

Non-Food GM Crops: New Dawn or False Hope?



Part 1: Drug Production

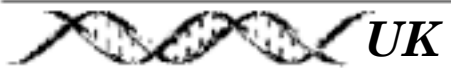
Non-Food GM Crops: New Dawn or False Hope?

Part 1: Drug Production

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Executive Summary

The development of GM crops to produce drugs and vaccines has received considerable investment and is relatively well advanced. The use of GM in this way was referred to in the Prime Minister's Strategy Unit's recent study as an example of the potential benefits of the technology whilst the Science Review pointed to possible risks. This report considers the research which is taking place, its potential to improve health, the possible negative effects and whether GM drug-producing crops are, in fact, likely to offer benefits for the UK biotechnology and farming industries.

Some high-value proteins for use in research and diagnostics are already in commercial production from GM plants. Currently, however, there are no drugs licensed for use that are produced in this way. GM plants are being investigated for the production of:

- vaccines;
- antibodies;
- therapeutic proteins.

Some high-value proteins for use in research and diagnostics are already in commercial production from GM plants

Vaccines

Plants are being genetically modified with genes from disease-causing viruses and bacteria which code for the proteins (known as antigens) that stimulate a protective immune response. It is hoped that the GM plants could then be used either as an edible vaccine, where the vaccine is eaten as part of the plant, or conventionally, where the vaccine is extracted from the GM plant and administered by injection or by mouth.

Human disease vaccines which have been the target of this research include hepatitis B, *E.coli*, rotavirus, Norwalk virus, measles virus and cytomegalovirus using GM potatoes, tobacco, maize, carrots and tomatoes. Animal vaccines include Foot and Mouth disease, Transmissible Gastroenteritis Virus (TGEV) in pigs, goats' plague, rabbit haemorrhagic disease, shipping fever in cattle, and porcine parvovirus.

Research has shown that antigens can be produced in plants and some can stimulate an immune response which can protect against disease. However, the potential for using GM plants as edible vaccines appears to have been exaggerated and it is more likely that they will be used to prepare conventional products. Levels of the antigen in the plant tend to be variable and are often low, and levels of antibody stimulated in the gut or blood stream do not match those following natural infection or conventional vaccination. Questions also remain about whether edible plant vaccines can be formulated to be used reliably and reproducibly. The antigens may be broken down in the stomach, for example, and thus be less effective. The dose and frequency of ingestion to attain the best results has still to be determined.

The potential for using GM plants as edible vaccines appears to have been exaggerated

Antibodies

Antibodies are an important part of the immune system. They are proteins that are produced by the body when it meets a potentially harmful organism or toxin. These proteins are then involved in the different processes that lead to

the organism being killed or the toxin being neutralised. Antibodies are used in diagnosis and therapy of infectious diseases and cancer. A variety of GM plants are being developed to produce antibodies, including tobacco, potato, alfalfa, rice and wheat. The antibodies are being investigated for their potential use in preventing sexually transmitted diseases (through inclusion in vaginal barrier preparations), as contraceptives (using antibodies to human sperm), to produce cancer 'vaccines' (where the antibody recognises the cancer cells and assists the immune system in destroying them), to treat dental caries and as diagnostic aids. One US company, Epicyte, holds a key patent on the production of antibodies in plants.

Therapeutic proteins

The use of GM plants for the production of therapeutic or diagnostic proteins is more advanced than for vaccines or antibodies, with several proteins now being produced commercially by this method in the USA. The company, ProdiGene, now produce avidin, b-glucuronidase and aprotinin commercially from GM maize for use in scientific research. They expect to be producing trypsin commercially in 2003. There is some hope that the plants could eventually be used directly as a way to administer a drug – known as 'food as pill'. As with plant food vaccines, this is unlikely to be successful. Proteins may be partly degraded in the stomach and intestine making it difficult to determine the appropriate dosage.

There does not appear to have been any research which directly considers the possible environmental impacts

Environmental risks

Field trials with GM plants producing pharmaceuticals have taken place in North America, France, Italy and Spain. These trials have been focused on establishing whether the product could be produced in the plant and at what levels. There does not appear to have been any research which directly considers the possible environmental impacts. These include:

- gene transfer to a wild, related plant, which may then behave differently in its ecosystem or be toxic to animals consuming it;
- altered behaviour of the GM plant, causing it to become a weed;
- the drug being toxic in soil or other parts of the ecosystem;
- the genes being transferred to microorganisms in the soil.

Health risks

Any drug produced from a GM plant will have to be tested according to normal protocols for drug development. However, in addition, there are other new issues for human safety:

- The GM plant could be eaten inadvertently and cause harm.
- The introduced genetic material could be transferred to neighbouring crops, which are then eaten.
- The genes may be transferred to microorganisms in the intestine after consumption if the GM plant itself is used to administer the drug.

The outcome of these scenarios is the inadvertent consumption of a biologically active compound. This could be extremely dangerous for the

individual involved, especially infants, people who have an illness, and the elderly. Contamination of a food crop by a GM crop producing an experimental vaccine occurred in the US in 2002. Soybeans were contaminated after being grown in a field used previously to grow GM maize. The maize contained genes to produce an experimental vaccine against a pig disease, Transmissible Gastroenteritis Virus.

Conclusions and recommendations

GM plants can produce drugs and vaccines, but their safety and efficacy remain to be determined. In particular, the hype surrounding edible vaccines and 'food as pill' is misplaced as this is both unrealistic and a potentially dangerous option - it will be difficult to control intake and distribution, particularly in developing countries where education levels and literacy may be low. Ultimately, GM crops will at best provide a different form of manufacture of a protein or vaccine component. Where these replace a protein isolated from an animal or human source, this will have human safety benefits. However, the inadvertent consumption of a drug-producing crop and the potential for gene flow to other crops mean that food crops should not be used.

There has been a dearth of research on the environmental impacts of GM drug-producing crops even though the presence of biologically active compounds in new places could have ecological impacts. If the technology is to proceed, more research is urgently needed.

The production of pharmaceuticals in crops is unlikely to offer much for UK farmers. Small areas under tightly controlled conditions will inevitably be required and thus only a few farmers or landowners will be able to participate should the technology be used commercially.

