

Plant-made Pharmaceuticals



The use of genetically modified (GM) plants to produce pharmaceutical drugs and vaccines is an emerging technology that offers a low-cost, large-scale alternative to current methods. This POSTnote looks at recent advances in, and the benefits of, the technology, and analyses the associated biosafety and regulatory issues.

Therapeutic Proteins

Proteins are large molecules, composed of long chains of subunits called amino acids. Proteins play a critical role in cell biology and are widely used in research and medicine for diagnostic, therapeutic and preventative applications. Because of their biological importance, many pharmaceutical drugs and vaccines are proteins or are derived from proteins. These include antibodies, viral or bacterial proteins (used mainly in vaccines) and human proteins (such as insulin). Pharmaceutical proteins are currently manufactured using biotechnology.

A simple, inexpensive system for large scale production of therapeutic proteins could have a significant impact on public health. For instance, in 2010, the World Health Organisation estimated that routine vaccination could prevent 2 million deaths in children under 5.¹ Furthermore, 347 million people worldwide have diabetes and diabetes-related deaths are projected to rise by two thirds by 2030,² increasing the global demand for insulin.

Plant-made Pharmaceuticals (PMPs)

'Biopharming' involves the use of genetically modified (GM) plants or plant cells to produce proteins of therapeutic value. It combines the disciplines of biopharmaceutical production with molecular genetics for agricultural biotechnology. Genes that encode high value pharmaceutical proteins are inserted into crops or plant cells, which are grown on a large

Overview

- The use of genetically modified (GM) plants to produce pharmaceutical proteins is known as 'molecular pharming' or 'biopharming'.
- Plants grown in fields, greenhouses or plant cell cultures grown in bioreactors can be used to produce protein drugs or vaccines.
- The cultivation of pharmaceutical GM crops may pose risks such as the contamination of the food or feed chain.
- A number of methods (such as growing crops in containment) can be employed to limit the risks but these can increase the cost of production.
- Current EU regulations for GM crops may affect this technology's development.

scale and the protein product is extracted and purified from the plant tissue. Biopharming is an alternative method for producing biotechnology-derived drugs and has advantages over current systems.

Cell-based protein production

Biotechnology-derived protein drugs are currently manufactured using microbial, insect or mammalian cell cultures grown in bioreactors (Box 1). Such methods can be complicated, costly and can pose some safety risks.³

In May 2012, the first PMP was approved for human use by the US Food and Drug Administration. The drug is a protein used in enzyme-replacement therapy to treat the symptoms

Box 1. Biotechnology-derived Drug Production

Many pharmaceutical drugs are relatively small molecules that can be produced by chemical synthesis. The most common way to produce large, complex molecules such as therapeutic proteins is through biotechnology. This involves insertion of a gene, which encodes a protein of interest, into a host organism. The host organism is grown to produce large quantities of the protein, which is subsequently purified. There are a number of production systems/host organisms which vary in their suitability and efficacy.

Simpler production systems, which use bacteria or yeast, are low-cost and can generate high yields of protein. However, they are unable to make complex human proteins such as antibodies. More sophisticated production systems such as insect or mammalian cell lines can produce complex human proteins, but are more expensive and can become susceptible to contamination with human viruses.

of the rare genetic condition, Gaucher's disease. It was developed by Protalix (an Israeli company) by inserting a gene encoding the protein into carrot cells, which were grown in contained bioreactors. By using a plant-based production system the drug can be produced for 25% less than its competitors, which use a mammalian system.⁴

Plant cell cultures have also been used to produce a vaccine against Newcastle disease virus (a major poultry pathogen) by Dow AgroSciences (USA),¹⁴ although this product was never marketed. Other plant cell-based approaches include using moss and algae grown in contained bioreactors. Biopharmaceuticals (including PMPs) made using cell culture are subject to EU regulations on medicinal products and contained use (Box 5, page 4).

Plant-based Protein Production

Researchers have also developed whole plant-based production systems for biopharmaceuticals. Plants have been a source of drugs and useful molecules for millennia but only since the advent of biotechnology has it been possible to use plants to produce proteins that are found elsewhere in nature. The first pharmaceutical protein made in plants was human growth hormone, which was produced in transgenic tobacco in 1986.⁸ In 1989, the first antibody was produced in tobacco⁹ and in 1992 plants were used to produce an experimental Hepatitis B vaccine.¹⁰ This research demonstrated that plants can produce complex proteins that could be used to treat and prevent a variety of diseases. While there are currently no whole plant-derived PMPs commercially available, there are a number at various stages of development (Box 2).

