5-2001

Medical Molecular Farming: Production of Antibodies, Biopharmaceuticals and Edible Vaccines in Plants

Henry Daniell
University of Pennsylvania

Stephen J. Streatfield

Keith Wycoff

At the time of publication, author Henry Daniell was affiliated with the University of Central Florida. Currently, he is a faculty member at the School of Dental Medicine at the University of Pennsylvania

Medical Molecular Farming: Production of Antibodies, Biopharmaceuticals and Edible Vaccines in Plants

Abstract

The use of plants for medicinal purposes dates back thousands of years but genetic engineering of plants to produce desired biopharmaceuticals is much more recent. As the demand for biopharmaceuticals is expected to increase, it would be wise to ensure that they will be available in significantly larger amounts, on a cost-effective basis. Currently, the cost of biopharmaceuticals limits their availability. Plant-derived biopharmaceuticals are cheap to produce and store, easy to scale up for mass production, and safer than those derived from animals. Here, we discuss recent developments in this field and possible environmental concerns.

Disciplines

Dentistry

Comments

At the time of publication, author Henry Daniell was affiliated with the University of Central Florida. Currently, he is a faculty member at the School of Dental Medicine at the University of Pennsylvania

HHS Public Access

Author manuscript

Trends Plant Sci. Author manuscript; available in PMC 2017 July 04.

Published in final edited form as:

Trends Plant Sci. 2001 May ; 6(5): 219–226.

Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants

Henry Daniell[*],

Dept Molecular Biology and Microbiology and Center for Discovery of Drugs and Diagnostics, University of Central Florida, 12 722 Research Parkway, Orlando, FL 32826, USA

Stephen J. Streatfield, and

ProdiGene, 101 Gateway Boulevard, College Station, TX 77845, USA

Keith Wycoff

Planet Biotechnology, 2462 Wyandotte Street, Mountain View, CA 94043, USA

Abstract

The use of plants for medicinal purposes dates back thousands of years but genetic engineering of plants to produce desired biopharmaceuticals is much more recent. As the demand for biopharmaceuticals is expected to increase, it would be wise to ensure that they will be available in significantly larger amounts, on a cost-effective basis. Currently, the cost of biopharmaceuticals limits their availability. Plant-derived biopharmaceuticals are cheap to produce and store, easy to scale up for mass production, and safer than those derived from animals. Here, we discuss recent developments in this field and possible environmental concerns.

Research in the past few decades has revolutionized the use of therapeutically valuable proteins in a variety of clinical treatments. Because most genes can be expressed in many different systems, it is essential to determine which system offers the most advantages for the production of the recombinant protein. The ideal expression system would be the one that produces the most safe, biologically active material at the lowest cost. The use of modified mammalian cells with recombinant DNA techniques has the advantage of resulting in products that are identical to those of natural origin; however, culturing these cells is expensive and can only be carried out on a limited scale. The use of microorganisms such as bacteria permits manufacture on a larger scale, but introduces the disadvantage of producing products that differ appreciably from the products of natural origin. For example, proteins that are usually glycosylated in humans are not glycosylated by bacteria. Furthermore, human proteins that are expressed at high levels in *E. coli* frequently acquire an unnatural conformation accompanied by intracellular precipitation, owing to lack of proper folding and disulfide bridges.

The production of recombinant proteins in plants has many potential advantages for generating biopharmaceuticals relevant to clinical medicine. First, plant systems are more economical than industrial facilities using fermentation or bioreactor systems. Second, the

[*] daniell@mail.ucf.edu.

technology is already available for harvesting and processing plants and plant products on a large scale. Third, the purification requirement can be eliminated when the plant tissue containing the recombinant protein is used as a food (edible vaccines). Fourth, plants can be directed to target proteins into intracellular compartments in which they are more stable, or even to express them directly in certain compartments (chloroplasts). Fifth, the amount of recombinant product that can be produced approaches industrial-scale levels. Last, health risks arising from contamination with potential human pathogens or toxins are minimized.

Antibody production in plants

In the decade since the expression and assembly of immunoglobulin (Ig) heavy and light chains into functional antibodies was first shown in transgenic tobacco, plants have proven to be versatile production systems for many forms of antibodies. These include full-sized IgG and IgA, chimeric IgG and IgA, secretory IgG and IgA, single-chain Fv fragments (scFv), Fab fragments and heavy-chain variable domains. Recently, this list has been extended to include bispecific antibodies, which are made by the genetic fusion of two different scFvs via a flexible peptide linker[1]. Plants have great potential as a virtually unlimited source of inexpensive monoclonal antibodies (dubbed ‘plantibodies’) for human and animal therapeutics (Table 1).