Key elements of the intellectual property relating to the production of drugs in crops lie in the hands of a few North American companies, including Epicyte and Prodigene, and one French company, Meristem Therapeutics. Therefore, opportunities for UK biotech companies to become involved in the technology are severely restricted.

Whilst the UK Government has emphasised the potential benefits of GM drug crops, it has not undertaken a comprehensive, realistic analysis of costs and benefits. To address the issues identified in this report, GeneWatch UK recommends that:

- Physical containment (in greenhouses) or reliable and proven biological containment (to prevent gene flow via pollen) must be required for the production of therapeutic compounds in GM plants.
- Only non-food crops should be used.
- Research into environmental impacts must be urgently undertaken.
- The Government must review the use of GM crops for drug production, including their safety and likely efficacy in relation to other disease control methods. Its aim should be to produce clear standards by which the industry would be expected to operate.

GM plants can produce drugs and vaccines, but their safety and efficacy remain to be determined

Opportunities for UK biotech companies to become involved in the technology are severely restricted

1. Introduction

The prospect of using GM crops to produce drugs is viewed with excitement by the biotechnology industry. The Prime Minister's Strategy Unit's report on the costs and benefits of GM crops¹ identified the use of GM in this way as a potential future opportunity. Often, such applications of GM crops are used to promote them more widely, particularly the potential for using vaccines from GM crops to tackle diseases in developing countries. Is the hype surrounding GM crops for drug production being used to justify GM food crops more generally?

Of all the non-food GM crops under development, this area has attracted the highest level of investment and is well advanced. Some high-value proteins for use in research and diagnostics are already in commercial production.

The possible advantages of using plants to produce proteins for use as drugs or as diagnostic aids are given as^{2,3}:

- cheaper production than in other systems using fermenters;
- large scale growing, harvesting and processing are technically feasible;
- direct administration as food (e.g. edible vaccines) to avoid costly purification;
- stable storage systems for proteins such as in chloroplasts;
- reduced potential for contamination with human or animal pathogens compared to extraction of proteins from animal or human sources.

However, to reap these benefits it will be necessary to produce enough of the drug in the plant to be economically feasible. The clinical effectiveness and safety of the final drug also has to be demonstrated. Which applications are explored will also depend on who owns the key patents surrounding the technology. This report reviews what chemicals are being produced and what research and development is taking place. It discusses the prospects for the future and the safety issues that will have to be addressed. It considers whether the use of GM in this way will bring benefits to the UK's farming and biotechnology industries that some have envisaged.

Of all the non-food GM crops under development, this area has attracted the highest level of investment and is well advanced

2. Who's involved

There are three main areas where the use of GM plants for the production of pharmaceutical proteins is under development:

- vaccine production;
- antibody production;
- therapeutic proteins.

Much of the research is being carried out by private companies, often in partnership with public universities or institutes. The main companies and their areas of interest are described in Table 1. One feature of GM crops being used for the production of pharmaceuticals is that, with the exception of Monsanto and Dow, the companies involved are very different from those developing GM crops for food use. Most are specialist biotechnology companies, who tend to own the intellectual property rights on certain techniques and genes, and who have links to larger pharmaceutical companies. Epicyte has an unusually comprehensive patent (US 6,417,429) covering the production of antibodies in GM plants and Prodigene has several patents (e.g. US 6,136,320) covering edible vaccines and the production of proteases in plants (e.g. US 6,087,558).

The scope of these patents will mean that others interested in producing such products in plants will have to pay licensing fees to the company holding the patent⁴. There are many licensing and other agreements between companies as seen in Table 1. For example, Japan Tobacco license their transformation technology known as 'PureIntro' to both Meristem Therapeutics and Ventria Bioscience. The Japan Tobacco system claims to allow more accurate genetic modification of monocotyledonous plants (such as rice, maize and barley) using the soil bacterium, *Agrobacterium tumifaciens*⁵. There is an irony that Japan Tobacco's patented technology may ultimately be used to produce drugs to treat certain lung conditions that may be a result of smoking.

Almost all of the leading companies are based in the USA and Meristem Therapeutics, based in France, is currently the only major European company involved. The only current UK involvement in the technology appears to be a Guy's Hospital collaboration with Plant Biotechnology in the USA and some work at the John Innes Institute.

Much of the research is being carried out by private companies, often in partnership with public universities or institutes

Table 1: Companies involved in producing pharmaceuticals in crops

Company	Partners	Applications being developed	Notes
<p>ProdiGene Based in Texas. www.prodigene.com</p>	<p>Spin-off from Pioneer Hybrid. Collaborations/alliances with: · Stauffer Seeds, who market all their 'Identity Containment System' seeds; · Eli Lilly (enzyme production for manufacturing); · Avant Immunotherapeutics to produce recombinant therapeutic compounds; · Large Scale Biology to produce therapeutic antibodies; · Genencor to produce enzymes; · EPlycte to produce antibodies; · NeKtar to produce the artificial sweetener, Brazzein.</p>	<p>Vaccines: research on developing AIDS and TEGV vaccines. Antibody production. Enzymes: · avidin (for research and diagnostics - marketed by Sigma); · β-glucuronidase (for research and diagnostics - marketed by Sigma); · laccase (textiles and pulp and paper); · trypsin (medical uses).</p>	<p>With NIH, are developing a sub unit vaccine in maize that is expected to produce an immune response to simian immunodeficiency virus (SIV): SIV gp120⁶. Commercial production of trypsin expected by end of 2003. U.S. Patents: · 6,136,320 - edible vaccines; · 6,087,558 - production of proteases in plants; · Hepatitis and TEGV vaccine production in plants.</p>
<p>EPlycte Pharmaceutical Based in California. www.epicyte.com</p>	<p>Current/past agreements with: · Dow Chemical and Dow AgroSciences for animal based antibody uses; · Dow and Centocor (Johnson and Johnson subsidiary) for human monoclonal antibodies, including against herpes; · Biovation (part of Merck group) for therapeutic antibodies; · NIH - total grant of over \$1million for viral diseases including human papilloma virus; · ReProtect - contraceptives; · DARPA grant for biological weapons defence research; · Alliance for Microbicide Development - for prevention against sexually transmitted diseases; · Scripps, Cornell, Boyce Thompson, John Hopkins and Oklahoma Universities.</p>	<p>Use maize to produce human antibodies (Plantibodies™). Developing topically applied antibodies - HX8 - or Herpes simplex (in phase 1 clinical trials); as topical contraceptives; for prevention of Respiratory Syncytial Virus and <i>Clostridium difficile</i> diarrhoea.</p>	<p>Epicyte's founding scientists, Dr Andrew Hiatt and Dr Mich Hein, originally developed the Plantibodies™ technology at The Scripps Research Institute (TSRI). In September 1997, TSRI granted Epicyte the exclusive licence to develop Plantibodies™ technology, which includes patents and patent applications covering the expression of any antibody in plants (US 6,417,429). Also, patent US 6,355,235 on the use of antibodies for contraception and use against sexually transmitted diseases.</p>
<p>Large Scale Biology (LSBC) www.lsbcb.com</p>	<p>Formed from Biosource Technologies. Alliances with: · GlaxoSmithKline plc; · The Procter & Gamble Co; · Novartis AG; · Genentech, Inc; · Gemini Genomics plc; · BioSite Diagnostics, Inc; · The Dow Chemical Co; · Dow AgroSciences; · University of Cape Town - AIDS vaccine; · Scottish Crop Research Institute to find new viral vectors.</p>	<p>Personalised non-Hodgkin lymphoma vaccines in phase 1 clinical trials. Therapeutic proteins: Alpha-galactosidase, an enzyme replacement therapy for Fabry's disease.</p>	<p>Use GENEWARE® system - viral vectors modified to produce protein and then infect plant and produce the protein. Vectors are disabled and do not modify the plant itself.</p>

