur during the screening of the final product (van der Laan et al. 2006). For example, Kentucky BioProcessing, LLC (KBP; Owensboro, KY), is a facility specialized for the expression, extraction, and purification of recombinant proteins from plants, from bench to commercial scale, using its proprietary Geneware® expression technology under the GMP conditions (Yusibov et al. 2011).

The evaluation of vaccines by traditional methods can be followed for plant-based vaccines. However, plant containment is a particular issue for the production of this type of vaccines. In terms of production and clinical assessment, plant-based vaccines must apply for the Investigational New Drug (IND) application and all applicable regulatory requirements (Yusibov et al. 2011). These guidelines are contained in draft documents defined by the FDA and US Department of Agriculture (USDA), which address quality aspects in the production of plant-based biopharmaceuticals for human and animal use, and present the points related to product safety and efficacy, environmental issues, and manufacturing control (Center of Veterinary Medicine et al. 2002).

In particular, FDA establishes that development stages must comprise evaluation of: (1) the presence of potential allergenic or toxic compounds, (2) the method of plant production and propagation, (3) the characterization of recombinant DNA, and (4) genetic stability for those cases based on stable transformation events. Environmental concerns should be taken into consideration by means of implementing confinement mechanisms in order to not only control the spread of the bioengineered pharmaceutical plants but also meet regulations for transnational commercialization.

In addition, facilities and procedures should be designed to prevent cross-contamination of the source material during harvest and processing, which implies establishing procedures for appropriate cleaning, maintenance, and sanitization of equipment and utensils. Thus, malfunctions or contaminations that would alter the safety, identity, strength, quality, or purity of the products are prevented. On the other hand, testing the presence and identity of potentially harmful constituents is a requirement for performing preclinical trials. These comprise toxins, pathogens, pesticides, herbicides, fungicides, heavy metals, anti-nutrients, and allergens, which are assessed by in vitro and in vivo assays. In addition, unintended immunogenicity due to plant-specific posttranslational modifications must be assessed.

Once passed to clinical trials, existing guidelines for the clinical evaluation of drugs and biologics for humans are considered, and additionally, specialized advice can be obtained by communication with regulatory agencies, such as the Center for Drug Evaluation and Research of the FDA (CDER) or Center for Biologics Evaluation and Research of the FDA (CBER; Center of Veterinary Medicine 2002).

Toxicity, dose, lot-to-lot consistency, possible allergic responses, and immune tolerance must be evaluated during the development of plant-based biopharmaceuticals intended for commercialization. However, in spite of the inherent potential of plants as bioreactors, robust proofs would be provided regarding efficacy, ease of delivery, and cost. Because of these stringent regulatory requirements for human
Despite the fact that oral vaccination is considered to be an advantageous immunization approach, this approach faces a number of challenges. A suitable dosing regimen must be defined for each vaccine candidate; the formulation of oral vaccines requires, in general, high doses of antigen, which in turn may also be difficult to be accurately determined because of the complexity of the gastrointestinal tract (see Mucosal Immunology and Oral Vaccination). Therefore, formulation and delivery strategies should be optimized to attain immunoprotection under acceptable consistency (Streatfield and Howard 2003).

It is also a consideration that ethical issues for plant-based vaccines have not been subjected to sufficient analysis. Many of the ethical and social debates related to genetically modified (GM) plants have covered the topic of plant-based vaccines in a superficial manner, if at all. However, the negative perception of GM foods may in fact influence the social acceptability of plant-based vaccines. The main reason that GM foods have not been widely accepted by civil society is the perception that their consumption carries unknown risks, and that the major beneficiaries are farmers and seed companies, not the final consumer.

In contrast to these examples, under humanitarian projects such as those of the Pharma-Planta consortium, the major beneficiary of plant-based vaccines and therapeutics will be patients in the developing and poor countries. However, substantial transfer of the technology, its principles, and its benefits will definitively favor a positive perception of the technology, and hopefully its eventual acceptance.