There is not yet a consensus as to the best plant species or tissue for commercial antibody production. Most antibodies expressed to date have been in tobacco, although recently potatoes, soybean, alfalfa, rice and wheat have also been used successfully[2–6]. The major advantage of using green tissue (tobacco, alfalfa, soybean) is sheer productivity. Both alfalfa and tobacco can support several crops (cuttings) per year, with potential annual biomass yields of 25 tonne ha^{-1} and >100 tonne ha^{-1}, respectively. By contrast, the maximum yields of wheat, rice and corn seed are ~3 tonne ha^{-1}, 6 tonne ha^{-1} and 12 tonne ha^{-1}, respectively. Other advantages of tobacco include its relative ease of genetic manipulation, production of large numbers of seeds (up to a million per plant) and an impending need to explore alternate uses for this hazardous crop. However, seeds are likely to have fewer phenolic compounds and a less complex mixture of proteins and lipids than green leaves, which might be an advantage in purification. Another advantage of seeds or tubers is their ability to be stored for long periods. Levels of scFv in rice seeds did not show a significant decline after storage at room temperature for six months[5]. Potato tubers in cold storage for 18 months lost only 50% of functional antibody[2]. For short periods of time (five to seven days), dried tobacco and alfalfa leaves can also be stored with little loss of scFv (Ref. 7) or IgG antibody[4]. Purification of antibody from stored plant material has the advantages that the processing facility need not be near the field and can be used continually all year, rather than for just a few large batches.

To date, only four antibodies have been made in plants that are potentially useful as human therapeutics. Only one of these has been tested in humans: a chimeric secretory IgG–IgA antibody against a surface antigen of *Streptococcus mutans*, the primary causal agent of tooth decay. This tobacco-produced antibody was applied topically to teeth and found to be as effective as an IgG produced in a murine hybridoma at preventing recolonization by *S. mutans*[8]. The second antibody, a humanized anti-herpes-simplex virus (HSV) antibody made

in soybean, was effective in the prevention of vaginal HSV-2 transmission in a mouse model[3]. Its activity was indistinguishable both *in vitro* and *in vivo* from the monoclonal antibody produced in cell culture. A third antibody, against carcinoembryonic antigen (CEA), has recently been expressed in rice and wheat[5]. CEA, a cell-surface glycoprotein, is one of the best-characterized tumor-associated antigens. Antibodies against CEA are used for *in vivo* tumor imaging, as well as in antibody-based cancer therapy. Levels of scFv in seeds did not show a significant decline after storage at room temperature for six months. This same antibody has been expressed in a rice cell culture[6].

The fourth antibody is an example of both a novel use of plant-produced antibodies and an alternative production system. A plant virus vector has been used to produce a tumor-specific vaccine transiently in tobacco for the treatment of lymphoma[9]. The antibody genes for expression of an scFv were derived from a mouse B-cell lymphoma. The plant-produced scFv was used to immunize mice, which generated anti-idiotypic antibodies (antibodies against the binding portion of the antibody). These mice were protected against infection by the lymphoma that produced the original antibody. Other groups have used modified plant viral vectors to produce therapeutically useful antibodies in plants, including an antibody against the colorectal-cancer-associated antigen GA733-2 (Ref. 10). Although these vectors might find limited usefulness if the rapid production of an antibody is necessary (perhaps in greenhouse production), their acceptability to regulatory agencies (e.g. the US Food and Drug Administration, Dept of Agriculture and Environmental Protection Agency) has not been tested.

There are no plantibodies yet in commercial production, therefore estimates of cost are difficult to find and involve many assumptions. The costs of producing an IgG from alfalfa grown in a 250 m^2 greenhouse are estimated to be US$500–600 g^{-1}, compared with US $5000 g^{-1} for the hybridoma-produced antibody[4]. Planet Biotechnology (Mountain View, CA, USA) has compared the cost per gram of purified IgA made by cell culture, transgenic goats, grain (7.5 tonne ha^{-1}) and green biomass (120.0 tonne ha^{-1}) (Fig. 1). Expression levels will have a significant impact on the costs but, at the best expression level reported [500 μg g^{-1} leaf for a secretory IgA (Ref. 11)], the final cost should be well below US$50 g^{-1}. This significantly undercuts the costs of cell culture (US$1000 g^{-1}) or transgenic animal production systems (US$100 g^{-1}). The biggest component of cost with plantibodies will be purification. However, expression in seeds of rice and wheat[5] opens up the possibility of oral administration of some therapeutic antibodies without the need for expensive purification.

Some of the properties of Igs depend on their glycosylation (e.g. binding to monocyte Fc receptors). There is one conserved *N*-glycosylation site in the CH_2 domain of IgG. The structures of *N*-linked glycans on plant- and murine-produced Guy's 13 (an IgG1) have been determined and compared[12]. The plantibody *N*-glycans were more structurally diverse, with 40% being of the high-mannose type. The other 60% of the plantibody oligosaccharides had β-(1,2)-xylose and α-(1,3)-fucose linked to the $Man_3GlcNAc_2$ core. These linkages are typical of plants but are not found in mammalian *N*-glycans. The plantibody also lacked sialic acid, which represented ~10% of the sugar content of the mouse monoclonal antibody. These differences in glycan structure appear to have no effect on antigen binding or affinity

in vitro[3,4,11,13] and might not be significant *in vivo* either. An IgG produced in alfalfa had a serum half-life in Balb/c mice that was indistinguishable from that of the hybridoma-produced antibody[4]. However, there is some concern about the potential immunogenicity and allergenicity of plantibodies used as human therapeutics. For mucosal applications, this is not likely to present problems for most people because plant glycoproteins are ubiquitous in the human diet. There has been no evidence of allergic reaction or of a human anti-mouse antibody (HAMA) response in 60 patients receiving topical oral application of a secretory IgA specific to *S. mutans*[8].