Table 1: Companies involved in producing pharmaceuticals in crops (continued)

Company	Partners	Applications being developed	Notes
<p>Meristem Therapeutics www.meristem-therapeutics.com</p>	<p>Owned by Limagrain.</p> <p>Licensing agreement with:</p> <ul style="list-style-type: none"> · Japan Tobacco for use of plant transformation technology; · Solvay Pharmaceuticals for gastric lipase. <p>Acquired Sedaherb, which supplies plant starter materials to pharmaceutical industry.</p> <p>Alliances with:</p> <ul style="list-style-type: none"> · Quintiles - pharmaceutical industry supplier; · Goodwin Biotechnology for monoclonal antibodies; · Mitsubishi Pharma to produce a therapeutic protein; · Eli Lilly to produce therapeutic compound in tobacco. 	<p>Therapeutic proteins (under Molecular Pharming trademark):</p> <ul style="list-style-type: none"> · dog gastric lipase (for medical use in e.g. cystic fibrosis) in phase 2 clinical trials; · human serum albumin, lactoferrin, collagen. <p>Monoclonal antibodies.</p>	<p>US patent 6,344,600 to produce human haemoglobin in plants.</p>
<p>Crop Tech Based in South Carolina. www.croptech.com</p>	<p>Agreements with:</p> <ul style="list-style-type: none"> · Amgen for therapeutic antibody production; · Immunex for therapeutic protein production; · ToBio (tobacco farming organisation) to develop use of tobacco for therapeutic protein production. 	<p>Use GM tobacco to produce therapeutic proteins such as glucocerebrosidase for Gaucher's disease.</p>	<p>Use MeGA-PharMTM technology (Mechanical Gene Activation-Post-harvest Manufacturing). A control gene, triggered by shredding of the leaf, is linked to the genes that control the production of the therapeutic protein. Therefore, the protein is only produced post harvest.</p>
<p>Planet Biotechnology Based in California. www.planetbiotechnology.com - restricted site access.</p>	<p>Collaborate with Guy's Hospital, London.</p>	<p>Using tobacco to produce antibodies for use against dental caries - in Stage 2 clinical trials.</p>	
<p>Monsanto Protein Technologies Based in Madison, Wisconsin. www.mpt.monsanto.com/asp/default.asp</p>	<p>Via past acquisition of Agracetus.</p>	<p>Using maize to produce high value therapeutic proteins in plants.</p>	<p>cGMP (a human protein important in cellular energy systems) already in production.</p>
<p>Ventria Bioscience (formerly Applied Phytologics Inc) Based in Sacramento, California. www.ventriabio.com</p>	<p>Recent agreement with Japan Tobacco to license plant transformation technology.</p> <p>Other alliances with:</p> <ul style="list-style-type: none"> · Sigma Chemicals; · Procter and Gamble; · Sioux Pharm. 	<p>Therapeutic proteins for humans and animals, using rice and barley to produce human lysozyme, lactoferrin, alpha-1-antitrypsin, thioreductase, fibrinogen.</p>	<p>Investigating the production of lactoferrin in rice for use in infant formulas⁷.</p>
<p>Chlorogen Based in St Louis at Nidus Centre for Scientific Expertise. www.chlorogen.com</p>	<p>Dr Daniell, the founder, has a laboratory at University of Central Florida and all intellectual property is licensed to Chlorogen.</p>	<p>Therapeutic proteins: Using tobacco to produce:</p> <ul style="list-style-type: none"> · human serum albumin; · interferon (insulin-like growth factor). 	<p>Direct production of the protein to the chloroplast (a cell organelle).</p> <p>Dr Daniell, founder of Chlorogen, holds key patents on targeting protein production to the chloroplast.</p>

3. Vaccines

The main centres for GM plant-based vaccine research are in the USA at Loma Linda University in California and the Boyce Thompson Institute for Plant Research in Ithaca, New York. ProdiGene is the main company interested in plant-based vaccines and owns the main patents in the area.

For use in vaccine production, plants are genetically modified with genes from the disease-causing organism which code for the proteins (known as antigens) that stimulate a protective immune response. The GM plants could then be used in one of two ways – either as an edible vaccine, where the vaccine is eaten as part of the plant, or the vaccine component is extracted from the GM plant and used to prepare a conventional vaccine for administration by injection or by mouth. The vaccine could be developed for human or animal use.

Plants are also being used to grow GM plant viruses which are then used as vaccines⁸. A plant virus is modified with antigen genes from the disease-causing organism and a plant is then infected with the GM virus, which multiplies in the plant. The plant is harvested and the GM virus extracted for use as a vaccine. These have been investigated as potential sources of vaccines to prevent parvovirus diseases in mink, dogs and cats⁹, and *Staphylococcus aureus*¹⁰ and *Pseudomonas aeruginosa*¹¹ infections in humans. However, research on this approach appears to have declined in recent years.