The systems under development vary in their choice of plant species, growth conditions and nature of genetic modification.³ A broad range of plant species has been studied and tested for suitability for biopharming:

- **Tobacco** is a leafy, non-food crop with a high biomass yield (for high protein production) and most advanced procedures for PMP production. Large-scale protein production in contained greenhouses is a viable option for this crop.
- **Maize** has the largest biomass yield for a cereal, is simple to genetically manipulate, and easy to scale up in the field.
- **Safflower** is an oilseed crop that is self-pollinating, scalable and genetically tractable. It is only a minor crop in USA and Canada and is not cultivated in Europe.⁵

Box 2. Product Applications for PMPs

Therapeutic proteins are used as both treatment and prevention for a diverse range of diseases. Examples of current applications of PMPs in development include the following:

- **Vaccines.** Medicago (Canada) has engineered tobacco plants, using transient technology, to produce influenza vaccines, which can be manufactured in a short time-frame in response to an epidemic.
- **Antibodies.** The Pharma-Planta project (EU) has engineered tobacco to produce anti-HIV antibodies, which could be used as an active component in a topical anti-HIV microbicide cream.
- **Therapeutic proteins.** Sembiosys (Canada) developed insulin and ApoAI (cholesterol-reducing therapy) production in safflower.

- **Rice** is similar to maize in potential for scalability and genetic tractability but has higher production prices. It carries a lower biosafety risk because it is self-pollinating, making it less likely to cross with related plant species.

Systems that use whole plants can be cultivated in:

- **Greenhouses**, in which case the process is subjected to 'contained use' regulation as well as biopharmaceutical regulation (Box 5, page 4).
- **Open fields**, using conventional agricultural practices. In this case the process is subject to 'environmental release' regulation in addition to biopharmaceutical regulation (Box 5, page 4).

Pros and Cons of Plant-based Systems

The PMP production platform must be selected on a case-by-case basis, depending on the nature of the protein, the required speed, scale and purity of production, and the environmental and biosafety considerations.⁷ The choice of production system often involves a compromise: the cheapest system may increase biosafety risk but the safest system may not be cost-effective.

Cells and Whole Plants

Economics

One of the main challenges of synthesising complex biotechnology-derived proteins is producing them on a large scale at a low cost. Plant cell cultures can be used to produce complex human proteins and are often cheaper to maintain than mammalian cells because they require less sophisticated growth media.¹¹ Both plant and mammalian cell culture systems are limited by the capacity of bioreactors, which are less suitable for large-scale production of biopharmaceuticals. In contrast, cultivation of whole plants in greenhouses or open fields is relatively 'low-tech' and requires comparatively little resource input or capital investment.

The inherent low-cost and scalability of using whole plants makes biopharming a convenient alternative for the production of proteins that have a global demand exceeding 10 tonnes per year.⁵ For instance, it is estimated that the entire global demand for insulin could be supplied by 15,000 acres of biopharmed crops.²⁸ Therefore, the use of farmland for PMP production is unlikely to have a detrimental impact on agricultural food production (see the 'Food vs fuel' debate, POSTnote 410)³³.

Developing Country Perspective

The low cost of a plant-based system makes this technology particularly applicable to developing countries. The issue of how to deliver perishable drugs and vaccines requiring refrigeration to remote areas with poor roads and storage systems is a major obstacle to the treatment of many diseases associated with developing countries (e.g. rabies). Establishing PMP production facilities in close proximity to populations in need of them could limit the requirement for a 'cold chain' to preserve the drug between the point of production and point of delivery. In 2009, Kentucky Bioprocessing (USA) signed an agreement with the South

African-based Council for Scientific and Industrial Research to develop a commercial plant-derived anti-rabies antibody for use in post-exposure prophylaxis.³¹

Safety of Production Systems

A further advantage of plant-based systems is that they do not support the growth of human pathogens such as bacteria or viruses. Such pathogens do grow in mammalian and microbial systems and can be harmful unless removed during purification. Plant systems can harbour plant pathogens but these are not thought to be harmful to humans.¹²

Risks of PMP production

Despite the benefits of PMPs there are a number of challenges that have so far hindered their commercialisation in Europe. The establishment of good manufacturing procedures and risk assessment within an appropriate regulatory framework is essential to reduce the risks associated with their production.¹⁴ Such risks include contamination of the food chain and the transfer of genes to related plants.

