

# Pharma-Planta: Road testing the developing regulatory guidelines for plant-made pharmaceuticals

Penelope Amelia Claire Sparrow · Judith A. Irwin ·  
Phil J. Dale · Richard M. Twyman · Julian K. C. Ma

Received: 7 November 2006 / Accepted: 11 January 2007 / Published online: 7 February 2007  
© Springer Science+Business Media B.V. 2007

**Abstract** Significant advances over the last few years have seen plant-made pharmaceuticals (PMPs) move from the exploratory research phase towards clinical trials, with the first commercial products for human use expected to reach the market by 2009. Europe has yet to witness the commercial application of PMP technology, although at least one product has begun phase II clinical trials with others following close behind. These emerging products are set to challenge the complex and overlapping regulations that currently govern GM plants and ‘conventional’ pharmaceutical production. The areas of responsibility are being mapped out between the different EU regulatory agencies, with specific guidelines currently being drawn up for the

regulation of PMPs. This article discusses issues surrounding the development of robust risk-assessment and risk-management practices based on health and environmental impact, while working with EU regulatory authorities to ensure appropriate regulatory oversight.

**Keywords** Pharma-planta · PMP · Plant made pharmaceuticals · Biosafety · Regulations

## Introduction

The use of plants to produce pharmaceutical and industrial proteins is likely to be the next major commercial development in biotechnology. Plants offer a number of benefits over conventional mammalian or bacterial cell culture systems (Twyman et al. 2005). These include lower start-up costs, increased flexibility in terms of scale and storage, and the potential to produce high product volumes at relatively low cost. For some high-demand pharmaceutical products, particularly those desperately needed in developing countries, plant production may be the only viable option to ensure lower costs and wider availability.

In January 2006 the first registration for a plant-derived vaccine was granted to Dow AgroSciences by the USDA Centre for Veterinary Biologics (<http://www.dowagro.com/animal-health/>). The product was a poultry vaccine

---

P. A. C. Sparrow (✉) · J. A. Irwin  
John Innes Centre, Norwich Research Park, Norwich  
NR4 7UH, UK  
e-mail: penelope.sparrow@bbsrc.ac.uk

P. J. Dale  
Emeritus Fellow, John Innes Centre, Norwich  
Research Park, Norwich NR4 7UH, UK

R. M. Twyman  
Department of Biology, University of York,  
Heslington, York YO10 5DD, UK

J. K. C. Ma  
St George’s Hospital Medical School, University of  
London, London SW17 0RE, UK

against Newcastle disease, produced in tobacco cells within sealed and sterile containers. This was a milestone for the industry as it validated the safe use of genetically modified plant cells to produce therapeutic proteins. In April 2006, Cuba's Biotechnology and Genetic Engineering Center (CIGB) won approval from Cuba's Medication Quality Control Agency to produce the monoclonal antibody CB-Hep.1 in tobacco plants ([www.cigb.edu.cu](http://www.cigb.edu.cu)). This monoclonal antibody is used in the manufacturing process for a Hepatitis B vaccine. This product represents the first commercial use of whole plants as a production vehicle for reagents used in a clinical manufacturing process, and moves away from the bioreactor conditions that may be more familiar to the current pharmaceutical industry and regulators.

Thus far there has been no commercial application of PMP technology in Europe, although several products have reached the clinical trials stage. These include gastric lipase and lactoferrin, produced in maize by Meristem Therapeutics ([www.meristem-therapeutics.com](http://www.meristem-therapeutics.com)), and human intrinsic factor produced in *Arabidopsis thaliana* by Cobento Biotech ([www.cobento.dk](http://www.cobento.dk)). The regulation of these crops currently falls under the authority of a number of different bodies, and to some extent their involvement will depend on a variety of points including the choice of host plant and where it is grown, the choice of product, the final formulation and target population.

## Pharma-Planta

Pharma-Planta ([www.pharma-planta.org](http://www.pharma-planta.org)) is an EU-funded academic research consortium established in 2004 to address a number of concerns surrounding the use of plants as production platforms for pharmaceuticals. The consortium's objectives are:

1. To build a plant-based production platform for pharmaceuticals relevant to the European market, and to help the development of appropriate regulatory oversight in the EU.
2. To produce recombinant antibodies in transgenic plants, that will be developed through regulatory requirements, Good Manufactur-

ing Practice (GMP), pre-clinical toxicity testing and phase I human clinical trials in Europe.

3. To demonstrate a practical commitment to the humanitarian use of PMPs and the associated production technology in developing countries. This has been achieved by establishing a PMP licensing strategy for humanitarian purposes and instituting a novel, consortium-wide Statement of Intent on the use of Pharma-Planta intellectual property for humanitarian purposes in developing countries.

A Pharma-Planta sub-group responsible for the analysis of biosafety issues carried out a consultation exercise to define the latest thinking on the pros and cons of different plant species for the production of recombinant pharmaceuticals, and the latest ideas on appropriate international regulatory oversight. Several reviews have been published on this topic recently (Commandeur et al. 2003; Mascia and Flavell 2004; Petersen and Arntzen 2004; Ma et al. 2005a, b). The aim of this paper is not to reproduce these earlier articles, but rather to focus on wider, generic implications that are likely to be of interest to all parties considering the production of recombinant pharmaceuticals in plants. The choice of product, production hosts, and the environmental conditions for plant growth are discussed. For the industry, choosing the right product will be essential to ensure the success and regulatory acceptance of this emerging technology.