Edible vaccines

Proteins of microbial and viral pathogens were some of the earliest examples chosen to show the feasibility of transgenic plant expression systems[14–17]. The rationale was that key immunogenic proteins of major pathogens could be synthesized in plant tissues and then fed as edible subunit vaccines to humans or commercially important animals. The proof of this concept has since been shown using several bacterial and viral proteins (Table 2). The practical aspects of choosing particular foodstuffs in which to deliver defined doses of a vaccine are being explored, and efforts are under way to establish clear regulatory paths for the development of edible vaccines.

Oral delivery of vaccines is an attractive alternative to injection, largely for reasons of low cost and easy administration. The chances of acquiring mucosal immunity against infectious agents that enter the body across a mucosal surface are also increased with oral vaccines. However, a major concern with oral vaccines is the degradation of protein components in the stomach and gut before they can elicit an immune response. To guard against degradation, several delivery vehicles have been developed to ferry intact proteins to the gut. These include recombinant strains of attenuated microorganisms, bioencapsulation vehicles such as liposomes and transgenic plant tissues.

Early work with plant-based subunit vaccines used the readily transformed species tobacco, potato and tomato[14–18]. However, the most attractive species for expressing subunit vaccine components should have high levels of soluble protein that is stable during storage; seed crops such as cereals are particularly suitable. The embryo fraction is rich in soluble protein and can easily be separated from other seed tissue to increase the concentration of antigen and thus decrease the dose size. The choice of crop defines the type of material to be fed. Many plant tissues can be consumed raw but others must be processed. Processing facilitates the creation of a homogeneous sample, enabling a defined dose size, but it is important that any heat or pressure treatments involved do not destroy the antigen. Alternative processing steps have been applied to a candidate vaccine component against enterotoxigenic strains of *E. coli* that consists of the B subunit of the heat-labile toxin (Lt-B) expressed in corn. A typical 1 mg dose of Lt-B could be delivered in an embryo fraction, to decrease the volume of the dose, or in a ‘cooked’ whole corn snack, to increase palatability and enhance stable storage (Fig. 2). In this case, neither treatment degrades the antigen. For commercial animal vaccines, the relevant protein can be expressed in a plant tissue that constitutes a major proportion of the diet, and heat and pressure treatments are not necessary.

Some key examples that illustrate the range of candidate proteins under investigation and plant expression systems being used are given in Table 2. Plant-expressed antigens have been shown to able to induce mucosal and serum immune responses when administered parenterally or orally to experimental animals and, in some test cases, they have offered protection against a subsequent pathogen challenge or challenge model[14,16,19–24]. A few of these vaccine candidates have been successfully tested in clinical trials or, where appropriate, in commercial or native animal trials[25–30]. Thus, edible vaccines delivered in plant tissues or processed plant products show great potential for efficacy in target organisms. The bioencapsulation of Lt-B in transgenic corn material results in an increased mucosal immune response compared with that achieved with naked antigen when fed to mice[30]. Presumably, this is because the antigen is protected from degradation in the gut, and it augurs well for the development of plant-based edible vaccines.

The quantity of plant tissue constituting a vaccine dose must be of a practical size for consumption. Thus, achieving a high level of expression is crucial. The expression of vaccine components in plants has been increased by using a range of leader and polyadenylation signals[31] and by optimizing codon usage for plants[22,29,30]. Expression could also be raised through crosses of transformed lines to various genetic backgrounds, an approach that has been successfully applied to boost protein production in corn. It is also important that any vaccine component should be present in its native form in the transgenic plant tissue. This has been assessed in several cases by examining the size of the synthesized protein, its ability to form higher-order complexes that mirror microbial or viral structures and, where relevant, by showing an enzymatic or receptor-binding activity[14,16–18,22,24,29].

The stability of heterologous proteins and the assembly of multisubunit structures depend on the cellular environment and therefore on the subcellular location. Favored locations for the expression of selected subunit vaccine components are the cell surface and the endoplasmic reticulum and Golgi body[14,18,29,31,32]. As with antibodies, transient expression systems (in which candidate vaccine sequences are incorporated into plant viral surface proteins) have also been investigated extensively and high levels of expression have been achieved. A related strategy to that of edible vaccines uses transgenic plants expressing autoantigens, whereby a large oral dose of an autoantigen can inhibit the development of an autoimmune disease through the mechanism of oral tolerance. This approach has been successful in a mouse model for diabetes[33].

Plant-derived biopharmaceuticals and human proteins

Generally, levels of pharmaceutical proteins produced in transgenic plants have been less than the 1% of total soluble protein that is needed for commercial feasibility if the protein must be purified[34]. Plant-derived recombinant hepatitis-B surface antigen induced only a low level serum antibody response in a small human study, probably reflecting the low level of expression (1–5 ng g^{-1} fresh weight) in transgenic lettuce[27]. In spite of recent improvements in expression levels in potato with a view to clinical trials[31], expression levels should be increased further for practical purposes. Also, even though Norwalk virus capsid protein expressed in potatoes caused oral immunization when consumed as food, expression levels are too low for large-scale oral administration (0.37% of total soluble protein)[16,26].

Expression of genes encoding other human proteins in transgenic plants has been disappointingly low: human serum albumin, 0.020% total soluble protein; human protein C, 0.001% total soluble protein; erythropoietin,~0.003% total soluble protein; and human interferon-β, <0.001% fresh weight (Table 3). A synthetic gene coding for the human epidermal growth factor was expressed only up to 0.001% of total soluble protein in transgenic tobacco[35,36]. In spite of several successful reports of high-level expression of non-human proteins (e.g. phytase, glucanase) via the nuclear genome, there is a great need to increase expression levels of human blood proteins to enable the commercial production of pharmacologically important proteins in plants.

