Research Article

Transgenic plants as sole source for biopharmaceuticals

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Abstract

Within a very short time period, the use of genetically modified plants for the production of therapeutic compounds has moved from being an experimental system with significant potential to a commercially viable process poised to deliver products useful in animal and human therapies. In roads have been made not only in more traditional areas of therapeutic development (e.g., the identification and isolation of bioactive secondary metabolites), but also in relatively uncharted areas such as the production of novel bioactive peptides and proteins, antibody production for passive immunization therapy, and edible oral vaccines. The rapid pace of development witnessed thus far is likely to accelerate in the very near future as additional, novel uses of transgenic plants as production systems for human therapeutics are explored. The limitations for the use of genetically modified plants will likely arise from our still somewhat unsophisticated knowledge of how plant gene expression is controlled and how various metabolic pathways within a plant interact and regulate themselves. The use of plants as production factories is already seen as an economically attractive alternative for the production of clinically important compound.

Keywords: Plants, transgenic, biopharmaceuticals, therapy, vaccine.

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INTRODUCTION

The use of plants or their extracts for the treatment of human disease predates the earliest stages of recorded civilization, from past at least to the Neanderthal period. By the 16th century, botanical gardens provided a wealth of material for teaching and therapeutic use, and herbal medicine flourished until the 17th century when more scientific 'pharmacological' remedies were discovered. Subsequently, the active principle in many medicinal plants was identified and, in many cases, purified for therapeutic use. Even today, about one fourth of current prescription drugs have a botanical origin[1]. Modern biotechnology has led to a revival of interest in obtaining new medicinal agents from botanical sources. Constantly genetic engineering (GE), plants are nowadays used to produce a variety of proteins, including mammalian antibodies, blood substitutes, vaccines and other therapeutic entities[2]. Latterly, the production of foreign proteins in GE plants has become a viable alternative to conventional production systems such as microbial fermentation or mammalian cell culture. GE plants, acting as bioreactors, can efficiently produce recombinant proteins in larger quantities than those produced using mammalian cell systems[3]. Considerable quantities of biomass can be easily grown in the field, and may permit storage of material prior to processing. So, plants offer the potential for efficient, large scale production of recombinant proteins with increased freedom from contaminating human pathogens. During the last two decades, generally 95 biopharmaceutical products have been approved by one or more regulatory agencies for the treatment of various human diseases including diabetes mellitus, growth disorders, neurological and genetic diseases, inflammatory conditions, and blood dyscrasias. Fairly 500 agents are believed to be in development world-wide, with some 370 biopharmaceuticals in the

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United States, including 178 agents directed against cancer or cancer related conditions, 47 among infectious diseases, and the remainder for a variety of other important medical conditions[4]. Among all these therapeutic entities are recombinant proteins, monoclonal antibodies, antisense oligonucleotides, and a variety of other protein agents such as hormones and immunomodulating drugs. Here, rapid increase in the number of new protein and peptide drugs reflects rapid advances in molecular biology, highlighted by the success of the Human Genome Project that, in turn, will help to identify many additional opportunities for therapeutic intervention. Regrettably, our capacity to produce these proteins in the quantities needed is expected to fall far short of demand by the end of the current decade[5]. While none of the commercially available products are currently produced in plants, biotechnology products which are comprised of proteins, and possibly also DNA-based vaccines, are potential candidates for plant-based production. Advances in plant biotechnology have already resulted in plants that produce monoclonal antibodies or other therapeutic proteins, or that may serve as a source of edible vaccines. Investigations underway will almost certainly result in GE plants designed to produce other therapeutic agents including hormones (e.g. insulin, somatotropin, erythropoietin), blood components, coagulation factors, and various interferons, and may well avoid critical limitations in production capacity. Transgenic pharmaceutical plants are primarily modified by the introduction of novel gene sequences which drive the production of 'designer' proteins or peptides. These proteins or peptides possess therapeutic value themselves, have properties that allow them to be used as precursors in the synthesis of medicinal compounds, or may serve as technical enzymes in pharmaceutical production. This review will attempt to catalogue the potential therapeutic applications of plant biotechnology and to address concerns related to the safety and efficacy of these agents in relation to human health and to specific disease states[4].