## What are the issues?

To produce PMPs successfully, many technical and regulatory factors must be addressed (Horn et al. 2004; Ma et al. 2003). These factors include whether or not the desired product can be synthesized, assembled and stored in the host plants and subsequently extracted efficiently (Gomord 2004; Tekoah 2004). In addition, the choice of production host, the achievable yield of pharmaceutical product per hectare, and the cost of inputs, harvesting, transport and processing must also be taken into consideration (Fischer et al. 2004; Stoger et al. 2005; Giddings et al. 2000). From a regulatory perspective, the production system must comply not only with the

strict regulatory requirements covering other GM crops, such as those laid down by the 2001/18 EU regulations and the USDA/APHIS permit application policies for the USA (for field grown plants), but also the regulations set out by agencies that oversee the production of pharmaceuticals. Draft documents addressing quality aspects of the production of medicinal products in GM plants were published in 2002 by both the FDA (US Food and Drug Administration, URL: <http://www.fda.gov/cber/gdlns/bioplant.pdf>) and EMEA (The European Agency for the Evaluation of Medicinal Products, URL: <http://www.emea.eu.int/pdfs/human/bwp/076402en.pdf>).

One of the most important determinants of commercial viability is the ability to achieve adequate expression and accumulation of the recombinant protein in plants. This is a critical step towards achieving the advantage of agricultural scalability that plants provide (Ma et al. 2003; Twyman et al. 2003; Hood et al. 2002). Absolute yields are partly affected by the plant species used for production, but the choice of production crop is influenced by many other factors. The principal questions addressed in the consultation were: what are the advantages and disadvantages of different crop production systems; and what are the critical biosafety and regulatory issues?

### Choice of production system

The biology of the production host needs to be considered from both the production perspective and according to how it impacts on the environment, food safety and human health. It is unlikely that any single plant species will satisfy all of the criteria required. There are sometimes conflicting considerations that need to be balanced in order to select the best species for a particular application.

Three ‘classes’ of plant species could potentially be used: non-cultivated species, non-food crops and food crops.

#### (i) Non-cultivated species

Non-cultivated species are essentially wild plants that are not generally cultivated by man. Like

non-food crops, they have the advantage that they are not part of the human food chain. On the other hand, less is known about the genetics and biology of such species, including whether or not they produce toxins, and their potential for outcrossing. Little, if any, work will have been done to domesticate such species in order to make field cultivation more feasible. This lack of domestication often means low potential seed yields, and as a result it is more likely that leaf material will be the harvestable target tissue.

It is possible that non-domesticated crops could be grown in containment, or in bioreactors. Biolex (<http://www.biolex.com/>), who acquired Lemnagene (<http://www.lemnagene.com>) in 2005, report good scale-up ability in duckweed (*Lemna minor*). The cultivation of this species in containment can also be seen as an advantage.

The domestication of a currently non-cultivated species is unlikely to be practicable within the near future. In the long term, it may be feasible to develop new ‘pharmaceutical crop’ species, but the relevant methodology should be evaluated in parallel with investigations to exploit current domesticated species rather than instead of them.

#### (ii) Non-food crops

The main advantage of non-food crops is that, although they have been developed and bred as crops, they are not used for food or feed. Consequently, it should be relatively easy to keep them separate from crop products used in the human or animal food chain. The main species being considered in the non-food category are tobacco and falseflax. Tobacco is a strong candidate for the commercial production of recombinant proteins since it already has a track record in PMP research (Stoger et al. 2002) and has recently been used in Cuba for the commercial production of a recombinant antibody against hepatitis B (Ramirez et al. 2003; Valdés et al. 2003; Pujol et al. 2005). The major advantages of tobacco include efficient, well-established methods of gene transfer and expression, high biomass yields (due to multiple cropping ability), plentiful seed production and a

well-established infrastructure for large-scale processing. It is unlikely that tobacco material would inadvertently mix with material destined for the human or animal food chain, unless it is grown in rotation with a food crop (*cf.* the ProdiGene maize incident, discussed later). The further development of strict Good Agricultural Practice should overcome these issues.

Many cultivars of tobacco produce high levels of toxic alkaloids (which need to be removed during downstream processing) but there are low-alkaloid varieties that could be exploited for pharmaceutical production (Fischer and Emans 2000; Ma et al. 2003). Alternatively these alkaloids are reportedly not present in cell suspension cultures, which could also be used to produce recombinant proteins (Doran, 2000; Hellwig et al. 2004), although not on the scale required for the antibodies chosen as target molecules in the Pharma-Planta programme. Another possibility is the targeting of proteins to the secretory pathway so that they are exuded from the roots or leaves (Drake et al. 2003; Kormarnytsky et al. 2000; Borisjuk et al. 1999). Tobacco also contains phenolic substances that are released during grinding and protein extraction, which can interfere with downstream processing. However, developments in downstream processing, such as the use of smart membranes during the clarification and capture stages, will potentially enable manufacturers to target and eliminate these undesirable molecules, making them no more a problem than the removal of any other protein in the purification process.

Falseflax (*Camelina sativa*) is being developed by the Finnish Biotech Company UniCrop ([www.unicrop.fi](http://www.unicrop.fi)) to produce recombinant proteins for the pharmaceutical industry. In this instance, transgenic seedlings are grown in air-lift bioreactors under full containment and protein is extracted from the soft sprout material, therefore avoiding the need to remove fibres and oil during downstream processing.