One of the major claims for development of edible vaccines in plants is that they would provide a safe, cheap production method, especially for use in developing countries as expensive processing and refrigeration would not be needed and administration would not require needles and syringes. Because the immunity against intestinal diseases is much more effective when a vaccine is given by mouth rather than by injection (the type of immune response is different and more appropriate to how the disease organism is encountered by the body), this would be another advantage.

However, there are obstacles to overcome before GM plants can be simply used as edible vaccines. Whilst orally induced immunity is best, it requires much more antigen than when given by injection – the antigen proteins may be broken down in the stomach or shielded from the immune system by food. Therefore, the GM plant will have to supply sufficient vaccine to be effective. Doses will have to be standardised and the vaccine will have to be produced by a plant which does not have to be cooked before eating as cooking can damage the proteins that make up the vaccine. Whilst storage problems may be less than for refrigerated vaccines, certain conditions will have to be met to ensure that the food does not deteriorate or become contaminated. Repeated, uncontrolled administration could lead to tolerance (where a protein is no longer seen as foreign) and not immunity. The vaccine, even if it is part of a food, would inevitably have to be given under medical supervision.

Table 2 reviews the progress which has been made so far with GM plant vaccines. Firstly, several research groups have shown that it is possible to modify crops - including potatoes^{15,18,23,27}, tobacco^{13,14,21,29}, maize^{19,20}, carrots³⁰ and tomatoes²⁵ - to produce antigens which could be used as a vaccine. Human disease vaccines which have been the target of this research include

Repeated, uncontrolled administration of edible plant vaccines could lead to tolerance and not immunity

hepatitis B^{13,14,15}, *E.coli*^{16,17,20}, rotavirus^{26,27}, Norwalk virus^{22,23}, measles³⁰ and cytomaglovirus²⁹. Much work has also been conducted using a cholera toxin subunit^{24, 25,26,27}. This latter work is not solely directed towards producing a vaccine to prevent cholera - a vaccine based only on the cholera toxin subunit B (CTB) used in these experiments would not give protective immunity because other antigens are also needed to stimulate a protective response. However, CTB acts as a general stimulant of the gut immune response and is being investigated as it could be coupled with other antigens to improve their ability to stimulate a response and be useful in vaccines to protect against a range of diseases¹².

Questions remain about whether edible plant vaccines can be formulated to be used reliably and reproducibly

Animal diseases which have been investigated include Foot and Mouth disease²⁸, which can affect cattle, pigs and sheep; Transmissible Gastroenteritis Virus (TGEV) in pigs^{20,21}; peste des petits ruminants (goats' plague)⁷⁸; rabbit haemorrhagic disease⁷⁹; porcine parvovirus⁸⁰; and shipping fever (pasturellosis) in cattle⁸¹.

The next stage of the research is to determine whether the antigen produced by the plant causes an immune response when eaten by, or injected into, an animal. Many studies have shown that an immune response can be provoked and levels of specific antibodies and antibody-forming cells have been shown to increase in mice and humans. However, although an immune response has been stimulated, this does not mean that it will be of the right type or strength to protect against infection with the disease-causing organism. Therefore, studies are now beginning to determine whether the immunity stimulated by the plant vaccine is protective. It has been shown that mice can be protected against the effects of the cholera toxin subunit B24, rotavirus²⁷ and *E.coli* toxin LT-B19, and piglets have been protected from TGEV20. However, rabbits were not protected from rabbit haemorrhagic disease⁷⁹.

The research conducted to date has demonstrated that antigens can be produced in plants and these can stimulate an immune response which may be protective. However, levels of the antigen in the plant tend to be variable and are often low, and levels of antibody stimulated in the gut or blood stream do not match those from natural infection or vaccination by other methods. Questions remain about whether edible plant vaccines can be formulated to be used reliably and reproducibly. The dose and frequency of ingestion to attain the best results has to be determined.

Environmental and broader public safety questions will influence the decision about which GM plants to use

One of the most important questions will be in which plant to produce a vaccine if it is to be administered orally. Because cooking could damage the vaccine, something which is eaten raw may have advantages, but fruits like tomatoes would not store as well as maize kernels or potato tubers. Tobacco will never be a suitable plant for an edible vaccine because of the many toxins it contains. If tobacco is to be used, the vaccine components will have to be extracted from the plant and processed as for a conventional vaccine. As well as the medical demands of safety and efficacy which will inform the choice of GM plant used, there will also be environmental and broader public safety questions which will influence the decision (see Sections 6 and 7 below).

One of the main arguments for the use of edible plant-based vaccines is that they would be heat stable and therefore suitable for use in developing countries. However, even food needs to be stored carefully and heat stable vaccines do already exist. For example, the heat stable rinderpest vaccine currently used in Sudan and elsewhere has formed an important part of the disease control programme in these areas³¹.