TRANGENIC PRODUCTION SYSTEMS

Biopharmaceuticals have traditionally been produced using a variety of transgenic systems, including cultured mammalian cells, bacteria, and fungi [6,7,8]. In the future, demand for existing biopharmaceuticals (e.g., erythropoietin to treat anemia and insulin to treat diabetes), as well as new therapeutic proteins discovered through genomics efforts, is expected to rise considerably[7]. It is prudent, therefore, to evaluate alternative transgenic production systems and determine how the future availability of safe recombinant biopharmaceuticals can be ensured in a cost effective manner. Producing therapeutic proteins in plants has many economic and qualitative benefits, including reduced health risks from pathogen contamination, comparatively high yields, and production in seeds or other storage organs[7]. The cultivation, harvesting, storage, and processing of transgenic crops would also use an existing infrastructure and require relatively little capital investment[7,8,9] making the commercial production of biopharmaceuticals an exciting prospect. Plants are potentially a cheap source of recombinant products[9,10,11]. Kusnadi *et al*.[12] have estimated that the cost of producing recombinant proteins in plants could be 10 to 50 fold lower than producing the same protein by *Escherichia coli* fermentation, depending on the crop. Many recombinant therapeutic proteins are produced using mammalian expression systems. A big advantage of these, and of insect tissue culture systems, is that they correctly synthesize and process mammalian products. However, product yields are generally low, and the requirement for fetal bovine serum in the growth medium makes production expensive[7]. In addition, cultured mammalian cells are sensitive to shear forces that occur during industrial scale culture, and to variations in temperature, pH, dissolved oxygen, and certain metabolites and makes it necessary to control culture conditions carefully, because variation in cell growth can affect fermentation and product purity. Although bacterial and fungal systems are more potent, they are not ideal for synthesizing many mammalian proteins because of differences in metabolic pathways, protein processing, codon usage, and the formation of inclusion bodies[8]. Although some differences exist in post-translation processing and in codon usage between plants and mammals, these are few compared with differences between mammals and microorganisms[7,13,14]. Where differences in processing do represent a problem, it may be possible to engineer plants with altered protein maturation pathways[7,15] Biopharmaceuticals produced in cell culture systems have to be purified from the culture supernatant, an expensive process. Plants can be made to store proteins in seed endosperm, from where they can be easily extracted[7,16]. Nevertheless, purification is potentially an expensive step, and various methods are being developed to overcome this problem, including the expression of proteins as fusions with oleosin[9,10,11]. An alternative approach is to cover the costs of purification with the income from the extraction of conventional products, such as meal, oil, or starch. The costs of isolating human serum albumin from starch potatoes, for example, could be largely covered by concomitant starch production[9,10,17]. In addition, purification may not always be necessary, for example, in the case of edible vaccines. Plant-derived products, whether purified or not, are less likely to be contaminated with human pathogenic

microorganisms than those derived from animal cells, because plants do not act as hosts for human infectious agents[18].

PHARMACEUTICAL CROPS

Pharmaceutical crops is an ambiguous term used by scientists of varying disciplines referring to different categories of plants and their utilization. Biologists often define *pharmaceutical crops* as genetically modified (GM) or engineered crops to produce vaccines, antibodies, and other therapeutic proteins[19-22], but sometimes, other terms for such a class of crops or proteins are used. For example, *pharma crops* is used to designate transgenic plants for the production of pharmaceuticals (e.g., antibiotics, diagnostic compounds, antibodies, vaccines, etc.) or industrially-useful biomolecules (e.g., biodegradable plastics, engine oils, food processing enzymes, etc.), rather than for the production of food, feed or textile fibers[19]. *Biopharming* means a practice of using GM or engineered crops (e.g., tobacco, maize, soybeans, tomato, rice, wheat, potato, safflower, alfalfa, and leaf mustard) as bioreactors to produce large therapeutic molecules[23]. However, natural product chemists occasionally use the term *pharmaceutical crops* for a different class of plants, those that produce pure small molecules as active pharmaceutical ingredients, although there is not any clear definition[24, 25]. These pharmaceutical ingredients are naturally occurring single entities of secondary metabolites in plants. Well known examples for this chemical definition are *Taxus* spp. (Taxaceae) and *Podophyllum* spp. (Berberidaceae) for production of anti-cancer drugs, and *Artemisia annua* L. (Asteraceae) for an anti-malarial drug. The different meanings of *pharmaceutical crops* as used by biologists and chemists may not only cause confusion in academia and industry, but also may often mislead the public. Thus, *Pharmaceutical Crops* should refer to those cultivated species that are used for the extraction or preparation of therapeutic substances such as active pharmaceutical ingredients (APIs), excipients used in pharmaceutical formulations, vaccines and antibodies, as well as other therapeutic proteins. Based on the type of pharmaceutical product, these crops can be classified into three distinct yet sometimes overlapping categories: crops for the production of small therapeutic molecules (STMs), large therapeutic molecules (LTMs), and standardized therapeutic extracts (STEs) (Table 1). Pharmaceutical crops can be either terrestrial or aquatic species. It is estimated that there are 400-500 plant species currently managed as pharmaceutical crops for production of STMs, fewer species are used for production of LTMs, and thousands of species are managed as crops for STEs[26].