Chloroplast transgenic system

One alternative approach is to express foreign proteins in chloroplasts of higher plants. Foreign genes have been integrated into the tobacco chloroplast genome, giving up to 10 000 copies per cell and resulting in the accumulation of recombinant proteins at up to 47% of the total soluble protein[37]. Chloroplast transformation uses two flanking sequences that, through homologous recombination, insert foreign DNA into the spacer region between the functional genes of the chloroplast genome, thus targeting the foreign genes to a precise location. This eliminates the ‘position effect’ upon expression that is frequently observed in transgenic plants with genes inserted into the nuclear genome. In addition, gene silencing has not been observed with chloroplast transformation, whereas it is a common phenomenon with nuclear transformation.

Chloroplast genetic engineering is an environmentally friendly approach, minimizing several environmental concerns[38,39]. Importantly, chloroplasts can process eukaryotic proteins, including enabling correct folding and the formation of disulfide bridges. Chaperonin proteins are present in chloroplasts and might function in the folding and assembly of non-native proteins of both prokaryotic and eukaryotic origins. Also, chloroplast proteins are activated by disulfide bond oxidation–reduction cycles using the plastid thioredoxin system[40] or protein disulfide isomerase[41]. Accumulation of large quantities of a fully assembled form of human somatotropin with the correct disulfide bonds (7% total soluble protein)[42] provides strong evidence for hyperexpression and assembly of pharmaceutical proteins using this approach. Such folding and assembly of foreign proteins should eliminate the need for expensive *in vitro* processing of pharmaceutical proteins produced in recombinant organisms. For example, 60% of the total operating cost for the commercial production of human insulin in *E. coli* is associated with *in vitro* processing (formation of disufide bridges and cleavage of methionine)[43].

Novel biopharmaceutical purification strategies

Purification is likely to represent most of the cost of biopharmaceutical production in plants. For the commercial production of insulin in *E. coli*, chromatography accounts for 30% of operating expenses and 70% of equipment costs[43]. Therefore, new approaches are necessary to minimize or eliminate chromatography in the production of pharmaceutical proteins. One successful recent approach is targeting pharmaceutical proteins to seed oil bodies. This was shown with hirudin, an anticoagulant first isolated from the leech *Hirudo medicinalis*. An

oleosin–hirudin fusion protein has been targeted to oil bodies of *Brassica napus* seeds and purified by flotation centrifugation for commercial production in Canada[44].

Another novel approach is the use of GVGVP as a fusion protein to facilitate single-step purification without the use of chromatography. GVGVP is a protein-based polymer encoded by synthetic genes. At low temperatures, it exists as an extended molecule but, upon raising the temperature above the transition range, the polymer hydrophobically folds into dynamic structures called β-spirals that further aggregate by hydrophobic association to form twisted filaments[45]. Using this approach, single-step purification of an insulin–polymer fusion has recently been shown. Inverse temperature transition offers several advantages, including facilitating the scale-up of purification from grams to kilograms (O. Carmona-Sanchez and H. Daniell, unpublished). Yet another recent approach is the use of a chaperonin protein to fold foreign proteins into cuboidal crystals, allowing their purification in a single step by centrifugation[37]. One additional advantage of this method is the protection of foreign proteins from cellular proteases.

Future perspectives

Plant-derived biopharmaceuticals should meet the same standards of safety and performance as other production systems. However, many herbal medicines are now exempt from such close scrutiny and are not required to meet the same standards because of their classification as nutritional supplements. Because several environmental concerns have been raised by interest groups to confuse public perception, it is of paramount importance that regulating agencies distinguish between real and perceived public concerns (scientific versus non-scientific). If biopharmaceuticals that are potentially harmful are capable of persisting in the environment and might accumulate in non-target organisms, precautionary measures should be taken. Induction of biopharmaceutical production after harvesting (as was done in the case of glucocerebrosidase[36]) might be one approach to minimize environmental exposure, provided that the use of viral vectors does not introduce additional environmental or regulatory concerns. Expression of potentially harmful proteins in a form that must be treated for activation might minimize the risk of exposure. For example, hirudin is produced as a fusion protein and is inactive in this form; it is activated only after it is purified from seeds[36].

Another hotly debated environmental concern has been the outcrossing of transgenic pollen to weeds or related crops[38,39]. Expression of harmful pharmaceutical proteins in non-target plants resulting from such outcrosses might create public concern and negative perception. Several gene containment methods are currently being investigated, including apomixis, incompatible genomes, transgenic mitigation, control of seed dormancy or shattering, suicide genes, infertility barriers, male sterility and maternal inheritance. Engineering foreign genes via the chloroplast genome has been shown to contain transgenes effectively, although there are a few exceptions in which the chloroplast genome shows biparental inheritance (e.g. pines)[46]. As an example of an alternative strategy, RNAse genes have been expressed under the control of a tissue-specific promoter to destroy the tapetum selectively during anther development, resulting in male sterile plants[47].