### (iii) Food crops

A wealth of knowledge surrounds the cultivation of food crops and transformation procedures are

also well defined for a number of major crop species. The use of plants with GRAS (Generally Recognised as Safe) status may also have advantages from a regulatory perspective. However, it should be noted that GRAS status takes only oral administration into account, and would not cover topical or injection formats (EMEA draft guidance document on PMPs, [www.emea.eu.int/pdfs/human/bwp/076402en.pdf](http://www.emea.eu.int/pdfs/human/bwp/076402en.pdf)).

Food crops can be divided into three major groups (a) seed crops, (b) vegetables and (c) fruit/green leaf crops. The main difference between the three categories is the length of time the plant tissue containing the pharmaceutical molecule can be stored after harvest without the need for special preservation measures such as desiccation or freezing.

#### (a) Seed crops

Several seed crops are being investigated as potential production systems including cereals, (rice, wheat, barley and maize) legumes (peas and soybean) and oilseeds (safflower, oilseed rape) (Stoger et al. 2005). The expression of proteins in seeds enables long-term storage, even at room temperature, because seeds have the appropriate natural biochemical processes to enable stable protein accumulation, dehydration and preservation. Antibodies expressed in seeds have been shown to remain stable for at least 8 years at ambient temperatures with no detectable loss of activity (Eva Stoger, pers comm). It is attractive to consider field-grown crops such as maize, soybean or rice as production hosts because very efficient systems are already established for production and processing. The targeted expression of recombinant proteins in seeds also has bio-safety advantages, in that the recombinant protein is not present in leaves, shoots or roots, thereby avoiding the exposure of herbivores and other non-target organisms to the product. However, the transgenic plants must go through a flowering cycle to produce the seeds, and hence issues of pollen transfer need to be addressed. Procedures also need to be adopted to control seed spillage and seed mixing during transportation and handling, as inadvertent entry into the

food chain is the biggest perceived risk for PMPs produced in seed crops. Transparency and clear documentation is vital not only at the production stage but also throughout the development and breeding stages.

## Maize

Maize has many benefits for PMP production including familiarity, economics, technical know-how, germplasm resources, a wealth of genetic knowledge, and a well-developed agricultural infrastructure. Compared to other seed crops, maize is advantageous because the seed is protected by a husk, and thus does not shatter. It is also enclosed in an environment with little or no microbial burden. As with other field-grown row crops, production can easily be scaled up. Maize already has a track record in molecular farming, having been used for the commercial production of recombinant avidin,  $\beta$ -glucuronidase and trypsin by ProdiGene Inc. in the US. Furthermore, the production of recombinant antibodies (Hood et al. 2002) and further technical enzymes such as aprotinin have been explored using maize ([www.aphis.usda.gov/brs/ph\\_permits](http://www.aphis.usda.gov/brs/ph_permits), e.g. ProdiGene Permits 04-121-01r, 04-114-01r on [http://www.aphis.usda.gov/brs/ea\\_pubs.html](http://www.aphis.usda.gov/brs/ea_pubs.html)). It is generally considered impractical to grow maize in containment beyond the research phase because of low yields, the cost of pollen filtration, capital expenditure, and/or high maintenance. Therefore, this approach is only viable for high-value products. In 2005, Meristem Therapeutics was granted authorization for an experimental field release of PMP maize, covering more than 20 ha (50 acres) in France. The authorization permitted the use of maize to produce gastric lipase (for the treatment of exocrine disorders caused by cystic fibrosis) and monoclonal antibodies for the treatment of various types of cancer ([www.meristem-therapeutics.com](http://www.meristem-therapeutics.com)). The company's purification capacity is currently 500 g of lipase per week from almost 1400 kg of disgermed corn ([www.meristem-therapeutics.com](http://www.meristem-therapeutics.com)). Meristem Therapeutics plans to increase its capacity progressively to reach one tonne of gastric lipase per annum. This quantity

would be sufficient to meet the worldwide demand for gastric lipase.

The main disadvantage of maize is that it is open pollinated and the pollen can be spread by wind (Luna et al. 2001). In most countries, maize has no known weedy relatives with which to cross, but it is the potential to cross with other corn crops that provides the greatest perceived cause for concern. Any potential impact will depend on the field location and the specific pharmaceutical molecules involved. For example, unless appropriate confinement measures can be defined, it would not be good practice to grow pharmaceutical maize in areas that already grow maize for food or feed purposes, where the potential for gene flow through cross-pollination or seed mixing is likely to be a critical issue. In areas where maize is a large commodity crop, such as across the US, mitigation measures such as restricting pollen flow and physical and temporal isolation would need to be built into any production strategy.

Pollen movement has been studied for decades and a great deal of information is available on isolation zones. The USDA (<http://www.aphis.usda.gov/>) currently recommends at least a 1 mile buffer zone for relatively light pollen crops such as maize, and distances of 50 to several hundred feet for self-pollinating crops with relatively heavy pollen such as rice and barley (Elbehri 2005). In practice, PMP production is likely to be carried out under contract and the land area required for production will mostly be small. Thus it should be practical and straightforward to limit the growth of pharmaceutical maize to land areas that comply with these restrictions.

## Rice

Rice has a number of advantages as a production crop including its well-developed gene transfer system and the fact that it is self-pollinating. The expression of recombinant proteins in rice has been demonstrated successfully (Clarapols et al. 2004; Yang et al. 2003), and the biopharmaceutical company Ventria Bioscience is currently testing rice for the production of lysozyme and lactoferrin ([www.ventria.com](http://www.ventria.com)).