Table 1: Three Types of Pharmaceutical Crops[26]

	Pharmaceutical Crops for the Production of		
	Small Therapeutic Molecules (STMs)	**Large Therapeutic Molecules (LTMs)**	**Standardized Therapeutic Extracts (STEs)**
Therapeutic Substances			
Molecule Type	Basically secondary metabolites	Basically primary metabolites	Both
Molecular Weight	Low molecular weight (usually <1,000)	High molecular weight (usually10,000 to 100,000)	Usually of low molecular weight
Molecular Origin	Endogenous	Endogenous or exogenous	Endogenous
Purity	Pure	Pure	Mixture
In vitro Production	Possible but most are not commercially feasible yet	Possible but most are not commercially feasible yet	May be unable to produce the same quality products
Biotransformation	Possible and relatively easy	No data	No data
Quality Control	Relatively easy	Relatively easy	Relatively difficult
Crops			
Type	Traditional (possible Transgenic in the future)	Traditional or Transgenic	Traditional
Cultivation History	< 100 years	Non-transgenic crops: <100 yrs Transgenic crops: <20 yrs	Many cultivated for centuries
Induction	Possible by stresses	No data	Traditional
Ethnobotanic Uses	Many are used in traditional medicines	Not used in traditional medicines	Usually used in traditional medicines

There are over 70,000 plant species thought to be of medicinal value in the world[27]. Most of the medicinal plants in the world are still harvested in the wild and have not been developed as crops. In China, for example, there are approximately 31,000 plant species[28]. A total of 11,146 plant species have been recorded in Chinese

herbal or medical literature according to a national survey conducted in 1995[29]. Only 10% (1,200 species) are marketed as commercial medicines[30], of which 300 species are often cultivated as crops[31]. This trend is consistent with the use of the angiosperms as food; while over 3,000 are known to be used as foods only about 150 are in the economic system and of those only a handful are major crops with global nutritional significance. Most of the natural products in clinical use today were discovered through a routine examination of terrestrial plants and microorganisms[32]. Most pharmaceutical crops have their origins in previously known medicinal plants. However, some pharmaceutical crops emerged from the discovery, through established bioactivity-guided screens, of molecules in plants that have not previously been used in traditional medicine or considered as medicinal plants (Table 2)[26].

Table 2: Useful applications of some transgenic plants

Transgenic plants	Useful application
Bt cotton	Pest resistance, herbicide tolerance and high yield. it is resistant to bollworm infection
Wheat	Resistance against the herbicide
Brassica napus	A gene encoding hirulidin (a protein that prevents blood clotting) is synthesized chemically and then transferred into *Brassica napus*. This gene is activated in the plant and starts synthesizing hirudin, which accumulates in seeds. The hirudin is then extracted and purified to be used in medicine.
Tobacc	CPTI (Cow Pea Trypsin Inhibitor) gene has been introduced on tobacco to show resistance against pets
Flavr Savr Tomato	Increased Shelf- life (delayed ripening) and better nutrient quality
Golden Rice	Vitamin A- Rich
Potato	Higher protein content
Banana	Transgenic Bananas act as edible vaccine to protect children against diarrhoea
Soyabean, Maize	Herbicide resistance