Table 2: Experimental findings with GM plant vaccines

Disease	Crop	Results	Reference
Hepatitis	Tobacco + hepatitis B surface antigen gene.	GM tobacco was shown to produce the hepatitis B surface antigen.	Mason <i>et al</i> (1992) ¹³
Hepatitis	Tobacco + hepatitis B surface antigen gene.	Antigen purified from GM tobacco leaves was shown to produce an immune response in mice when injected into the abdomen.	Thanavala <i>et al</i> (1995) ¹⁴
Hepatitis	Potato + hepatitis B surface antigen (HbsAg) gene.	Mice fed on raw GM potatoes only developed a weak immune response to HbsAg unless they had previously been injected with HbsAg extracted from the GM potato.	Richter <i>et al</i> (2000) ¹⁵
<i>E.coli</i>	Tobacco and potato + <i>E.coli</i> heat-labile enterotoxin (LT-B) gene.	Antibodies produced in mice following consumption of raw GM potato.	Haq <i>et al</i> (1995) ¹⁶
<i>E.coli</i>	Potato + <i>E.coli</i> heat-labile enterotoxin (LT-B) gene.	Humans who ingested raw GM potatoes developed an immune response to LT-B.	Tacket <i>et al</i> (1998) ¹⁷
<i>E.coli</i>	Potato + <i>E.coli</i> heat-labile enterotoxin (LT-B) gene.	Mice fed on raw GM potatoes did not develop an immune response to LT-B unless they had previously been injected with LT-B extracted from the GM potato.	Lauterslager <i>et al</i> (2001) ¹⁸
<i>E. Coli</i>	Maize + LT-B enterotoxin gene.	Mice fed on raw GM maize developed an immune response to LT-B and had reduced severity of LT-B induced diarrhoea.	Chikwamba <i>et al</i> (2002) ¹⁹
<i>E.coli</i> and Porcine Transmissible Gastroenteritis Virus (TGEV)	Maize + LT-B enterotoxin gene or TGEV spike protein gene.	Mice fed on GM maize developed antibodies to LT-B and were protected against LT holotoxin effects. Piglets fed on GM maize showed reduced incidence, duration and severity of TGEV induced diarrhoea.	Streatfield <i>et al</i> (2001) ²⁰
Porcine Transmissible Gastroenteritis Virus (TGEV)	Tobacco + TGEV spike protein gene.	Piglets injected with tobacco leaf extract developed antibodies to TGEV spike protein.	Tuboly <i>et al</i> (2000) ²¹
Norwalk Virus	Tobacco and potatoes + Norwalk virus capsid protein (NVCP) gene.	Tobacco and potato produced NVCP and induced an immune response when fed to mice.	Mason <i>et al</i> (1996) ²²
Norwalk Virus	Potatoes + Norwalk virus capsid protein (NVCP) gene.	Humans who ingested raw GM potatoes developed an immune response to NVCP.	Tacket <i>et al</i> (2000) ²³
Cholera*	Potato + cholera toxin B (CTB) gene.	Mice fed on GM potatoes had reduced duration and severity of cholera toxin induced diarrhoea.	Arakawa <i>et al</i> (1998) ²⁴

Table 2: Experimental findings with GM plant vaccines (continued)

Disease	Crop	Results	Reference
Cholera*	Tomato + cholera toxin B (CTB) gene.	The CTB protein was produced in the GM tomatoes.	Jani <i>et al</i> (2002) ²⁵
Cholera* + rotavirus	Potato + cholera toxin B (CTB) + rotavirus enterotoxin (NSP4) gene.	The CTB-NSP4 fusion protein was produced in the GM potato. The CTB protein is used to improve the immune response.	Arakawa <i>et al</i> (2001) ²⁶
Cholera*, <i>E.coli</i> and rotavirus	Potato + cholera toxin B (CTB) and A2 subunit genes + rotavirus enterotoxin (NSP4) gene + <i>E.coli</i> fimbrial colonisation factor (CFA/1) gene.	Multi-component GM raw potato tissue fed to mice stimulated immune response to CTB, NSP4 and CFA/1. Rotavirus diarrhoea duration and severity were reduced in mouse pups that acquired antibodies from their mothers.	Yu & Langridge (2001) ²⁷
Foot and Mouth Disease (FMD)	<i>Arabidopsis</i> + FMD virus structural protein (VP1) gene.	Plant extracts injected into the abdomen of mice stimulated antibody production and protected against experimental FMD.	Carrillo <i>et al</i> (1998) ²⁸
Human cytomegalovirus (HCMV)	Tobacco + surface glycoprotein, gB, of HCMV expressed in protein storage vesicles in seed.	A promoter and signal sequence from the seed storage protein, glutenin, were used to direct the gB protein production to the seed. The gB protein was stable for several months in seed stored at room temperature.	Wright <i>et al</i> (2001) ²⁹
HIV/AIDS	Maize + simian immunodeficiency virus (SIV) protein gp120 gene.	GM maize produced the SIV gp120 protein.	ProdiGene press release, April 2002 ⁶
Measles virus	Carrots + measles virus H protein.	GM carrots produced the H protein which stimulated an immune response when injected into the abdominal cavity of mice.	Marquet-Blouin <i>et al</i> (2003) ³⁰
Rabbit haemorrhagic disease	Potatoes + virus capsid protein VP60.	GM potatoes produced VP60 protein which induced a weak immune response in rabbits at high doses. This was not protective and 9/10 animals died following challenge infection.	Martin-Alonso <i>et al</i> (2003) ⁷⁹

* The cholera toxin subunit B used in these experiments would not give full immunity to cholera. Other antigens are also needed to stimulate a protective response. However, CTB acts to stimulate the gut immune response and is being investigated as it could be coupled with other antigens to improve their ability to immunise against a range of diseases.

4. Antibodies

Antibodies form an important part of the immune system. They are proteins that are produced by the body when it meets a potentially harmful organism or toxin. These proteins are then involved in the different processes that lead to the organism being killed or the toxins being neutralised.

Antibodies are also now being used in diagnosis and therapy of infectious diseases and cancer. Because they are very specific to an organism or toxin, they can be linked to a molecule which can be easily identified to aid in diagnosis. If given as a therapeutic agent, the antibody helps mark the invading organism (or disease cell when used in cancer therapy), which is then destroyed or inactivated by the host's immune system. In the past, antibodies have been produced largely by GM bacteria or in cell cultures, but plants are thought by some to be a better source because the final shape of the protein is more like the original protein produced by a person³². This means that it may be more effective when used therapeutically.

Antibodies have been produced in a variety of GM plants including tobacco^{33,34}, potato³⁵, alfalfa³⁶, rice and wheat³⁷. They are being investigated for:

- their use in preventing sexually transmitted diseases (through inclusion in vaginal barrier preparations) such as genital herpes³⁴;
- as contraceptives using antibodies to human sperm (see patent US 6,355,235);
- to produce cancer 'vaccines'³³ (where the antibody recognises the cancer cells and assists the immune system in destroying them);
- to treat dental caries³⁸;
- as diagnostics^{36,39}.

As well as demonstrating that antibodies can be produced by GM plants and the various chains making up different immunoglobulins can be assembled in plants⁴⁰, it has also been shown that an antibody against *Streptococcus mutans* (an organism involved in dental caries) produced in plants can prevent colonisation of the mouth by *S.mutans* in humans³⁸. However, because the antibody produced by plants may not be exactly the same as when produced in humans (the way in which sugars are added - glycosylation - varies between bacteria, plants and animals), it is possible that the human immune system may consider a plant-produced antibody to be 'foreign' and produce antibodies to attack it. Studies in mice did, however, show that mouse antibodies produced in plants did not lead to antibody production in mice⁴¹.