## Barley

Cultivated barley is normally self-pollinating, and hybrid formation with related wild species is rare. Barley outcrosses with other barley crops at a very low level (Mäkinen and Nuutila 2004; Ritala et al. 2002), and the production of hybrids with other cultivated crops (e.g. barley-wheat and barley-rye) results in sterile plants that can only be rendered fertile using tissue culture techniques.

Barley is currently being tested as a host for PMP production by a number of companies. Ventria Bioscience is carrying out field trials with transgenic barley engineered to produce lactoferrin and lysozyme ([www.ventria.com](http://www.ventria.com)), while Maltagen ([www.maltagen.de](http://www.maltagen.de)) and ORF genetics ([www.orfgenetics.com](http://www.orfgenetics.com)) are using barley to produce a number of recombinant proteins in Iceland. This country has an unusually low number of plant species in local/indigenous flora, none of which are sufficiently related to barley to allow cross pollination. The Icelandic environment therefore provides a convenient containment system for transgenic barley.

## Oilseeds

SemBioSys ([www.sembiosys.ca](http://www.sembiosys.ca)) have developed a technology based on fusing recombinant proteins to the endogenous protein oleosin, which allows recombinant proteins to be targeted to oil bodies, simplifying the extraction and purification process (Abell et al. 2004; SemBioSys, US patent 6509453). Their primary crop is safflower, which is generally considered to be self-pollinating (Classen 1950). SemBioSys is currently using this technology to produce a number of products; including insulin and proteins for use in cardiovascular therapies. These products are set to enter clinical trials in 2007/2008. Safflower-derived insulin could potentially be one of the first PMPs on the market for human use, in 2009/2010 ([www.sembiosys.ca/News1.aspx](http://www.sembiosys.ca/News1.aspx) press release 18/7/06).

Oilseed rape/canola has also been considered for PMP production. However, the principal disadvantage of this crop is that it is open pollinated and has compatible wild relatives.

APHIS (the USDA Animal and Plant Health Inspection Service) has stated that crops with multiple year seed dormancy, which are bee pollinated and which are sexually compatible with weedy or feral species that grow close to field sites, are inappropriate for use for the production of pharmaceuticals in the field. The Canadian regulatory authorities have recommended against the use of oilseed rape/canola and alfalfa (see later) for field production.

## Soybean

The economic argument for the use of soybean is very strong, given the potential yield, the familiarity with cultivation and the general crop infrastructure. Soybean has the additional advantage of using atmospheric nitrogen, which reduces the need for chemical fertilizers. It is also self-pollinating, and has no wild relatives (at least in the US, South America, Africa and Europe), which reduces the risk of gene flow. Soybean has been explored as a possible system for the production of recombinant antibodies (Zeitlin et al. 2003). However, soybean is unlikely to be adopted widely as a commercial platform due to intellectual property constraints particularly covering the transformation procedure.

## (b) Vegetable crops

### Potato

Potatoes can be used for the production of PMPs and, like seeds, have the advantage that the product remains stable due to the specialized molecular environment in the tuber. Interest has focussed on the development of potatoes as a system for oral vaccine production, and transgenic potato tubers have been fed to humans in several clinical trials (Walmsley and Arntzen 2003; Streatfield and Howard 2003). Although these trials have been successful, the widespread development of potato for oral vaccines is hindered by the necessity to cook the tubers to destroy toxins, which leads to the degradation of thermolabile products. A problem common to all fruit or vegetable crops used for oral vaccination

is variability in the amount of the product between individual tubers, roots or fruits. If the pharmaceutical product is not extracted and purified, but given in a semi-processed or processed form, transgenic tissue would need to be homogenized to provide an even dose. An EU experimental field release for potatoes was granted in 2005 to the University of Rostock, Germany (<http://gmoinfo.jrc.it>), to assess the technological properties, potential influence on the environment, ingredients and specific characteristics of different transgenic potatoes with pharmaceutical and technical traits.

### Carrot

Carrots may be useful as a vaccine-producing crop because the taproot is a natural storage organ and can be consumed raw. Several antigens have already been expressed in carrot, including the *Mycobacterium tuberculosis* MPT64 protein (Wang et al. 2001), glutamic acid decarboxylase (Porceddu et al. 1999) and derivatives of measles haemagglutinin (Bouche et al. 2003; Marquet-Blouin et al. 2003). Protalix Biotherapeutics recently entered phase 1 clinical trials, after gaining FDA approval for the production of the human recombinant glucocerebrosidase, in carrot cell cultures ([www.protalix.com](http://www.protalix.com)). As is the case for potato, oral vaccination using carrot tissue will require measures to ensure dose equivalence.