PLANTS AS A MAJOR SOURCE OF DRUG DISCOVERY

Plants have formed the basis for traditional medicine systems which have been used for thousands of years in China, India, and Egypt and later by the Greeks and Arabs. Botanical medicines still contribute to improving public health of the majority of the world's population[27,33]. Plants have also proven to be a major source for the discovery of modern drugs, particularly in the cancer field[34]. Of 155 small molecules developed as anti-cancer drugs worldwide from the 1940s to the present time, 72.9% are naturally-inspired, with 47% being either the natural products or semi-synthetic derivatives. Many well-known anti-cancer drugs are of plant origin, e.g., CPTs, taxanes, podophyllins, and vinca alkaloids. Several experimental plant based drugs have also shown promising potential: homoharringtonine[26] (alkaloid from *Cephalotaxus harringtonia*) for leukemia; 4-ipomeanol (a pneumotoxic analog of furan isolated from *Ipomoea batatas* for lung cancer; elliptinium (a semi-synthetic analog of ellipticine from *Bleekeria vitiensi*s for advanced breast cancer; and flavopiridol (a synthetic flavones derived from alkaloid rohitukine isolated from *Amoora rohituka* and *Dysoxylum binectariferum* for colorectal cancer[87]. As Drs. Wall and Wani stated, "undoubtedly, there are other highly active natural products from plant, marine, and fungal sources as yet unknown which, when discovered, will have therapeutic utility. Cancer is not one, but several hundred diseases and will require many different types of agents"[36]. Plants are the obvious choice for future research of drug development because they contain an almost infinite variety of novel molecules[37]. Many compounds have very complex structures that are too difficult and expensive to synthesize on an industrial scale. The global market for botanical and plant-derived drugs is expected to increase from $19.5 billion in 2008 to $32.9 billion in 2013[38]. However, insufficient supply of source material has been one of the major problems for bulk production of plant-based pharmaceuticals[39]. Major portion made till dated is highlight in Tables 3-5, while advantages of transgenic plants as protein expressing systems are enlisted in Table 6.

Table 3: Proteins with applications for human or animal vaccines and expressed by transgenic plants[40]

Source of the protein and the vaccine target species for	Protein or peptide expressed	Plant system expression	Integrity, immunogenicity and protective capacity of the vaccine
Enterotoxigenic *E. coli* (humans)	Heat-labile toxin B-subunit	Tobacco	Intact protein forms multimers and is immunogenic when administered orally
Enterotoxigenic *E. coli* (humans)	Heat-labile toxin B-	Potato	Receptor-binding activity and immunogenic

	subunit		and protective when administered orally
Enterotoxigenic *E. coli* (humans)	Heat-labile toxin B-subunit	Maize	immunogenic and protective when administered orally
Vibrio cholerae (humans)	Cholera toxin B-subunit	Potato	Intact protein forms multimers, has receptor- binding activity and is immunogenic and protective when administered orally
Hepatitis B (humans) virus	Envelop protein surface	Tobacco	Virus-like particles form and extracted protein is immunogenic when administered by injection
Hepatitis B (humans) virus	Envelop protein surface	Potato	Immunogenic when administered orally
Hepatitis B (humans) virus	Envelop protein surface	Lupin (Lupinus spp.)	Immunogenic when administered orally
Hepatitis B (humans) virus	Envelop protein surface	Letttuice	Immunogenic when administered orally
Norwalk virus (humans)	Capsid protein	Tobacco	Intact protein and virus-like particles form, immunogenic when administered orally
Norwalk virus (humans)	Capsid protein	Potato	Virus-like particles form and immunogenic when administered orally
Rabies virus (humans)	Glycoprotein	Tomato	Intact protein
Human cytomegalovirus (humans)	Glycoprotein B	Tobacco	Immunologically related protein
Rabbit hemorrhagic disease virus (rabbits)	VP60	Potato	Immunogenic and protective when administered by injection
Foot and mouth disease virus (agricultural domestic animals)	VP1	Arabidopsis	Immunogenic and protective when administered by injection
Foot and mouth disease virus (agricultural domestic animals)	VP1	Alfalfa	Immunogenic and protective when administered by injection or orally n
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Arabidopsis	Immunogenic when administered by injection
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Tobacco	Intact protein and immunogenic when administered by injection
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Maize	Protective when administered orally

Table 4: Different antigens expressed in plants[41]