There is also concern over the expression of harmful proteins in transgenic pollen. For example, the controversial observation of the toxic effect of *Bacillus thuringiensis* (*Bt*) corn pollen on milkweeds (*Asclepias* spp.) fed to monarch butterfly larvae had a significant impact on public perception, even though the validity of this study has been repeatedly questioned. Engineering biopharmaceuticals via the chloroplast genome might be a solution. Although the Cry protein of *Bt* was expressed at high levels in leaves (up to 47% of total soluble protein), no toxicity was observed when milkweeds dusted with transgenic pollen were fed to monarch butterfly larvae[37]. However, to date, chloroplast genetic engineering has been shown only in tobacco and potato. More recently, several academic and industrial laboratories have initiated projects to extend this technology to other useful crops. Also, there are no reports of the production of glycoproteins in transgenic chloroplasts. Another public concern is the presence of antibiotic resistance genes or their products (which are used as selective markers) in edible parts of genetically modified crops. However, several approaches are now available to generate plants with transgenes in their nuclear[48] or chloroplast[49] genomes without the use of antibiotic selection.

Practical considerations will dictate the choice of biopharmaceutical proteins and the crop in which they are to be produced. These include yield, storage conditions, containment properties, initial set up and running costs, purification strategies, size of the market, environmental concerns, public perception and competing technologies. Access to several alternative approaches to optimize protein synthesis in plants in an environmentally sound manner augurs well for the safe production of biopharmaceuticals in transgenic plants and for greater availability of these proteins to populations requiring them.

References

1. Fischer R, et al. Expression and characterization of bispecific single chain Fv fragments produced in transgenic plants. Eur J Biochem. 1999; 262:810–816. [PubMed: 10411643]
2. Artsaenko O, et al. Potato tubers as a biofactory for recombinant antibodies. Mol Breed. 1998; 4:313–319.
3. Zeitlin L, et al. A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes. Nat Biotechnol. 1998; 16:1361–1364. [PubMed: 9853620]
4. Khoudi H, et al. Production of a diagnostic monoclonal antibody in perennial alfalfa plants. Biotechnol Bioeng. 1999; 64:135–143. [PubMed: 10397849]
5. Stoger E, et al. Cereal crops as viable production and storage systems for pharmaceutical scFv antibodies. Plant Mol Biol. 2000; 42:583–590. [PubMed: 10809004]
6. Torres E, et al. Rice cell culture as an alternative production system for functional diagnostic and therapeutic antibodies. Transgenic Res. 1999; 8:441–449. [PubMed: 10767987]
7. Fiedler U, et al. Optimization of scFv antibody production in transgenic plants. Immunotechnology. 1997; 3:205–216. [PubMed: 9358273]
8. Ma J, et al. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med. 1998; 4:601–606. [PubMed: 9585235]
9. McCormick A, et al. Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants. Proc Natl Acad Sci U S A. 1999; 96:703–708. [PubMed: 9892697]
10. Verch T, et al. Expression and assembly of a full-length monoclonal antibody in plants using a plant virus vector. J Immunol Methods. 1998; 220:69–75. [PubMed: 9839927]

11. Ma JK, et al. Generation and assembly of secretory antibodies in plants. Science. 1995; 268:716–719. [PubMed: 7732380]
12. Cabanes-Macheteau M, et al. *N*-glycosylation of a mouse IgG expressed in transgenic tobacco plants. Glycobiology. 1999; 9:365–372. [PubMed: 10089210]
13. Vaquero C, et al. Transient expression of a tumor-specific single-chain fragment and a chimeric antibody in tobacco leaves. Proc Natl Acad Sci U S A. 1999; 96:11128–11133. [PubMed: 10500141]
14. Haq TA, et al. Oral immunization with a recombinant bacterial antigen produced in transgenic plants. Science. 1995; 268:714–716. [PubMed: 7732379]
15. Mason HS, et al. Expression of hepatitis B surface antigen in transgenic plants. Proc Natl Acad Sci U S A. 1992; 89:11745–11749. [PubMed: 1465391]
16. Mason HS, et al. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. Proc Natl Acad Sci U S A. 1996; 93:5335–5340. [PubMed: 8643575]
17. McGarvey PB, et al. Expression of the rabies virus glycoprotein in transgenic tomatoes. Biotechnology. 1995; 13:1484–1487. [PubMed: 9636308]
18. Arakawa T, et al. Expression of cholera toxin B subunit oligomers in transgenic potato plants. Transgenic Res. 1997; 6:403–413. [PubMed: 9423288]
19. Arakawa T, et al. Efficacy of a food plant-based oral cholera toxin B subunit vaccine. Nat Biotechnol. 1998; 16:292–297. [PubMed: 9528012]
20. Carrillo C, et al. Protective immune response to foot-and-mouth disease virus with VP1 expressed in transgenic plants. J Virol. 1998; 72:1688–1690. [PubMed: 9445079]
21. Gomez N, et al. Expression of immunogenic glycoprotein S polypeptides from transmissible gastroenteritis coronavirus in transgenic plants. Virology. 1998; 249:352–358. [PubMed: 9791026]
22. Mason HS, et al. Edible vaccine protects mice against *Escherichia coli* heat-labile enterotoxin (LT): potatoes expressing a synthetic LT-B gene. Vaccine. 1998; 16:1336–1343. [PubMed: 9682399]
23. Thanavala Y, et al. Immunogenicity of transgenic plant-derived hepatitis B surface antigen. Proc Natl Acad Sci U S A. 1995; 92:3358–3361. [PubMed: 7724566]
24. Wigdorovitz A, et al. Induction of a protective antibody response to foot and mouth disease in mice following oral or parenteral immunization with alfalfa transgenic plants expressing the viral structural protein VP1. Virology. 1999; 255:347–353. [PubMed: 10069960]
25. Tacket CO, et al. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nat Med. 1998; 4:607–609. [PubMed: 9585236]
26. Tacket CO, et al. Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. J Infect Dis. 2000; 182:302–305. [PubMed: 10882612]
27. Kapusta J, et al. A plant-derived edible vaccine against hepatitis B virus. FASEB J. 1999; 13:1796–1799. [PubMed: 10506582]
28. Castanon S, et al. Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. J Virol. 1999; 73:4452–4455. [PubMed: 10196345]
29. Tuboly T, et al. Immunogenicity of porcine transmissible gastroenteritis virus spike protein expressed in plants. Vaccine. 2000; 18:2023–2028. [PubMed: 10706964]
30. Streatfield SJ, et al. Plant-based vaccines: unique advantages. Vaccine. 2000; 19:2742–2748.
31. Richter LJ, et al. Production of hepatitis B surface antigen in transgenic plants for oral immunization. Nat Biotechnol. 2001; 18:1167–1171.
32. Tackaberry ES, et al. Development of biopharmaceuticals in plant expression systems: cloning, expression and immunological reactivity of human cytomegalovirus glycoprotein B (UL55) in seeds of transgenic tobacco. Vaccine. 1999; 17:3020–3029. [PubMed: 10462237]
33. Ma S-W, et al. Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. Nat Med. 1997; 3:793–796. [PubMed: 9212110]
34. Kusnadi A, et al. Production of recombinant proteins in transgenic plants: practical considerations. Biotechnol Bioeng. 1997; 56:473–484. [PubMed: 18642268]
35. May, GD., et al. Application of transgenic plants as production systems for pharmaceuticals. In: Fuller, G., et al., editors. Agricultural Materials as Renewable Resources: Nonfood and Industrial