**Commercialization of Plant-Based Vaccines**

Interest by and investment from the pharmaceutical industry are seen as key factors for the benefits of the plant-based technology for biopharmaceutical production to become a reality. During the last two decades, the production of biopharmaceuticals has increased notably. In the period from 1982 to 1991, 15 biologics were approved by the FDA, while 54 biopharmaceuticals were approved from 1992 to 2001. During the last two decades, a total of 95 biopharmaceutical products have been approved by regulatory agencies for treating several human diseases (Goldstein and Thomas 2004; Rader 2009; Wong 2009).

Recombinant proteins produced in plants have found a preferential niche in the biopharmaceutical field, rather than in industrial products such as enzymes and polymers. However, pharmaceutical companies have not completely adopted biopharmaceuticals as the next-generation drugs (Davies 2010). Molecular farming approaches have been technically successful, and possess the potential for being implemented on a large scale, as evidenced through many publications, patents, and field tests. These studies have yielded positive results in efficacy trials in animals, and some of them have been evaluated in clinical trials with promising insights (Tiwari et al. 2009; Yusibov et al. 2011).
Plant-based vaccines have a high potential for improving global health, although this will depend on demonstrations of efficacy, safety, and feasible commercialization, for pharmaceutical companies, philanthropic organizations, or the governments of developed or developing countries. Sadly, and despite their promise, breakthroughs in the development of oral vaccines have been few and far between, due partly to the complexity of the approach. Sustained collaborations between plant scientists, immunologist, and vaccinologists are considered of particular importance in maintaining the advancement in the development of effective and cost-effective oral vaccine candidates. This requires not only innovative research goals to address important questions related to safety and strategies to improve immunogenicity but also significant economic resources through public and non-profit organizations, as well as investments from industry in order to invigorate this field (Tiwari et al. 2009).

Notably, a vaccine for Newcastle virus produced in a suspension-cultured tobacco cell line by Dow AgroSciences, India, was successfully tested as a purified injectable product in chickens, and was approved by the USDA with full licensure for animals in the USA (Rybicki 2009; Melnik and Stoger 2013). The most advanced approach for plant-based human vaccines is in development by Medicago Inc. (USA and Canada), consisting of influenza candidate vaccines targeting seasonal H5N1 and seasonal and H1N1pdm influenza (Landry et al. 2010; Penney et al. 2011; Medicago Inc 2013).

The support of public funds is needed in order to advance the development of plant-based vaccines to the stage at which corporate investors will become involved. However, it is also necessary that they be evaluated by clinical trials if this technology is to prove itself worthy of the major disbursement of funds granted to target particular diseases in specific locations (Robert and Kirk 2006).

The Outlook for Developing Countries It is envisioned that plant-based biopharmaceutical industry will be consolidated in the next decade. Recombinant protein vaccines, including subunit-based or VLP-based vaccines, are good candidates for these new production systems, and plant-based pharmaceutical companies have responded to regulatory requirements in order to demonstrate that plants are an appropriate platform for production (Davies 2010; Scotti and Rybicki 2013).

In a realistic view, translational companies will favor financial support for developing vaccines with a significant market. Thus, in spite of achieving significantly at the preclinical level, it is considered of particular relevance to advance this technology in developing countries where the need for new vaccines against diseases is great, and is neglected by the larger companies.

A humanitarian focus is an indispensable factor in opening the path for exploitation of plant-based vaccines. As an example, the Pharma-Planta consortium was established in Europe in order to promote the production of anti-HIV microbical antibody candidates meeting all regulatory requirements, GMP standards, and preclinical toxicity testing, with a statement of intent for humanitarian use, which guaranteed access by poor countries to the plant-based products to be developed (www.pharma-planta.net/). Of particular relevance are the objectives of this consortium that comprised (1) the development of robust risk-assessment practices
for plant-made pharmaceuticals, based on health and environmental impact, collaborating with regulatory authorities within the EU and public groups to guarantee safety and acceptance of the production systems based on biosafety regulations and (2) design and implementation of a program intended for securing and managing intellectual property, and thus facilitation of the availability of high-priority plant-derived recombinant pharmaceuticals to poor countries, while at the same time allowing the products to be developed commercially in Europe and North America. This example illustrates the kind of efforts that may allow for a massive exploitation of the technology, and which will aid in overriding the “Valley of Death” that blocks the wider use of technologies for humankind benefit (Obembe et al. 2011).