Antigen	Pathogen or disease	Plant species
Hepatitis B surface Antigen (HbsAg)	Hepatitis B	Tobacco
LT-B	*E. coli* heat-labile enteroxin B subunit	Potato, Tobacco
SIgA-G	Streptococcus mutans	Tobacco
Glycoprotein G	Rabies virus	Rabies virus Tomato
Capsid protein	Norwalk virus	Tobacco, Potato
CT-B	*Vibrio cholera*	Potato
Insulin	Diabetes (autoimmune)	Potato
Structural protein VP 1	Foot and mouth disease	Arabidopsis
Spike (S) glycoprotein	Swine transmissible gastroenteritis coronavirus (TGEV)	Arabidopsis
s-LT-B	Heat-labile enterotoxin B	Potato
Structural protein VP60	Rabbit hemorrhagic disease virus (RHDV)	Potato
HBsAg	Hepatitis B	Lupin, Lettuce
Glycoprotein B	Human cytomegalovirus	Tobacco
HBsAg	Hepatitis B	Potato
F protein	Respiratory syncytial virus (RSV)	Tomato
S-glycoprotein	TGEV	Tobacco
Capsid protein 2L21	Canine parvovirus	Arabidopsis
Hemagglutinin protein	Measles virus (MV)	Tobacco
Human acetyl-choline esterase (AchE)	Organophosphate poisoning	Tomato
LT-B	Heat-labile enterotoxin B subunit	Corn
S	TGEV	Corn
CT-A2:CFA/I-CT-B:NSP4	Cholera, enterotoxigenic *E. coli*	Potato
Protective antigen	*Bacillus anthracis*	Tobacco

Table 5: Important plant-derived secondary metabolites of pharmaceutical value[41]

Active compound	Plant source	Proposed therapeutic use
Alkaloids Atropine, hyoscyamine, spp. Scopolamine	Solanaceous	Anticholinergic
Vinblastine, Vincristine	*Catharanthus roseus L.*	Antineoplastic
Nicotine	Nicotiana spp.	Smoking cessation
Codeine, morphine	*Papaver somniferum L.*	Analgesic, antitussive
Quinine	Cinchona spp.	Antimalarial
Quinidine	Cinchona spp.	Cardiac depressant
Terpenes and steroids		
Artemisinin	*Artemisia annua L.*	Antimalarial
Diosgenin, hecogenin, stigmasterol	Dioscorea spp.	Oral contraceptives hormonal drugs
Taxol and other taxoids	*Taxus brevifolia* Nutt.	Antineoplastic
Glycosides		
Digoxin, digitoxin Sennosides A and B	Digitalis spp. *Cassia angustifolia Vahl*	Cardiotonic Laxative
Others		
Ipecac	*Cephaelis ipecacuanha* (Brot.) A. Rich	Emetic
Podophyllotoxin	*Podophyllum peltatum L.*	Antineoplastic

Table 6: Advantages of transgenic plants as protein expression systems[42]

Cost effective
Able to produce complex proteins
High level of accumulation of proteins in plant tissues
Low risk of contamination with animal; pathogens
Proper folding and assembly of protein complexes
Relatively simple and cheap protein purification
Easy and economical scale up

PLANTIBODIES

Monoclonal antibodies (mAbs) have been critical both for the development of biotechnology itself and as products for both therapeutic and diagnostic purposes. Traditional therapeutic monoclonal antibodies have been derived from mice. These proteins were readily identified by the human immune system as foreign, limiting the utility of these antibodies for therapeutic use, especially with repeated dosing. Even in the absence of anaphylaxis or serum sickness, the occurrence of neutralizing antibodies which inactivate the drug often precluded further therapeutic use. However, recombinant technologies have allowed murine antibodies to be replaced with partially humanized or chimeric antibodies, and now allow the production of fully human antibodies.[43] Currently, there are over a dozen FDA-approved mAbs, and as many as 700 therapeutic Abs may be under development.[44] Plants now have potential as a virtually unlimited source of mAbs, referred to by some as 'plantibodies' (Table 7).