Applications (ACS Symposium Series 647). American Chemical Society; Clarendon Hills, Il, USA: 1996. p. 196-204.

36. Cramer C, et al. Transgenic plants for therapeutic proteins: linking upstream and downstream technologies. Curr Top Microbiol Immunol. 1999; 240:95–118. [PubMed: 10394717]
37. DeCosa B, et al. Hyper-expression of the Bt Cry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals. Nat Biotechnol. 2001; 19:71–74. [PubMed: 11135556]
38. Daniell H. GM crops: public perception and scientific solutions. Trends Plant Sci. 1999; 4:467–469. [PubMed: 10562729]
39. Daniell H. Environmentally friendly approaches to genetic engineering. In vitro. Cell Dev Biol–Plant. 1999; 35:361–368.
40. Reulland E, Miginiac-Maslow M. Regulation of chloroplast enzyme activities by thioredoxins: activation or relief from inhibition. Trends Plant Sci. 1999; 4:136–141. [PubMed: 10322547]
41. Kim J, Mayfield PS. Protein disulfide isomerase as a regulator of chloroplast translational activation. Science. 1997; 278:1954–1957. [PubMed: 9395399]
42. Staub JM, et al. High-yield production of a human therapeutic protein in tobacco chloroplasts. Nat Biotechnol. 2000; 18:333–338. [PubMed: 10700152]
43. Petridis D, et al. Computer-aided process analysis and economic evaluation for biosynthetic human insulin production: a case study. Biotechnol Bioeng. 1995; 48:529–541. [PubMed: 18623518]
44. Boothe JG, et al. Molecular farming in plants: oil seeds as vehicles for production of pharmaceutical proteins. Drug Dev Res. 1997; 42:172–181.
45. Daniell H, et al. Hyperexpression of an environmentally friendly synthetic polymer gene. Methods Mol Biol. 1997; 63:359–371. [PubMed: 9113662]
46. Daniell H, et al. Containment of herbicide resistance through genetic engineering of the chloroplast genome. Nat Biotechnol. 1998; 16:345–348. [PubMed: 9555724]
47. Mariani C, et al. Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature. 1990; 347:737–741.
48. Puchta H. Removing selectable marker genes: taking the shortcut. Trends Plant Sci. 2000; 5:273–274. [PubMed: 10871898]
49. Daniell H, et al. Engineering chloroplast genome without the use of antibiotic resistance genes. Curr Genet. in press.
50. Giddings G, et al. Transgenic plants as factories for biopharmaceuticals. Nat Biotechnol. 2000; 18:1151–1155. [PubMed: 11062432]
51. Ruggiero F, et al. Triple helix assembly and processing of human collagen produced in transgenic tobacco plants. FEBS Lett. 2000; 469:132–136. [PubMed: 10708770]
52. Chong DKX, et al. Expression of full length bioactive antimicrobial human lactoferrin in potato plants. Transgenic Res. 2000; 9:71–78. [PubMed: 10853271]