Contamination of the Food/feed Chain

Unlike GM crops intended for use as food or feed, biopharming crops are engineered to produce high levels of biologically active, potentially toxic compounds. Depending on the nature of the pharmaceutical, small amounts of these compounds may harm people or animals that inadvertently consume them. Such a scenario can occur if biopharming crops or seeds are accidentally mixed with food/feed crops. The Prodigene case (Box 3) is an example of where a biopharming crop (maize) contaminated a food crop (soybean) grown in the same plot the following season.

Gene Transfer

Cultivation of GM field crops also poses the risk of transferring genes for pharmaceutical proteins to non-GM crops. Gene transfer into non-GM food crops increases the risk of contaminating food/feed chains. Issues about growing GM crops in the EU are discussed in POSTnote 386, GM Crops and Food Security.²⁷

Box 3. Prodigene Case- A 'Zero Tolerance' Policy

In 2002, the US biotechnology company ProdiGene developed a veterinary vaccine in GM maize. Following a standard crop rotation practice, farmers planted conventional soybeans on land previously used to test Prodigene's GM maize. As a result, maize seed left from the GM crop grew in the soybean fields. In this case, Prodigene was fined \$250,000 and instructed to carry out a \$3million clean-up operation. As part of the cleanup more than half a million bushels of soybeans were bought and destroyed. The penalty issued was the maximum possible, and reflected a 'zero tolerance' policy to the perceived risk associated with consumption of a pharmaceutical product. However, it was never demonstrated that the maize plants found in the soy bean crop were transgenic or that they produced viable seed containing the vaccine. The US regulatory authorities are moving towards a tiered regulatory approach that better reflects the actual risks posed.¹⁶

Addressing the Risks

Use of Cell Culture

The use of transgenic plant cell cultures to produce PMPs increases the safety of biopharming.¹¹ Production takes place in a closed bioreactor and does not use whole plants, eliminating the risks associated with food chain contamination. However, this technology remains limited to only a few commonly used plant cell lines and cannot be implemented on the same scale as whole plants.

Choice of Crop

Food crop genetics are well understood and their cultivation practices are long established. However, the use of food crops to produce PMPs carries a risk of contamination of the food chain. In addition, the risk of gene transfer from the biopharming crop to a farmed crop of the same (or closely-related) species is increased by using food crops. The use of non-food crops, such as tobacco, drastically reduces the risk of accidental contamination.¹⁵

Growth of Plants in Containment

Growing plants in contained physical structures, such as greenhouses, plastic tunnels, laboratories or underground facilities,¹⁷ are examples of preventative measures to avoid unwanted exposure of PMP crops to the food chain or the environment. Growing, harvesting and purifying the pharmaceutical product in a closed system decreases the likelihood of accidental contamination of the food chain or gene transfer to native plant species. However, the requirement to build containment structures increases the cost of production. A number of other complementary containment options that can be employed to limit risk of environmental exposure are outlined in Box 4.

Stable and Transient Systems

Stable production systems involve permanently inserting genes into the plant's genetic make-up and the traits are passed onto the next plant generation. However, there are also transient production systems where the genes are not inserted into the plant's heritable genetic make-up. Instead, a plant virus can be introduced into the plant, which 'hijacks' the plant's biological machinery and produces large amounts of the desired pharmaceutical protein. Transient

Box 4. Complementary Methods to Limit Biosafety Risk of Biopharming

A number of containment methods, some of which should be used in combination, can be employed to limit the risk associated with cultivation of GM crops.

Physical methods:

- **Physical containment**, such as greenhouse facilities.⁷
- **Spatial containment** involves using dedicated land for PMP crops in locations where no-similar crops are grown and the use of buffer zones.

Biological methods:

- **Biological confinements**, such as male sterility, prevents gene transfer to other plants.¹⁵
- **Targeted expression** of the protein to specific plant parts such as roots¹⁸, seeds¹⁹ or edible parts²⁰ aids harvesting and can reduce the likelihood of unintended exposure.
- **Temporal confinement** involves engineering the plant to produce the protein only at certain points in the plant's life cycle, such as after it has been harvested.²¹

production systems have the potential for much higher protein yields than stable genetic systems.²² Transient systems potentially avoid the issue of field release as high protein yields can be obtained from relatively little plant material, which can be grown in large greenhouses.

Regulatory Issues

Public attitudes to PMPs are predominantly positive, but there are concerns about the use of food crops, open-field cultivation and how PMP production is regulated.³² Each production method is associated with a certain degree of risk, which in turn incurs a particular series of regulatory requirements to adhere to: the greater the risk the more stringent the regulatory requirements.