Whilst it has been demonstrated that GM plants could provide a source of antibodies for therapeutic and diagnostic use, one important commercial consideration will be whether enough can be produced consistently. It has already been found, for instance, that 'gene silencing' has caused instability in gene expression and decreasing levels of production in later generations of *Arabidopsis* modified to produce antibodies⁴².

In 1990, the cost of producing antibodies in plants was estimated as \$100 per kilogram of product if the antibody was produced in the plant at a level of 1% of total protein⁴³. Levels of antibody production in GM plants vary according to the particular modification and where the antibody accumulates in the plant. Typically, levels of production currently vary from 0.1% to 2% of total soluble

Antibodies have been produced in a variety of GM plants including tobacco, potato, alfalfa, rice and wheat

The human immune system may consider a plant-produced antibody to be 'foreign' and produce antibodies to attack it

The economic viability of producing any drug in a GM plant is not yet clear

protein. The economic viability of producing any drug in a GM plant is not yet clear. Levels may be too low or unstable and the costs of research and development have to be added to the production costs. Other drug production methods using plant cell cultures, GM microorganisms or chemical techniques, for example, may outperform plants economically.

5. Therapeutic proteins

The use of GM plants for the production of therapeutic or diagnostic proteins is more advanced than for vaccines or antibodies and several proteins are being produced commercially by this method in the USA. ProdiGene - together with Stauffer Seeds, who market the seed - now produce avidin⁴⁴, b-glucuronidase⁴⁵ and aprotinin⁴⁶ commercially from GM maize. They hope to be producing trypsin commercially in 2003⁴⁷. Although the products are sold commercially, the area used for growing the plants is currently small. In 2001, it involved seven fields in five US states⁴⁸ and all the avidin production comes from GM maize grown on less than 5 acres of land on one farm⁴⁹. However, areas are expected to increase markedly as production is scaled up.

Most of these compounds are normally extracted from animal products. Aprotinin usually comes from the lungs of cattle and pigs and is used in scientific research on proteins because it inhibits protein breakdown and in surgery to reduce bleeding and promote wound healing. Avidin comes from chickens' eggs and is used in medical diagnostic kits. b-glucuronidase (GUS) comes from bacteria and is used in scientific research. Trypsin comes from cattle pancreas and is used in pharmaceutical production, research, and the leather and detergent industries. Research has shown that GUS and avidin levels remain stable in whole maize kernels for at least 3 months at 10°C and up to 2 weeks at 25°C, with the products being concentrated in the embryo portion of the seed^{50,51}.

Other products being developed by ProdiGene - the market leader and owner of key areas of the intellectual property - include laccase, an enzyme used in fibreboard production and currently isolated from a fungus, and brazzein, a natural sweetener.

Another leading company is Meristem Therapeutics, owned by the French multi-national seed company, Limagrain. Meristem Therapeutics have developed methods using tobacco (where production of the protein takes place in the leaves). However, they intend to use edible plants such as maize and potato because of the toxic compounds that are present in tobacco and the ease of extraction when production can be targeted to potato tubers or maize kernels⁵². They are developing GM plants to produce the following therapeutic proteins:

- dog lipase⁵³ - for use in patients who produce insufficient of this digestive enzyme;
- human collagen⁵⁴ – for use in wound healing;
- human lactoferrin⁵⁵ – for nutritional use or as an antibacterial;
- human haemoglobin⁵⁶ – for use in blood substitutes.

The Canadian company, SemiBioSys, is targeting production of the therapeutic proteins to the parts of the seed which accumulate oil - the seed oil bodies - to maximise production and facilitate extraction. They have directed the production of an anticoagulant, hirudin, from the medical leech, *Hirudo medicinalis*, to the seed oil bodies in oilseed rape through linkage to a protein, olesin, which accumulates naturally in the seed oil bodies⁵⁷. Other researchers, at the University of Calgary, have used the same approach to produce the enzyme, zylanase, used in the pulp and paper industry and as an animal feed additive to aid food breakdown, in GM oilseed rape⁵⁸.

The land used for growing GM plants to produce therapeutic proteins is expected to increase markedly as production is scaled up

A range of other proteins for therapeutic or industrial use have been engineered into plants including:

- human serum albumin in potatoes⁵⁹;
- human lysozyme in rice^{60,61};
- human lactoferrin in potatoes⁶²;
- human growth hormone in tobacco⁶³;
- human granulocyte-macrophage colony stimulating factor in tobacco⁶⁴.

These studies have shown that it is possible to engineer plants to produce and assemble complex human proteins for research and therapeutic use and enzymes for industrial applications which have the appropriate biological activity. Before they are used as pharmaceuticals, there will have to be clinical trials, and the industrial and research enzymes and other proteins are likely to enter the market most quickly. However, it is being suggested that some proteins introduced by GM could be used to 'improve' or fortify foods. For example, lysozyme is a protein which has antibacterial activity and is found in human milk. It has been proposed that the flour or extract from GM lysozyme-producing rice could be used in baby foods⁶¹. Another, more bizarre application is seen in Korean research on GM cucumbers which produce an anti-ageing compound, superoxide dismutase, for use in cosmetics including face packs⁸².

It has been proposed that the flour or extract from GM lysozyme-producing rice could be used in baby foods

It will not necessarily prove straightforward to gain approval for medical uses of proteins from GM plants as the experiences of PPL Therapeutics in Scotland have shown. They produce their proteins in the milk of sheep but have had to find a partner to engage in the expensive clinical trials required. However, Bayer have recently mothballed their agreement with PPL to produce alpha-1 antitrypsin⁶⁵ because of poor performance in early clinical trials. Developing new drugs is a costly and time-consuming business - however they are produced - with many products failing during the testing phase.

Whilst most research is aimed at producing the therapeutic protein in the plant itself, tobacco has also been genetically modified so that it excretes the protein from its roots⁶⁶. The intention would be for the GM plants to be grown in hydroponic systems and the product extracted from the growing medium.

6. Environmental impacts

Field trials with GM plants producing pharmaceuticals have taken place in many countries including North America, France, Italy and Spain (see Tables 3 and 4). Without exception, these trials appear to have been focused on establishing whether the product could be produced in the plant and at what levels. There does not appear to have been any research which directly considers the possible environmental impacts.