### (c) Fruit/green leaf crops

A major advantage of protein expression in fruit and edible green leaf crops is that the edible part can be consumed as uncooked, unprocessed or partially processed material. However, PMPs are unlikely to be administered in this way as it would be virtually impossible to maintain batch-to-batch consistency and dosage. Conversely, one of the biggest disadvantages of green leaf crops is that the recombinant proteins are synthesized and maintained in an aqueous environment and are often unstable, and this can result in low yields (Ma et al. 2003). To achieve stability of the pharmaceutical product, tissue needs to be processed soon after harvest. This often involves cold

treatment (refrigerated transport, or freezing the material), or drying. The logistics of such harvesting and processing could also be seen as a disadvantage for large-scale production. The expression of recombinant proteins in leaves or other vegetative parts of the plant could also potentially interfere with plant growth and development. Additional biosafety issues, compared to the use of seed crops, include the potential exposure of herbivores to pharmaceutical products expressed in the leaves and the leaching of recombinant proteins into the environment (the latter also applies to vegetable crops). However, one advantage of harvesting leaf tissue, rather than seeds or fruit, is that the plants do not need to flower, thereby reducing the risk of pollen and seed dispersal.

### Alfalfa

Alfalfa is a perennial plant that does not die after the leaves are harvested, but can be grown again to vegetative maturity without flowering, sexual crossing or seed production. Alfalfa is being used by the Canadian company Medicago Inc. to develop a multiple-cut, glasshouse-based production facility ([www.medicago.com](http://www.medicago.com)). However, its use in the field would be discouraged by current regulatory guidelines on open-pollinated species with compatible weedy or feral relatives (see oilseed rape/canola above).

### Lettuce and spinach

Lettuce has been developed as a production host for edible recombinant proteins, such as vaccines against hepatitis B virus (Kapusta et al. 1999). Spinach has been used as a vehicle for the expression of a rabies virus surface antigen, which subsequently entered human trials (Yusibov et al. 2002), but in this case viral vectors were used to produce the protein, rather than transgenic plants. Familiarity with the production scale-up of these crops may be seen as an advantage.

### Banana

Bananas have many advantages for oral vaccine distribution. They are widely grown in Africa

where vaccines are most needed. They can be eaten raw or as a puree by both adults and children. However, it has been difficult to develop a workable transformation system (Sala et al. 2003), especially in genotypes that are more likely to be eaten in countries such as Africa (i.e. not dessert bananas). Controlling vaccine dose is also considered to be the major problem with vaccines in edible plant parts (Sala et al. 2003; Mor et al. 1998).

### Tomato

Tomatoes, unlike potatoes, are palatable and can be eaten without cooking. They also have other advantages including high biomass yield and suitability for glasshouse production for increased containment. Tomatoes were first used for the production of a rabies candidate vaccine (Walmsley et al. 2003; Sandhu et al. 2000; McGarvey et al. 1995) and have been used to produce antibodies, although yields were lower than 3 µg per gram fresh weight. Tomatoes are good for vaccine production because the ripe fruit does not contain toxins and can be dried to a powder and administered in capsule form (Coghlan 2006). The best method emerging for the use of tomatoes is production in hydroponics. However, the problem of low yields may make this system only suitable for high-value products where lower yields can be tolerated.

### Target recombinant protein and mode of delivery

The choice of target protein and delivery method will also have an impact on the level of regulatory oversight. For example, an injectable antigen (vaccine) for human use is likely to require tighter regulation than a monoclonal antibody used in the oral cavity. Regulatory oversight will also be influenced by how the product will be used, i.e. as a product administered directly to patients, or as a reagent in the manufacturing process. Existing knowledge of the target molecule will be important. A simple, aglycosylated protein that has already been produced in CHO cells or bacteria and has completed clinical trials, will probably benefit from abbreviated testing

when produced in plants. Conversely, if it is a new protein produced for the first time in plants, or a complex glycoprotein only previously expressed in mammalian cells, a complete regulatory profile will be required. The production scale-up and downstream processing issues will also need to be addressed—how pure must the product be, are bioequivalence tests necessary, is the mode of action known? Finally the use of the product is also important, with veterinary products subject to less intense scrutiny than those destined for human use.

In the Pharma-Planta programme, the main target molecule is an anti-HIV monoclonal antibody that is to be administered as a topical agent (in the vagina or rectum). When produced in CHO cells, the target molecule has been shown to have HIV-neutralising properties and to be in safe in clinical trials (Armbruster et al. 2002). We aim to take the plant-derived antibody to the same clinical stage as its CHO-derived counterpart.

### What are the critical biosafety and regulatory issues?

The regulation of PMPs in Europe falls to several regulatory bodies whose authorities overlap. PMPs will need to adhere to both the current GM plant regulations, as well as those controlling pharmaceutical production. The involvement of these authorities is determined by the choice of host plant (food or feed) and growing location (containment or field grown), as discussed in more detail below.

### Regulation of plants for pharmaceutical production (US and EU)

Most of our experience with regulating the field release of pharmaceutical plants has been gained in North America. In the EU, regulators are striving to consider pharmaceutical crops on a case-by-case basis, as for standard agricultural crops. However, most regulatory processes do not readily accommodate pharmaceutical crops because the regulations have largely been devel-