Regulation of Biopharmaceuticals

The relevant EU regulatory framework (Box 5) predated this technology and as a result there was uncertainty as to how current directives should be applied to PMP production. For example, the current regulations for biotechnology-derived drugs were developed for cell-based production systems and can be difficult to follow when applied to whole plants. Cell-based systems are sterile environments with precisely controlled growth conditions. In contrast, whole plants are non-sterile and their variable environment can lead to batch-to-batch variation.¹⁴ In an effort to address this, the European Medicines Agency published guidance to achieve satisfactory quality and safety for PMP products.²³

Environmental Release

The 'environmental release' of field grown biopharming crops presents a slightly different challenge. In 2009, the European Food Safety Authority (EFSA) published guidance on the risk assessment issues associated with GM plants

Box 5. Regulation of PMP Production in the European Union

PMPs present two major challenges for regulatory bodies. It presents regulators of biotechnology drugs with a novel drug-production concept and regulators of agricultural biotechnology with a novel type of crop use.

- **Regulation of biopharmaceuticals.** Biotechnology-derived pharmaceutical drugs are regulated by the European Medicines Agency and the Medicines and Healthcare products Regulatory Agency, in the UK. Pharmaceuticals from GM plants must adhere to the same regulation that covers all biotechnology-derived drugs: Directive 2001/83/EC and Regulation (EC) No 726/2004.
- **Regulation of PMP crops grown in containment.** The production of PMPs via whole plants in greenhouses or cell culture in bioreactors is regulated by the 'Contained Use' Directive 2009/41/EC. These regulations are far less stringent than an environmental release and do not necessitate a fully-fledged environmental risk assessment. Essentially, the site of manufacture is licensed for contained use and production proceeds in a similar nature to a conventional biotechnology production facility.
- **Regulation for cultivating of PMP crops.** Cultivation of GM field plants constitutes an 'environmental release' and is regulated by the European Commission under Directive 2001/18/EC and 1829/2003/EC if the crop can be used as food/feed. For example, the amflora (starch) potato was an industrial use crop not destined for the food chain, but because potato is a food crop it was assessed and deemed safe as food/feed. It is less clear what path an application for a tobacco-derived PMP would take. Decisions on field release are advised by the European Food Safety Authority (EFSA).

used for non-food/feed purposes.²⁴ However, only two GM agricultural crops have gained market approval in the EU in the last 14 years (see POSTnote 386)²⁷. The application procedure can cost €7-10million and can take several years²⁶, because of the large number of safety studies the applicant must present to EFSA. EU member states which oppose the field release can request further evidence of the safety of crops' exposure to the environment, which can delay approval. The large cost and time investment in gaining approval for a field release offsets any economic benefit of biopharmaceutical production by field crops, which limits incentives for commercial investment.

The Effect of Regulation on Investment

Although PMP production is possible through the existing regulatory framework the challenges of bringing unfamiliar products through the established regulation have so far deterred commercial investment in the industry. The products approaching commercial viability will road test the existing regulatory framework and may help reduce regulatory uncertainties. So far, this challenge has mostly been undertaken by publicly-funded academic research programs (Box 6).

Prospects for the PMP industry

Public sector R&D activities into PMPs are of a comparable scale in the EU and North America. However, most EU companies began PMP research several years after their North American counterparts so are in earlier stages of R&D.⁵ Partly because of the regulatory requirements associated with field crop release and the drawbacks of using food crops (Box 5), EU companies have adopted a stronger research interest on non-food crops and contained systems. Only companies aiming for large volume (and/or low cost) products are likely to consider open field-based production.

The UK has a strong life sciences (including agricultural and pharmaceutical biotechnology) industry.²⁹ However, the problem of bridging the gap between academic research and commercial application is ongoing. The Science and Technology Select Committee is conducting an inquiry into how Government and other organisations can improve the commercialisation of research.³⁰ The Committee intends to publish its findings in early 2013.

Box 6. The Pharma-Planta Project

In 2004, the Pharma-Planta Consortium was set up to establish a plant-based production platform for pharmaceuticals in Europe. It ran from 2004-2011 and was comprised of 30 academic and industry partners, funded by the EU sixth Framework Program. One of its aims was to work alongside regulatory authorities to establish good manufacturing procedure and risk assessment, based on health and environmental impact, for the manufacture of pharmaceutical proteins in plants. The project culminated in a Phase I clinical trial of a tobacco-derived antibody, grown in containment, which neutralises HIV. This proof of principle program laid out a manufacturing template for other organisations to follow.²⁵

Endnotes

For references, please see:

http://www.parliament.uk/documents/post/postpn424_plant-made-pharmaceuticalsreferences.pdf