It is envisioned that poor or developing countries must establish policies favoring increased connections between research institutes and universities with local companies or even government agencies: This will be a critical factor for exploiting the potential of plant-based vaccines in countries where the need for low-cost vaccines is great and urgent. Novel methods are needed to finance the increasing number of new vaccines that have the potential to save lives in countries that are too poor to afford them (Hefferon 2013; Levine et al. 2011). Selection of new vaccines that should be a priority for particular countries should be given under the advice of the WHO, in conjunction with a national immunization advisory committee, as adoption of plant-based vaccines could necessitate the modification of immunization schedules and delivery procedures.

It has recently been suggested (Rybicki et al. 2013) that developed country-firms and non-governmental organizations concerned with vaccine manufacture should team up with developing country-scientists and institutions for both research and development, as well as for clinical trials. There are already indications that this is happening: for example, Fiocruz/Bio-Manguinhos (Brazil) is collaborating with iBio Inc., (USA) to make yellow fever virus vaccine using plants and Ventria Bioscience (USA) has been growing transgenic rice-producing lactoferrin and lysozyme for some years now (see Rybicki et al. 2013). This kind of collaboration could open the door for the production of low-cost vaccines and other pharmaceuticals where they are needed, using local scientific and commercial resources, which would be a welcome change from the present model.

**Concluding Remarks**

Plant-based production has evolved into a robust approach for manufacturing vaccines under conditions that meet the requirement of a variety of regulatory systems. The benefits derived from this technology are expected to become evident in the coming years and to be realized by the introduction of the first vaccines into the market. Non-vaccine biopharmaceuticals already in the market are an indication of the potential for achieving this goal in the short term. Interesting aspects of the development of oral vaccines represent a research path that should be explored in a detailed and systematic manner in order to accelerate their development; these
constitute the ideal application of plant-based vaccines due to easy administration and low costs.

Humanitarian initiatives such as Pharma-Planta consortium are identified as key strategies for attaining the benefits of biofarming for developing countries. Future research efforts to expand the application of this technology to new target-relevant diseases will also be of key importance to exploit in a wider manner the advantages of plant-based vaccines.

References

Alvareza ML, Done SC, Cardineau GA (2013) Reverse vaccination and treatment of type 1 diabetes using plant-produced autoantigens and antiinflammatory cytokines. J Clin Cell Immunol S2:007

Bachmann MF, Jennings GT (2011) Therapeutic vaccines for chronic diseases: successes and technical challenges. Philos Trans R Soc Lond B Biol Sci 366:2815–2822

Berinstein NL (2002) Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. J Clin Oncol 20:2197–2207

Castro-Díaz N, Salaun B, Perret R, Siero S, Romero JF, Fernández JA, Rubio-Moraga A, Romero P (2012) Saponins from the Spanish saffron *Crocus sativus* are efficient adjuvants for protein-based vaccines. Vaccine 30:388–397

Center for Biologics Evaluation and Research (US), Center for Drug Evaluation and Research (US), Center for Devices and Radiological Health (US), Center for Veterinary Medicine (US), & United States (2002) Guidance for industry: drugs, biologics and medical devices derived from bioengineered plants for use in humans and animals. U.S. Dept. of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, Rockville

Davies HM (2010) Review article: commercialization of whole-plant systems for biomanufacturing of protein products: evolution and prospects. Plant Biotechnol J 8:845–861

Franconi R, Demurtas OC, Massa S (2010) Plant-derived vaccines and other therapeutics produced in contained systems. Expert Rev Vaccines 9:877–892

Fraunhofer (2011) Clinical tests for medicines made from genetically modified plants. http://www.ime.fraunhofer.de/en/press_medi/clinical_tests.html. Accessed 21 April 2014.