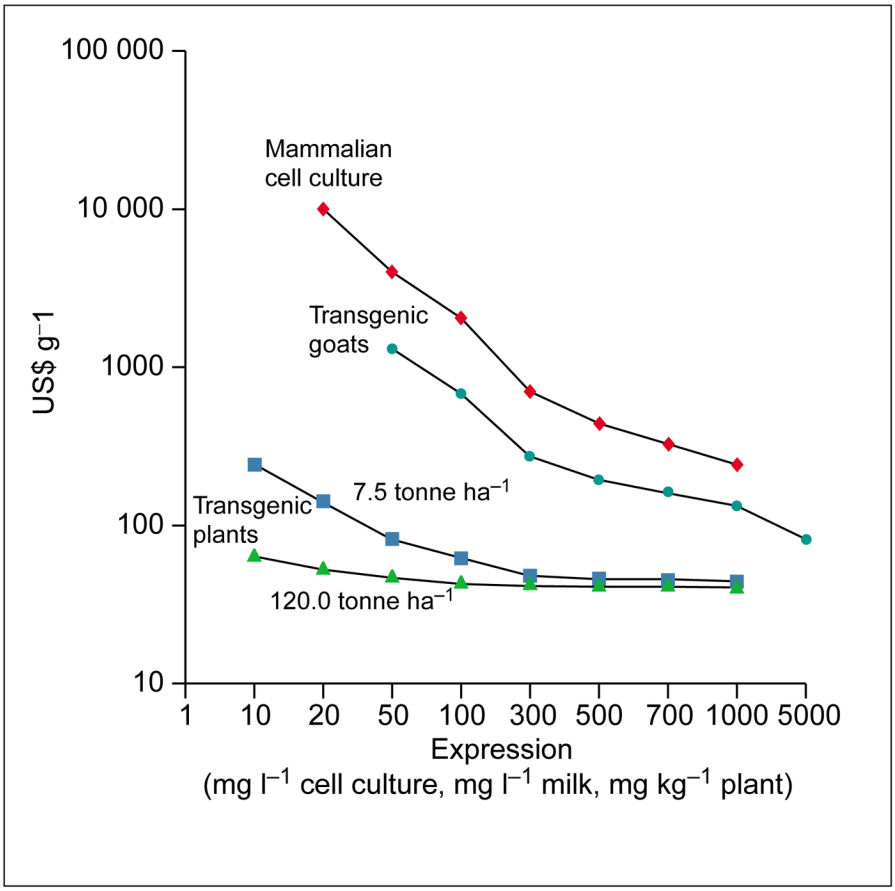

Fig. 1.

Costs per gram for purified immunoglobulin A produced by different expression systems. Costs for mammalian cell culture are derived from industry costs for cell culture and purification facilities. Costs for transgenic goats are derived from publicly available estimates from Genzyme Transgenics (Farmingham, MA, USA). Costs for plants compare green biomass (120.0 tonne ha^{-1}) and seed production (7.5 tonne ha^{-1}). Cost differences are based primarily on production costs, and it was assumed that purification costs and losses during purification will be the same for all systems.

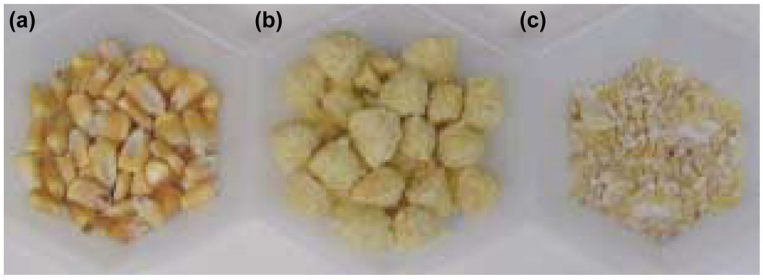

Fig. 2.

Alternative forms of delivery of a corn-based edible vaccine, produced by Prodigene (College Station, TX, USA). Transgenic corn kernels corresponding to a typical 1 mg dose of the B subunit of *E. coli* heat-labile toxin (Lt-B) (a), can be processed to generate a palatable whole corn snack of the same mass (b), or fractionated to yield an embryo or germ component in which the antigen is about six times more concentrated (c). The exact size of an administered dose depends on the expression level attained with a particular line of corn.

Table 1

Therapeutic and diagnostic plantibodies

Application and specificity	Promoter	Signal sequences	Antibody name or type	Plant	Expression levels	Refs
Dental caries; streptococcal antigen I or II	CaMV 35S	Murine IgG signal peptides	Guy's 13 (SIgA)[a]	*Nicotiana tabacum*	500 μg/g FW[b] leaves	8,11
Diagnostic; anti-human IgG	CaMV 35S	Murine IgG signal peptides	C5-1 (IgG)	Alfalfa	1.0% TSP[c]	4
Cancer treatment; carcinoembryonic antigen	Maize ubiquitin	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Wheat	900.0 ng/g leaves; 1.5 μg/g seed	5
Cancer treatment; carcinoembryonic antigen	Maize ubiquitin	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Rice	29.0 μg/g leaves; 32.0 μg/g seed; 3.8 μg/g callus	5,6
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Rice	27.0 μg/g leaves	5
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	TMV Ω leader; murine IgG signal peptides; KDEL	T84.66 (IgG)	*Nicotiana tabaccum* (transiently with *Agrobacterium* infiltration)	1.0 μg/g leaves	13
B-cell lymphoma treatment; idiotype vaccine	TMV subgenomic coat protein promoter	Rice α-amylase	38C13 (scFv)	*Nicotiana benthamiana*	30.0 μg/g leaves	9
Colon cancer; surface antigen	TMV subgenomic promoter U5 CP	Murine IgG signal peptide; KDEL	CO17-1A (IgG)	*Nicotiana benthamiana*	Not reported	10
Herpes simplex virus 2	CaMV 35S	Tobacco extensin signal peptide	Anti-HSV-2 (IgG)	Soybean	Not reported	3

[a] SIgA, secretory IgA.