oped for food and feed crops, and the assessment of potential impacts on the environment. There have been limited attempts to adapt these regulations for pharmaceutical crops, but at present there is no “natural home” for assessing pharmaceutical crops in the EU regulatory process. Currently, any field grown pharmaceutical crop grown within the EU would require notification under 2001/18/EC to the competent authority in the country of release. These regulations cover the intended release of both food and non-food crops. If commercial release is requested for a food crop, then EFSA (the European Food Standards Agency) would review the application under the 1829/2003/EU food and feed guidelines. EFSA would also have the deciding role if a non-food crop was proposed, but would usually only act in cases where Member States were not in agreement. EFSA is currently developing guidance documents specifically for PMPs. It should be noted that should a pharmaceutical crop be grown in containment, the contained regulations (Directive 90/219/EEC as amended by Directive 98/81/EC) would apply rather than the field release regulations. Whether grown in containment or not, pharmaceutical products from plants would also need to adhere to the 2309/93/EU regulations. These regulations are overseen by the relevant national authority during the early clinical trial phase, and by EMEA (European Medicines Evaluation Agency), which is broadly equivalent to the FDA in the US, at the point of commercial application. EMEA published draft guidance notes in 2002 (<http://www.emea.eu.int/pdfs/human/bwp/076402en.pdf>), which are still under revision (publication due by the end of 2006). Part of the delay in finalising these guidance notes has been due to the biological and semantic differences when comparing production in plants and conventional systems based on cells grown in bioreactors. For example, concepts that need to be defined properly for plants include those of the working and master bank stocks, batch-to batch consistency, standard operating procedures (inputs, downstream processing, QA etc). The stage at which each regulatory authority becomes involved, and the limits of their authority, is currently being established.

Field-release PMP crops grown in the US require a permit from APHIS (the Animal and Plant Health Inspection Service of the USDA). This must include a containment plan for the production, handling and movement of plants in and out of the field. APHIS reviews all plans for seed production, timing of pollination, harvest, crop destruction, shipment, confinement, and the storage and use of equipment. Field inspections may take place up to five times during the growing season, coinciding with critical phases of production. APHIS issues a field test permit either to an individual company or research institution which, in turn, may subcontract with growers. Subcontracters are required to undergo training in permit requirements and implementation (Elbehri 2005).

APHIS (USDA) currently has no plans to deregulate any pharmaceutical crop. Commercial as well as developmental crops are therefore likely to remain under experimental permit for the foreseeable future, with the associated additional oversight and monitoring. The FDA is particularly concerned about the potential adventitious mixing of pharmaceutical and food crops. The Star Link maize incident (although not a PMP crop) where GM maize approved for animal feed, entered the food chain and the ProdiGene case have had a profound impact on attitudes to the use of food crops to produce “drugs”. In certain cases the USDA may decide that specific permit applications may require an environmental assessment (EA). These EA’s may be triggered by the proposed location or growing conditions. The USDA released individual Environmental Assessments (EAs) for two of ProdiGene’s field trials using maize (Permits 04-121-01r, 04-114-01r on [http://www.aphis.usda.gov/brs/ea\\_pubs.html](http://www.aphis.usda.gov/brs/ea_pubs.html)). These documents were available for public comment and an extended closing date (beyond the normal 30 day period) was allowed for interested parties to comment. The applications were subsequently withdrawn.

### **International developments in regulation**

In 2004, the Canadian Food Inspection Agency (CFIA) hosted an international technical work-

shop on the segregation and handling of commercial PMP products and by-products. Participants included representatives from the PMP industry, federal government bodies, agricultural and agribusiness associations, and experts in grain handling and identity preservation. As many of the plants currently being developed for PMP production are known food and feed crops (e.g. safflower and alfalfa) the first step in developing a regulatory framework was to take a closer look at whether PMP products and by-products could be segregated adequately from other commodities, and more specifically, from commodities intended for the food and feed chains. The results of the workshop have been published on the CFIA web site ([www.inspection.gc.ca/english/plaveg/bio/mf/segrege.shtml](http://www.inspection.gc.ca/english/plaveg/bio/mf/segrege.shtml)).

Similarly, APHIS organised an international workshop on the “Confinement of Genetically Engineered Crops During Field Testing” in 2004. The main aim of this workshop was to review the results for crop plants already planted under APHIS field trial permits for the production of PMPs and plant made industrials (PMIs). The workshop considered a wide range of environmental impacts and confinement issues, summaries of which can be found on the APHIS website ([www.aphis.usda.gov/brs/confine\\_workshop\\_2004.html](http://www.aphis.usda.gov/brs/confine_workshop_2004.html)).

In Europe, both EFSA and EMEA are developing guidance notes for the regulation of PMPs. These regulations are likely to evolve further as products are taken through the new regulatory processes.

## Risk assessment and risk management

### General environmental issues

General environmental issues, such as gene transfer and harm to wildlife, are applicable to all field-grown GM plants (Mascia and Flavell 2004). Indeed many environmental issues need to be addressed whether or not the plants are grown for PMP production, food or feed, grown in greenhouses or even in underground caves or mines (Tackeberry et al. 2003). Many of the

considerations are equally relevant to the development of certain conventional (non-GM) crops where genetic admixtures can occur. For example, there is already experience in the EU and US in the production of High Erucic Acid Rape (HEAR), for the production of specialised oils for industrial processing. HEAR seeds could become mixed with seeds from food and feed oilseed rape, and therefore production protocols are tightly defined and controlled to prevent this. In North America, for example, producers are required to grow HEAR under contract registration and the Canadian Food Inspection Agency mandate this requirement at the time of varietal registration (Smyth and Phillips 2002).

A range of environmental biosafety issues needs to be assessed case by case. These include the potential for transgene spread by pollen, seed dispersal, or horizontal gene transfer, and toxicity and allergenicity of the recombinant proteins. In certain instances, it may be possible to take into account mitigation measures that have been used to reduce risks (discussed later). For example, the risk of transgene spread by pollen dispersal can be addressed by the use of physical and genetic barriers as well as by choice of crop species and production site.