Environmental impacts that could arise include:

- gene transfer to a wild, related plant, which may then behave differently in its ecosystem or be toxic to animals consuming it;
- altered behaviour of the GM plant, causing it to become a weed;
- the drug being toxic in soil or other parts of the ecosystem;
- the genes being transferred to microorganisms in the field or in the intestine after consumption.

The extent of gene flow and the behaviour of a GM crop will be affected by the species involved and the type of protein introduced. There may be little experience to draw upon to predict the impacts of new mammalian proteins in plants or the effects on animals consuming them. However, avidin (the first compound to be produced commercially in a GM crop) is toxic to insects as it acts as an 'antivitamin' by making biotin unavailable. This has led to the suggestion that it could be used in this way to protect crops against insect attack⁶⁷. The potential exists for beneficial insect species to be harmed if they feed on the maize or consume others that have done so. It is also possible that birds and small and large mammals could be affected by consuming a drug-producing crop. All consume crops to some degree and they could be exposed to a range of potentially dangerous compounds. If the trait was transferred into wild related species, it could also affect the performance of that wild plant.

Therefore, if such crops were grown on a large scale in the open, the presence of these active compounds in novel ecological settings could have environmental impacts. It is conceivable that these compounds could affect how plants interact with infecting viruses or other environmental stresses. For example, viral disease resistance in GM plants has been achieved by introducing a human gene coding for a protein (double stranded RNA-dependent protein kinase), which is triggered by interferon and gives viral resistance in humans⁶⁸. GM potatoes with genes from moths coding for cationic antimicrobial peptides (which have been proposed for human therapy) showed resistance to some bacterial and fungal pathogens⁶⁹. If such viral resistance was passed to wild relatives, they could become more problematic as weeds as they may be better able to survive natural disease outbreaks.

A report for the Canadian Food Inspection Agency⁷⁰ also discusses how the environment could be exposed to the products of GM drug-producing crops in indirect ways – through the decomposition of residues in soil and runoff into water courses. This will depend on the characteristics of the compound involved and how rapidly it is inactivated or degraded. However, short term changes to soil microflora have been recorded following the decomposition of GM tobacco which has a gene coding for a disease resistance protein⁷¹.

Disturbingly, very little is known about the potential environmental impact of the GM drug-producing crops which are currently in development and there is no indication that such research is being commissioned.

There does not appear to have been any research which directly considers the possible environmental impacts of growing GM drug-producing crops

7. Health impacts

The use of plants to produce biologically active proteins which are often intended for pharmaceutical use inevitably raises questions about human safety. Any drug produced from a GM plant will have to be tested according to normal protocols for drug development. However, in addition, there are three other new issues for human safety:

- The GM plant could be eaten inadvertently and cause harm – it could be toxic or cause an allergic reaction.
- The introduced genetic material could be transferred to neighbouring crops, which are then eaten.
- The genes may be transferred to microorganisms in the intestine after consumption if the GM plant itself is used to administer the drug.

The outcome of both of the first two scenarios is the inadvertent consumption of a biologically active compound. This could be extremely dangerous for the individual involved, especially vulnerable groups such as infants, people who have an illness, and the elderly. The effects could either be acute or not be detected for some time if small quantities are consumed over a prolonged period. Detecting causation may be very difficult.

Disturbingly, contamination of a food crop by a GM pharmaceutical crop in an experimental trial has already occurred. On November 12th 2002 in the USA, the Department of Agriculture (USDA) announced that it had quarantined over \$2.7 million worth of soybeans (500,000 bushels), destined for human consumption, at a Nebraska grain elevator after finding stalks of ProdiGene's GM maize mixed with the soybeans⁷². They later ordered their destruction. The field where the soybeans were grown had been used previously by ProdiGene to grow GM maize which contained genes to produce an experimental vaccine against a pig disease, Transmissible Gastroenteritis Virus (TGEV). The US Food and Drug Administration has fined Prodigene £2 million⁷³.

Horizontal gene transfer from plants to microorganisms in the human intestine is considered to be a very low frequency event, although there is limited research on this subject. The only study with human volunteers that ever appears to have taken place with a GM food was conducted in Newcastle. A single meal including GM soybean was given to seven ileostomists (people who had had an operation which left them without a functioning large bowel) and twelve other volunteers. One apparent incidence of transfer of a transgene into an intestinal bacteria gene was discovered in one of the ileostomists⁷⁴. The significance of this finding has tended to be downplayed despite the small numbers involved and the fact that only one meal of GM soybean was eaten. That gene transfer was detected at all in such circumstances can be seen as surprising and demands further inquiry. If gene transfer were to take place from a drug-producing GM plant and a therapeutic compound began to be produced in the intestines by local bacteria, this could have very harmful consequences. It is one factor which suggests that giving a GM plant as a food to supply a drug may be ill advised.

Any drug produced from a GM plant will have to be tested according to normal protocols for drug development

Contamination of a food crop by a GM pharmaceutical crop in an experimental trial has already occurred

8. Conclusions and recommendations

Research has shown that it is possible to produce complex non-plant proteins that are biologically active in GM plants. Proteins that could form the basis of vaccines, antibodies and other proteins which could be used therapeutically have all been successfully produced. However, many questions still remain. It is unclear whether the system will prove to be successful economically – whether the amount of protein produced will be sufficiently high and can be extracted easily enough and/or, if intended for direct consumption, whether the product will be stable and uniformly expressed.

There are also questions about the efficacy of the product. For example, in the case of vaccines, will they produce a protective immune response? If the product is intended to be 'seed as pill'⁶⁴, will it be reliably effective when taken orally? Many proteins are at least partly degraded in the intestine, which is why most protein-based drugs, such as insulin, have to be given by injection.

In reality, there has been excessive hype about the potential for edible vaccines and other drugs. Clinical trials will be required in the same way as for any other therapeutic product and processing will almost certainly be necessary. Furthermore, the research is targeted at the needs of the developed world, and restrictive intellectual property rights mean that it will only be available at considerable cost. It will therefore be largely inaccessible to the developing world.

The impact on the environment and public safety if other food crops or wild species are contaminated raises further major questions. Is it wise to use food crops to produce therapeutic proteins at all? Should such GM plants be required to have gene containment measures to prevent gene flow?