Fujkuyama Y, Tokuhara D, Kataoka K, Gilbert RS, McGhee JR, Yuki Y, Kiyono H, Fujihashi K (2012) Novel vaccine development strategies for inducing mucosal immunity. Expert Rev Vaccines 11:367–379

Garg NK, Mangal S, Khambete H, Tyagi RK (2010) Mucosal delivery of vaccines: role of mucoadhesive/biodegradable polymers. Recent Pat Drug Deliv Formul 4:114–128

Gebril A, Alsaadi M, Acevedo R, Mullen AB, Ferro VA (2012) Optimizing efficacy of mucosal vaccines. Expert Rev Vaccines 11:1139–1155

Goldstein DA, Thomas JA (2004) Biopharmaceuticals derived from genetically modified plants. QJM 97:705–716

Gouiaa S, Khoudi H, Leidi EO, Pardo JM, Masmoudi K (2012) Expression of wheat Na(+)/H(+) antiporter TNHXS1 and H(+)-pyrophosphatase TVP1 genes in tobacco from a bicistronic transcriptional unit improves salt tolerance. Plant Mol Biol 79:137–155

Ha SH, Liang YS, Jung H, Ahn MJ, Suh SC, Kweon SJ, Kim DH, Kim YM, Kim JK (2010) Application of two bicistronic systems involving 2A and IRES sequences to the biosynthesis of carotenoids in rice endosperm. Plant Biotechnol J 8:928–938