Table 7: Therapeutic and diagnostic plantibodies[40]

Application and specificity	Promoter	Signal sequences	Antibody name or type	Plant	Expression levels
Dental caries; streptococcal antigen I or II	CaMV 35S	Murine IgG signal peptides	Guy's 13 (SIgA)a	*Nicotiana tabacum*	500 µg/g FWb leaves
Diagnostic; anti-human IgG	CaMV 35S	Murine IgG signal peptides	C5-1 (IgG)	Alfalfa	1.0% TSPc
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
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
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	Murine IgG signal peptide KDEL	ScFvT84.66 (ScFv)	Rice	27.0 µg/g leaves
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	TMV Ω leader; murin signal peptides; KDELe IgG;	T84.66 (IgG)	*Nicotiana tabaccum* (transiently with Agrobacterium infiltration)	1.0 µg/g leaves
B-cell l idiotype vaccineymphoma treatment;	TMV subgenomic	Rice α-amylase	38C13 (scFv)	*Nicotiana Benthamiana*	30.0 µg/g leaves

Colon cancer; surface antigen	coat protein promoter TMV subgenomic promoter U5 CP	Murine IgG signal peptide; KDEL	CO17-1A (IgG	*Nicotiana Benthamiana*	Not reported
Herpes simplex virus 2	CaMV 35S	Tobacco extensin signal Peptide	Anti-HSV-2 (IgG)	Soybean	Not reported

aSIgA, secretory IgA. bFW, fresh weight. cTSP, total soluble protein.

Tobacco plants have been used extensively for antibody expression systems. However, several other plants have been used including potatoes, soybeans, alfalfa, rice and corn. Antibody formats can be full-size, Fab fragments, single-chain antibody fragments, bi-specific scFv fragments, membrane anchored scFv, or chimeric antibodies (see Table 2)[2]. Plant cells, unlike mammalian cell expression systems, can express recombinant secretory IgA (sIgA). sIgA is a complex multi-subunit antibody that may be useful in topical immunotherapy, and has been successfully expressed in the tobacco plant. Transgenic soybeans are capable of producing humanized antibodies against herpes simplex virus-2. GE corn reportedly is capable of producing human antibodies at yields of up to a kg per acre,[44] and has been demonstrated to preserve antibody function through five years of storage under ordinary conditions. Antibodies derived from plants have a multitude of applications, including binding to pathogenic CEA, carcino embryonicd with earlier organisms, binding to serum or body fluid effect or proteins (e.g. interleukins), binding to tumor antigens to deliver imaging or anti-tumor agents, or binding to a cellular receptor site to up- or down regulate receptor function. However, plant glycosylation patterns differ from those in mammalian systems, and glycosylation is essential for antibody mediated activation of complement or the initiation of cellular immune responses[43,45]. Plantibodies may carry plant glycolproteins or may be non-glycosylated as a result of genetically deleting glycosylation sites, but are incapable of inducing the latter phenomena in either case[43]. This does not appear to be a major limitation, however, since therapeutic applications of monoclonal antibodies are often mediated by binding and inactivation of proteins or receptor molecules and do not require complement or cell-mediated immunity. While glycosylation sequences are poorly immunogenic and hence unlikely to precipitate immunological adverse reactions,[46] the presence of mammalian glycosylation sequences not required for therapeutic function may only serve to produce undesired complement- or cell-mediated side effects. As of 2001, four antibodies expressed in plants had shown potential to be useful as therapeutics. 3A chimeric secretory IgG/IgA antibody effective against a surface antigen of *Streptococcus mutans* has been expressed in tobacco, and has been demonstrated to be effective against dental caries[47]. Soybeans can express a humanized anti herpes simplex virus (HSV), which has been effective in preventing the transmission of vaginal HSV-2 in animals[48]. Rice and wheat expression systems can produce antibodies against carcino embryonic antigen, which may be useful for *in vivo* tumor imaging[49]. Finally, a plant viral vector has been used to produce a transiently expressed tumor specific vaccine in tobacco for the treatment of lymphoma[50]. Currently, seven plant-derived antibodies have reached the advanced stages of clinical product development[42]. These include products directed at the treatment and/or diagnosis of cancer, dental caries, herpes simplex virus, and respiratory syncytial virus. No 'plantibodies' have currently reached the commercialized stage, although at least one product has been tested clinically, and several have been examined in vitro and in animal systems and appear to be equivalent to mammalian-cell-derived analogues. Given the high levels of production, purification cost, apparent efficacy, and low immunogenicity of recombinant human antibodies derived from plants, plants appear to hold great potential for future production of mAbs[4].