### Impact on soil and watercourses

Recently, ACRE (the UK Advisory Committee on Releases to the Environment) published draft guidance notes on assessing the environmental impact of genetically modified organisms on the soil environment ([http://www.defra.gov.uk/environment/acre/soilecology/acre\\_soilecology\\_guidance-draft.pdf](http://www.defra.gov.uk/environment/acre/soilecology/acre_soilecology_guidance-draft.pdf)). Although this document does not address PMPs, it does highlight the type of information that is required from notifiers in the environmental risk assessment and post-market monitoring of GMOs with respect to soil ecosystems.

### Human health issues

Certain potential hazards to human health from transgenic crops are also commonly recognized by the scientific and regulatory communities

(Kuiper et al. 2001; National Research Council 2001; The Royal Society of Canada 2001; GM Science Review 2003; the FDA 1992 policy statement on biotech-derived crops, and more recently the FDA et al. 2002 draft guidance notes). The potential risks generally relate to the possibility of introducing new allergens or toxins into food-plant varieties or into pollen, or the possibility that previously unknown protein combinations produced in food plants will have unforeseen secondary or pleiotropic effects (National Research Council 2001; Nordlee et al. 1996; Kuiper et al. 2001; Halsberger 2003).

Certain molecules produced in plants for pharmaceutical use would naturally raise questions about human safety. Any drug produced in a GM plant needs to be tested according to normal protocols for drug development that assesses clinical safety and efficacy. However, the production of pharmaceuticals in plants raises several additional issues.

- The GM plant could be eaten inadvertently and cause harm because of toxicity or allergenicity.
- The introduced genetic material could be transferred to sexually compatible neighbouring crops, weeds or feral species.
- The genes could be transferred to microorganisms in the intestine after consumption, if the GM plant is used to administer the drug (i.e. oral delivery) rather than injection of the purified pharmaceutical molecule.

Inadvertent consumption could potentially be harmful, especially for vulnerable groups such as infants and the elderly. Effects could be acute or cumulative if small quantities were consumed over a long period. Detecting consumption may be very difficult. However, relevant in this context are two considerations: (1) DNA and RNA are not toxic, and (2) proteins are frequently not toxic, and even in the case of certain toxic effects, the exposure in the case of accidental consumption will be very low ([www.pubresreg.org](http://www.pubresreg.org)).

## Adventitious presence of pharmaceutical crops in food crops

The containment of genes that encode pharmaceutical products can pose important challenges. Gene flow and the occurrence of volunteer plants in subsequent harvests are issues that have direct consequences for the environment and human health. Past actions by regulatory agencies and biotechnology companies reflect the importance and emphasis placed on this issue. In 2002, ProdiGene Inc. was at the centre of a highly publicized debate about protocols to contain pharmaceutical crops produced under field conditions (Hoag 2003). This widely reported case involved volunteer transgenic maize plants that grew in the soybean crop in the year following the pharmaceutical maize crop. The presence of maize plant material mixed with the soybean crop resulted in soybean in the storage silo being impounded and destroyed. In the resulting settlement, Prodigene accepted a \$250,000 civil penalty, and agreed to pay the cost of 500,000 bushels of soybeans, plus the cost of cleaning all the facilities and equipment. While this case shows that the safeguards were in place to prevent adulterated food products entering the food chain, it also demonstrates that the biology of the production host crop, and subsequent crops in the rotation, must be taken into account in regulation and containment measures. Although this should reduce the likelihood of contamination, it may not be realistic to guarantee no contamination at all. Elbehri (2005) reported that a coalition of food industries favoured the inclusion of a food-safety assessment by event prior to issuing a permit. In practice, such an approach might tilt current research and development away from food crops (such as maize) in favour of non-food crops (tobacco).

## Transparency

Transparency is an important requirement of many regulatory processes. In most countries that have biosafety regulations in place, release of GMOs into the environment requires a permit or approval from the competent authorities. In

countries that do not yet have biosafety regulations in place, but are nevertheless party to the Cartagena Protocol on Biosafety (CPB) (<http://www.biodiv.org/biosafety/default.aspx>), the transboundary movement of GMOs for release into the environment requires notification prior to such movement. The EU system specifically states that a summary of the notifier's application, and the assessment report, should be made available to the public when a product is released into the environment, either as an experimental release (2001/18/EC part B) or when commercialized (2001/18/EU part C) (<http://gmoinfo.jrc.it/>). In the US, some agencies (e.g., USDA) provide access to the application and notice to the public, while other agencies (e.g., FDA) provide information only when specifically requested by an individual. The GMO Act of South Africa specifically states that the description of the GMO, the purpose of the release and its location, the monitoring of the release, and the environmental impacts evaluation 'shall not be kept confidential.'

#### Mitigation measures

Most of the steps required to prevent pharmaceutical crops entering the food chain involve relatively low-tech measures, mainly consisting of meticulous planning and execution of each of the processes required. The crop must be grown in isolation from breeding materials to avoid genetic and mechanical mixing of material carrying the industrial trait into conventional breeding material. In practice, such mixing might be difficult to detect, so effective handling and labelling protocols are essential. Similarly, both small and large-scale field trials need be carried out in isolation from conventional agricultural crop trials. Parent seed for commercial production and the commercial crops themselves need be grown in isolation from other plants of the same species or wild relatives to avoid adventitious pollination.

The achievement of a sufficient level of isolation can be difficult, and is challenging for both wind and insect pollinated species. Realistically, if mixing with a food crop or wild weed relatives must be avoided then the novel crop should not be grown in

areas where the species is normally grown for food or where wild relatives are prevalent.