Halpin C, Cooke SE, Barakate A, Amrani AE, Ryan MD (1999) Self-processing 2A-polyproteins-a system for co-ordinate expression of multiple protein in transgenic plants. Plant J 17:453–459
Hammarstrom S (1999) The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 9:67–81
Hammond RW, Nemchinov LG (2009) Plant production of veterinary vaccines and therapeutics. Curr Top Microbiol Immunol 332:79–102
Hayden CA, Streatchfield SJ, Lamphear BJ, Fake GM, Keener TK, Walker JH, Clements JD, Turner DD, Tizard IR, Howard JA (2012) Bioencapsulation of the hepatitis B surface antigen and its use as an effective oral immunogen. Vaccine 30:2937–2942
Hefferon K (2013) Plant-derived pharmaceuticals for the developing world. Biotechnol J. doi:10.1002/biot.201300162
Landry N, Ward BJ, Trépanier S, Montomoli E, Dargis M, Lapini G, Vézina LP (2010) Preclinical and clinical development of plant-made virus-like particle vaccine against avian H5N1 influenza. PLoS ONE 5:e15559
Langridge W, Dénès B, Fodor I (2010) Cholera toxin B subunit modulation of mucosal vaccines for infectious and autoimmune diseases. Curr Opin Investig Drugs 11:919–928
Levine OS, Bloom DE, Cherian T, de Quadros C, Sow S, Wecker J, Dulos P, Greenwood B (2011) The future of immunisation policy, implementation, and financing. Lancet 378:439–448
Marillonnet S, Giritch A, Gils M, Kandzia R, Klmyuk V, Gébeka Y (2004) In planta engineering of viral RNA replicons: Efficient assembly by recombination of DNA modules delivered by Agrobacterium. Proc Natl Acad Sci USA 101:6852–6857
Mascola JR (2002) Passive transfer studies to elucidate the role of antibody-mediated protection against HIV-1. Vaccine 20:1922–1925
McCormick AA, Kumagai MH, Hanley K, Turpen TH, Hakim I, Grill LK, Tusé D, Levy S, Levy R (1999) Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants. Proc Natl Acad Sci USA 96:703–708
Medicago Inc (2013) Product pipeline. http://www.medicago.com/English/Products/product-pipeline/default.aspx. Accessed 15 Oct 2013
Melnik S, Stoger E (2013) Green factories for biopharmaceuticals. Curr Med Chem 20:1038–1046
Mestecky J, Nguyen H, Czerkinsky C, Kiyono H (2008) Oral immunization: an update. Curr Opin Gastroenterol 24:713–719
Michoux F, Ahmão N, McCarthy J, Nixon PJ (2011) Contained and high-level production of recombinant protein in plant chloroplasts using a temporary immersion bioreactor. Plant Biotechnol J 9:575–584
Mosolitis S, Nilsson B, Mellstedt H (2005) Towards therapeutic vaccines for colorectal carcinoma: a review of clinical trials. Expert Rev Vaccines 4:329–350
Nojima J, Ishii-Katsuno R, Futai E, Sasagawa N, Watanabe Y, Yoshida T, Ishiura S (2011) Production of anti-amyloid β antibodies in mice fed rice expressing amyloid β. Biosci Biotechnol Biochem 75:396–400
Obembe OO, Popoola JO, Leelavathi S, Reddy SV (2011) Advances in plant molecular farming. Biotechnol Adv 29:210–222
Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C (2010) Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects. J Ethnopharmacol 127:760–767
Pelosi A, Shepherd R, Guzman GD, Hamill JD, Meeusen E, Sanson G, Walmsley AM (2011) The release and induced immune responses of a plant-made and delivered antigen in the mouse gut. Curr Drug Deliv 8:612–621
Pelosi A, Piedrafita D, De Guzman G, Shepherd R, Hamill JD, Meeusen E, Walmsley AM (2012) The effect of plant tissue and vaccine formulation on the oral immunogenicity of a model plant-made antigen in sheep. PLoS ONE 7:e52907
Penney CA, Thomas DR, Deen SS, Walmsley AM (2011) Plant-made vaccines in support of the millennium development goals. Plant Cell Rep 30:789–798
Pharma-Planta (2011) First approval for production of an anti-HIV monoclonal antibody in plants. http://www.pharma-planta.net/images/file/Pharma-Planta_Press_release_July2011.pdf. Accessed 15 Oct 2013
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Pniewski T, Kapusta J, Bociąg P, Wojciechowicz J, Kostrzak A, Gdula M, Fedorowicz-Stróńska O, Wójcik P, Otta H, Samardakiewicz S, Wolko B, Plucienniczak A (2011) Low-dose oral immunization with lyophilized tissue of herbicide-resistant lettuce expressing hepatitis B surface antigen for prototype plant-derived vaccine tablet formulation. J Appl Genet 52:125–136

Potterat O, Hamburger M (2008) Drug discovery and development with plant-derived compounds. Prog Drug Res 65:47–118

Protalix (2011) Our Lead Product Candidate, taliglucerase alfa (prGCD). http://www.protalix.com/glucocerebrosidase.html. Accessed 15 Oct 2013

Rader R (2009) FDA biopharmaceutical product approvals and trends: significantly more approvals were granted in 2009. http://www.biopharma.com/approvals_2009.html. Accessed 15 Oct 2013

Robert JS, Kirk DD (2006) Ethics, biotechnology, and global health: the development of vaccines in transgenic plants. Am J Bioeth 6:W29–W41

Mendoza S, Govea-Alonso DO, Monreal-Escalante E, Fragoso G, Sciuotto (2012a) Developing plant-based vaccines against neglected tropical diseases: where are we? Vaccine 31:40–8

Rosaes-Mendoza S (2012b) Can a plant-based vaccine treat hypertension? Med Hypotheses 79:555–559