VACCINES

There has been considerable interest in developing low-cost, edible (i.e. oral) vaccines. Tradition al edible vaccines, as for polio, use whole, attenuated organisms or semi-purified materials to induce both systemic (Ig-G-mediated) and local membrane (Ig-A-mediated) immunity. Plant vaccines can express entire selected proteins, but the use of DNA encoding only desired antigenic sequences from pathogenic viruses, bacteria and parasites has received considerable attention.[4] Key immunogenic proteins or antigenic sequences can be synthesized in plant tissues and sub sequently ingested as edible subunit vaccines[51,52,53]. The mucosal immune system can induce protective immune responses against pathogens or toxins, and may also be useful to induce tolerance to ingested or inhaled antigens[51,52]. The production of secretory IgA (sIgA) and provocation of specific immune lymphocytes can occur in mucosal regions, and these regions take on special importance in the development of edible vaccines. Aside from intrinsic low production cost, plant-based vaccines offer a number

of unique advantages, including increased safety, stability, versatility, and efficacy[54]. Plant produced vaccines can be grown locally where needed, avoiding storage and transportation costs. Relevant antigens are naturally stored in plant tissue, and oral vaccines can be effectively administered directly in the food product in which they are grown, eliminating purification costs[51,54]. In many instances, it appears that refrigeration will not be needed to preserve vaccine efficacy, removing a major impediment to international vaccination efforts of the past[51,53]. Plants engineered to express only select antigenic portions of the relevant pathogen may reduce immune toxicity and other adverse effects, and plant-derived vaccines are free of contamination with mammalian viruses. Finally, the development of multi-component vaccines is possible by insertion of multiple genetic elements or through cross-breeding of transgenic lines expressing antigens from various pathogenic organisms. There are, however, some limitations associated with the use of transgenic plants for vaccine production. A major limitation of the expression of recombinant antigens in transgenic plants is obtaining a protein concentration adequate to confer total immunity, given varying protein expression among and within the various plant species. Tight control of expression yields will likely be necessary to reduce variability and assure consistent, effective immunization[55]. During the last decade, nearly a dozen vaccine antigens have been expressed in plants[2]. Transgenic potatoes can produce antigens of enterotoxigenic *E. coli* heat labile enterotoxin B subunit, and is effective in immunizing against viruses and bacteria that cause diarrhoea. Still other 'edible vaccines' are under development for rabies, foot and mouth disease (veterinary), cholera, and autoimmune diabetes. Transgenic lupin and lettuce plants can express hepatitis B surface antigen. Efforts are underway to develop an 'edible vaccine' against the measles virus using the tobacco plant. A plant based oral subunit vaccine for the respiratory syncytial virus (RSV) using either the apple or the tomato is under development[51]. The plant species to be used for the production and delivery of an oral vaccine can be specifically selected to achieve desired goals. A large number of food plants (e.g. alfalfa, apple, asparagus, banana, barley, cabbage, canola, cantaloupe, carrots, cauliflower, cranberry, cucumber, eggplant, flax, grape, kiwi, lettuce, lupins, maize, melon, papaya, pea, peanut, pepper, plum, potato, raspberry, rice, serviceberry, soybean, squash, strawberry, sugar beet, sugarcane, sunflower, sweet potato, tomato, walnut, and wheat) have been transformed [56]. Many of the high volume, high acreage plants such as corn, soybeans, rice, and wheat may offer advantages. Corn, since it is a major component in the diet of the domestic animal, is a good candidate for vaccine production. In humans, particularly infants, the plant of choice to produce the vaccine might be the banana. Bananas are a common component of many infant diets and can be consumed uncooked, thus eliminating the possibility of protein denaturation due to high temperatures. Unfortunately, it is relatively difficult to create transgenic bananas and the production time is longer than for certain other food crops. Cereals and other edible plants are advantageous for vaccine production overplant species such as tobacco because of the lower levels of toxic metabolites. It is evident that there are numerous opportunities to identify and develop low-cost plant derived vaccine materials, including edible plant-based vaccines[4].

CONCLUSIONS AND FUTURE PERSPECTIVES

As our level of understanding of the factors that impact transgene expression in plants improves, we will see improvement in levels of production of target molecules (peptide, proteins, antibodies), decreased costs of production, and greater overall exploitation of plant based production systems. The use of plant derived recombinant molecules for human therapeutic agents will likely not be fruitful without addressing the same regulatory issues that surround the use of any recombinant molecule, namely safety risks versus public benefit. Here, a close cooperation will be necessary between experts in the medical and plant engineering communities to address potential concerns about the purity and safety of plant derived therapeutics and to demonstrate their reliability and cost-effectiveness relative to conventional approaches.

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