[b] FW, fresh weight.

[c] TSP, total soluble protein.

Table 2

Proteins with applications for human or animal vaccines and expressed by transgenic plants

Source of the protein and target species for the vaccine	Protein or peptide expressed	Plant expression system	Maximum recorded expression level in *planta*	Integrity, immunogenicity and protective capacity of the vaccine	Refs
Enterotoxigenic *E. coli* (humans)	Heat-labile toxin B-subunit	Tobacco	<0.01% TSP[a]	Intact protein forms multimers and is immunogenic when administered orally	14
Enterotoxigenic *E. coli* (humans)	Heat-labile toxin B-subunit	Potato	0.19% TSP	Receptor-binding activity and immunogenic and protective when administered orally	14,22,25
Enterotoxigenic *E. coli* (humans)	Heat-labile toxin B-subunit	Maize	Not given	Immunogenic and protective when administered orally	30
Vibrio cholerae (humans)	Cholera toxin B-subunit	Potato	0.30% TSP	Intact protein forms multimers, has receptor- binding activity and is immunogenic and protective when administered orally	18,19
Hepatitis B virus (humans)	Envelope surface protein	Tobacco	<0.01% TSP	Virus-like particles form and extracted protein is immunogenic when administered by injection	15,23
Hepatitis B virus (humans)	Envelope surface protein	Potato	<0.01% FW[b]	Immunogenic when administered orally	31
Hepatitis B virus (humans)	Envelope surface protein	Lupin (*Lupinus* spp.)	<0.01% FW	Immunogenic when administered orally	27
Hepatitis B virus (humans)	Envelope surface protein	Lettuce	<0.01% FW	Immunogenic when administered orally	27
Norwalk virus (humans)	Capsid protein	Tobacco	0.23% TSP	Intact protein and virus-like particles form, immunogenic when administered orally	16
Norwalk virus (humans)	Capsid protein	Potato	0.37% TSP	Virus-like particles form and immunogenic when administered orally	16,26
Rabies virus (humans)	Glycoprotein	Tomato	1.00% TSP	Intact protein	17
Human cytomegalovirus (humans)	Glycoprotein B	Tobacco	<0.02% TSP	Immunologically related protein	32
Rabbit hemorrhagic disease virus (rabbits)	VP60	Potato	0.30% TSP	Immunogenic and protective when administered by injection	28
Foot-and-mouth disease virus (agricultural domestic animals)	VP1	*Arabidopsis*	Not given	Immunogenic and protective when administered by injection	20
Foot-and-mouth disease virus (agricultural domestic animals)	VP1	Alfalfa	Not given	Immunogenic and protective when administered by injection or orally	24
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	*Arabidopsis*	0.06% TSP	Immunogenic when administered by injection	21
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Tobacco	0.20% TSP	Intact protein and immunogenic when administered by injection	29
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Maize	<0.01% FW	Protective when administered orally	30

[a]TSP, total soluble protein.

[b]FW, fresh weight.

Table 3

The production of biopharmaceuticals for human health in transgenic plants

Potential application or indication	Plant host	Protein	Expression levels	Refs[a]
Human proteins				
Anticoagulant	Tobacco	Human protein C	<0.01% TSP[b]	36
Thrombin inhibitor	Canola (*Brassica napus*)	Human hirudin	0.30% seed protein	36
Neutropenia	Tobacco	Human granulocyte-macrophage colony-stimulating factor	Not reported	50
Growth hormone	Tobacco Tobacco	Human somatotropin, chloroplast Nuclear expression	7.00% TSP <0.01% TSP	42 42
Anemia	Tobacco	Human erythropoietin	<0.01 TSP	34
Antihyperanalgesic by opiate activity	*Arabidopsis*	Human enkephalins	0.10% seed protein	34
Wound repair and control of cell proliferation	Tobacco	Human epidermal growth	<0.01% TSP	36
Hepatitis C and B treatment	Rice, turnip (*Brassica rapa*) Tobacco	Human interferon-α Human interferon β	Not reported <0.01% fresh weight	36 34
Liver cirrhosis, burns, surgery	Tobacco	Human serum albumin	0.02% TSP	34
Blood substitute	Tobacco	Human hemoglobin α, β	0.05% seed protein	36
Collagen	Tobacco	Human homotrimeric collagen	<0.01% fresh weight	51
Cystic fibrosis, liver disease and hemorrhage	Rice	Human α-1-antitrypsin	Not reported	50
Trypsin inhibitor for transplantation surgery	Maize	Human aprotinin	Not reported	50
Antimicrobial	Potato	Human lactoferrin	0.10% TSP	52
Non-human proteins				
Hypertension	Tobacco, tomato	Angiotensin-converting enzyme	Not reported	50
HIV therapies	*Nicotiana bethamiana*	α-Tricosanthin from TMV-U1 Subgenomic coat protein	2.00% TSP	50
Gaucher's disease	Tobacco	Glucocerebrosidase	1.00–10.00% TSP	36

[a] Because of space limitation, reviews that cite original citations are provided.

[b] TSP, total soluble protein.