Rosaes-Mendoza S, Soria-Guerra RE, Moreno-Fierros L, Govea-Alonso DO, Herrera-Diaz A, Korban SS, Alpuche-Solis AG (2011) Immunogenicity of nuclear-encoded LTB:ST fusion protein from Escherichia coli expressed in tobacco plants. Plant Cell Rep 30:1145–1152

Rosaes-Mendoza S, Orellana-Escobedo L, Romero-Maldonado A, Decker E, Reski R (2013) The potential of Physcomitrella patens as a platform for the production of plant-based vaccines. Expert Rev Vaccines 13(2):203–212

Rybicki EP (2009) Plant-produced vaccines: promise and reality. Drug Discov Today 14:16–24

Rybicki EP (2010) Plant-made vaccines for humans and animals. Plant Biotechnol J 8:620–637

Rybicki EP, Hizeroth II, Meyers A, X Dus Santos MJ, Wigdorovitz A (2013) Developing country applications of molecular farming: case studies in South Africa and Argentina. Curr Pharm Des 19:5612–5621

Salazar-González JA, Rosaes-Mendoza S (2013) A perspective for atherosclerosis vaccination: is there a place for plant-based vaccines? Vaccine 31:1364–1369

Samanci A, Yi Q, Fagerberg J, Strigård K, Smith G, Rudén U, Wahren B, Mellstedt H (1998) Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. Cancer Immunol Immunother 47:131–142

Schepekkin IA, Quinn MT (2006) Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. Int Immunopharmacol 6:317–133

Scotti N, Rybicki EP (2013) Virus-like particles produced in plants as potential vaccines. Expert Rev Vaccines 12(2):211–224

Skarjinskaia M, Ruby K, Araujo A, Taylor K, Gopalasamy-Raju V, Musiyuchuk K, Chichester JA, Palmer GA, de la Rosa P, Mett V, Ugulava N, Streitfield SJ, Yusibov V (2013) Hairy roots as a vaccine production and delivery system. Adv Biochem Eng Biotechnol. doi:10.1007/10_2013_184

Streitfield SJ (2005) Regulatory issues for plant-made pharmaceuticals and vaccines. Expert Rev Vaccines 4:591–601

Streitfield SJ, Howard JA (2003) Plant production systems for vaccines. Expert Rev Vaccines 2:763–775

Tiwari S, Verma PC, Singh PK, Tuli R (2009) Plants as bioreactors for the production of vaccine antigens. Biotechnol Adv 27:449–467

van der Laan JW, Minor P, Mahoney R, Arntzen C, Shin J, Wood D, WHO Informal Consultation Group (2006) WHO informal consultation on scientific basis for regulatory evaluation of candidate human vaccines from plants, Geneva, Switzerland, 24–25 January 2005. Vaccine 24:4271–4278
Veazey RS, Shattock RJ, Pope M, Kirjian JC, Jones J, Hu Q, Ketas T, Marx PA, Klasse PJ, Burton DR, Moore JP (2003) Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat Med 9:343–346

Walmsley AM, Arntzen CJ (2000) Plants for delivery of edible vaccines. Curr Opin Biotechnol 11:126–129

Walmsley AM, Arntzen CJ (2003) Plant cell factories and mucosal vaccines. Curr Opin Biotechnol 14:145–150

WHO (2009) Global health risks: mortality and burden of disease attributable to selected major risks. World Health Organization

WHO (2010) First WHO report on neglected tropical diseases: working to overcome the global impact of neglected tropical diseases. World Health Organization. WHO/HTM/NTD/2010.1

WHO (2011) Global status report on noncommunicable diseases 2010. World Health Organization

Wong G (2009) Biotech scientists bank on big pharma’s biologics push. Nat Biotechnol 27:293–295

Yusibov V, Rabindran S (2008) Recent progress in the development of plant derived vaccines. Expert Rev Vaccines 7:1173–1183

Yusibov V, Streatfield SJ, Kushnir N (2011) Clinical development of plant-produced recombinant pharmaceuticals: vaccines, antibodies and beyond. Hum Vaccin 7:313–321