The potential for inadvertent consumption of a drug in food could lead to very large liabilities for the companies involved. In the USA, new rules are being introduced to reduce the potential for cross pollination and for inadvertent food contamination⁷⁵. For maize, a separation distance of 400 metres has been proposed and the GM crop must be planted two weeks before or after neighbouring maize crops so they are not flowering and fertile at the same time. The Biotechnology Industry Organisation (BIO) in the US has also published a reference document specifying the requirements for confining GM plants which produce pharmaceuticals and an identity preservation system stretching from the seed supplier (who should only deal with such seeds and not food crop seeds) to harvesting and marketing⁷⁶. However, there are questions about whether such rules are practicable or would be followed. Flowering times are not completely predictable and pollen flow distances can change according to the local weather and conditions. It is unlikely that genetic isolation is possible if fertile GM crops are grown on the large scale that will be needed for commercial production. Recognising the practical problems, Monsanto and Dow are reported to have moved their trials to areas where maize is not produced for food use including Arizona, California and Washington State⁷⁷. The special requirements mean that this is a use of GM which will not be relevant to the vast majority of farmers in the UK if the systems are ever applied commercially as they are unlikely to be able to comply with very extensive separation distances. Specialised, dedicated farms would be required.

There has been excessive hype about the potential for edible vaccines and other drugs

The potential for inadvertent consumption of a drug in food could lead to very large liabilities for the companies involved

The Government must review the use of GM crops for drug production, including their safety and likely efficacy

In the light of these findings, GeneWatch UK recommends that:

1. Physical containment (in greenhouses) or reliable and proven biological containment (to prevent gene flow via pollen) must be required for testing and production of therapeutic compounds in GM plants.
2. Only non-food crops should be used.
3. Research on environmental impacts must be undertaken urgently.
4. The Government must review the use of GM crops for drug production, including their safety and likely efficacy in relation to other disease control methods. Its aim should be to produce clear standards by which the industry would be expected to operate.

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Appendix: Field Trials

Table 3: Field trials which have been conducted with plants producing pharmaceutical vaccines or drugs in Europe

Company/Institution	Plant	Product	Country/Year
Biocem SA - Limagrain Group	Tobacco	human alpha-1 anti-trypsin	France 1995
		dog gastric lipase	France 1996
		rabies virus G glycoprotein	
	Maize	collagen	France 1997
		dog gastric lipase	
Seita - Institut du Tabac	Tobacco	rabies virus G glycoprotein cDNA	France 1997
		human lactoferrin	
Meristem Therapeutics*	Maize	putrescine methyl transferase	France 1999
		human albumin	
		human collagen	
	Tobacco	collagen	
R.A.G.T. - Rustica Prograin Génétique	Tobacco	yeast lipase	
Meristem Therapeutics*	Maize	dog gastric lipase	France 2000
		human lactoferrin	France 2001
Istituto Sperimentale per l'Orticoltura	Eggplant	tryptophan-2-monoxygenase	Italy 1997
	Tomato		
	Eggplant	tryptophan-2-monoxygenase	Italy 1999
	Melon		
Istituto Sperimentale per l'Orticoltura - Section of Montanaso Lombardo	Tomato	tryptophan-2-monoxygenase	Italy 2000
PLANTECHNO S.r.l. - Università Cattolica S. Cuore - Facoltà di Agraria - Istituto di Botanica e Genetica Vegetale	Tobacco	human glucocerebrosidase protein	Italy 2002
Biocem SA - Clause Iberica	Tobacco	dog gastric lipase	Spain 1997
		collagen	
Tézier Ibérica sl - Limagrain Group	Tobacco	dog gastric lipase	

* Meristem Therapeutics were a spin-off company formed by Limagrain in 1997.

Table 4: Field trials which have been conducted with plants producing pharmaceutical vaccines or drugs in the USA (CBI = confidential business information - the product and/or source of the gene not disclosed.)

Company/Institution	Organism	Product (Donor Organism)
Agracetus	Maize	Pharmaceutical proteins - CBI
	Soybean	Antibody production Beta-glucuronidase (<i>E.coli</i>)
Applied Phytologics	Barley	Novel protein produced
	Rice	Novel protein produced (CBI) Lactoferrin (human) Lysozyme (human) Laccase (forsythia) Pinorensinol reductase (forsythia) Anti-thrombin (human) Anti-trypsin (human) Albumin (human) AAT (human)
	Wheat	Novel protein produced - CBI
Biosource	Tobacco Etch Virus	Pharmaceutical proteins - CBI
	Tobacco Mosaic Virus	Alpha-amylase (rice) Alpha-haemoglobin (rice) Beta-haemoglobin (human) Trichosanthin (<i>Trichosanthes kirilowii</i>)
	Tobacco	Pharmaceutical proteins - CBI
Chlorogen	Tobacco	Pharmaceutical proteins - CBI
CropTech	Tobacco	Pharmaceutical proteins - CBI
Dow	Maize	Pharmaceutical proteins - CBI
Hawaii Agriculture Research Centre	Sugarcane	CBI (human)
Horan Bros. Agri. Enterprises	Maize	CBI
Iowa State University	Maize	Vaccines: · Enterotoxin subunit B (<i>E.coli</i>) Drugs: · Cecropin - CBI
	Oilseed rape	Pharmaceutical proteins - CBI
Meristem Therapeutics	Maize	Pharmaceutical proteins - CBI (human)
Noble Foundation	Alfalfa	Vaccine: · Cholera toxin B (<i>Vibrio cholera</i>)
ProdiGene	Maize	Proteins: · Aprotinin (pig) · Laccase (turkey tails) · Aprotinin (<i>Bos taurus</i>) Vaccines: · Surface antigen (Hepatitis virus B) · Surface antigen (TGEV) · gp120 (glycoprotein 120) (simian immuno-deficiency virus) · Enterotoxin subunit B (<i>E.coli</i>)
R J Reynolds	Tobacco Mosaic Virus	CBI
University of Kentucky	Tobacco	CBI CBI (human) CBI (mouse)
Washington State University	Barley	Amylase (barley) Antithrombin (human) Antitrypsin (human) Lactoferrin (human) Lysozyme (human) Serum albumin (human)

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Non-Food GM Crops: New Dawn or False Hope?

Part 1: Drug Production

**Part 2: Grasses, Flowers, Trees, Fibre Crops
and Industrial Uses**