The mitigation measures appropriate for a particular pharmaceutical crop will depend on many factors including the properties of the molecule, the biology of the crop and the nature of the environment in which it is grown. Some of the potential mitigation methods that have been proposed include the following (reviewed by Commandeur et al. 2003; Dunwell 2005).

- The use of **marker genes** e.g. DsRed (*Disco-soma* sp. red fluorescent protein; [www.clon-tech.com/](http://www.clon-tech.com/)) to make the crop or its products (e.g. seeds) visually distinctive from food and feed crops
- The introduction of a bitter or **undesirable taste** so the crop would be unpalatable
- Post-harvest expression
- **Physical isolation** by distance from sexually compatible crops, weeds and feral species
- **Barrier crops** to minimise cross pollination
- **Temporal isolation** to isolate crops genetically. Sowing different crops at different times can result in them flowering at different times and prevent the possibility of cross pollination
- **Physical removal of flowers** to prevent the possibility of pollination. A frequent isolation measure used in maize, for example, is removal of tassels from the female transgenic parent, to prevent pollen dissemination

Protocols should be in place to avoid mixing PMP crops with agricultural crops and their products. These might include: dedicated land with security fencing, dedicated agricultural machinery, dedicated storage facilities, secure methods of transporting seeds for establishing the crop, secure methods of transporting the pharmaceutical containing crop product (e.g. seeds) and crop residues, measures to prevent adverse consequences of volunteer plants growing in subsequent years, and partial processing of the pharmaceutical product on the production site.

## Conclusions

Many biotechnology companies have evaluated the use of a range of crops for pharmaceutical production, and while maize has many merits, no single crop has emerged as the clear leader thus far. Different companies have their preferences depending on their individual business plans. There are also socio-political influences on the choice of crop, which are likely to be most significant if a food crop is adopted. It appears that to make progress in the short term, maize has many advantages such as familiarity, infrastructure, ease of scale up, product stability and ease of processing. In the longer term, the use of other crops may simplify production and help with public perception. Gaining public acceptance is something that must be taken seriously in order to maximize the potential of GM technology. For this reason, non-food crops as well as food crops need to be evaluated carefully.

Within the Pharma-Planta programme, maize has been selected as the primary crop for producing an anti-HIV monoclonal antibody. The crop is to be grown in containment, and the product (a topical cream) entered into phase 1 clinical trials. Tobacco is being evaluated as a secondary crop. It is hoped that this programme will road test the developing regulatory guidelines for PMPs by addressing many of the points outlined in this paper.

Pharmaceutical crops potentially offer a substantial opportunity to provide a clear public benefit. However, with the current negative perceptions surrounding GM crops, any mishandling of pharmaceutical crops could be a major setback to their application in the future. The potential benefit to humanity from a production technology that could truly deliver global access to medicines, particularly for countries with significant poverty, needs to be examined very seriously.

**Acknowledgements** The Pharma-Planta programme is a consortium of 39 principal scientists from academic and industrial institutions in Europe and South Africa. Pharma-Planta is funded by the European Commission as part of the Sixth Framework Programme in the area of “Plant platforms for immunotherapeutic biomolecule production”.

## References

- Abell BM, Hahn M, Holbrook LA, Moloney MM (2004) Membrane topology requirements for oil body targeting of oleosin. *Plant J* 37:461–470
- Armbruster C, Stiegler GM, Vcelar BA, Jager W, Michael NL, Vetter N, Katinger HW (2002) A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1. *AIDS* 16:227–33
- Borisjuk NV, Borisjuk LG, Logendra S, Petersen F, Gleba Y, Raskin I (1999) Production of recombinant proteins in plant root exudates. *Nat Biotechnol* 17:466–469
- Claparols MI, Bassies L, Miro B, Del Duca S, Rodriguez-Montesinos J, Christou P, Serafini-Fracassini D, Capell T (2004) Transgenic rice as a vehicle for the production of the industrial enzyme transglutaminase. *Trans Res* 13:195–199
- Classen CE (1950) Natural and controlled crossing in Safflower, *Carthamus tinctorius* L. *Agron J* 42:381–384
- Coghlan A (2006) Killer tomatoes attack human diseases. *New Sci* 189:20
- Commandeur U, Twyman RM, Fischer R (2003) The biosafety of molecular farming in plants. *AgBiotech-Net* 5:1–9
- Doran PM (2000) Foreign protein production in plant tissue cultures. *Curr Opin Biotechnol* 11:199–204
- Drake PMW, Chargelegue DM, Vine ND, van Dollweerd JC, Obregon P, Ma JKC (2003) Rhizosecretion of a monoclonal antibody protein complex from transgenic tobacco roots. *Plant Mol Biol* 52:233–241
- Dunwell J (2005) Technologies for biological containment of GM and non-GM crops. DEFRA contract CPEC 47
- Elbehri A (2005) Biopharming and the food system: examining the potential benefits. *AgrBioForum* 8:18–25
- FDA, CBER, CDER, CFSAN, CDRH, CVM, APHIS, CVB, BRS (2002) Guidance for Industry: drugs, biologics and medical devices derived from bioengineered plants for use in humans and animals. Draft Guidance
- Fischer R, Emans N (2000) Molecular farming of pharmaceutical proteins. *Trans Res* 9:279–299
- Fischer R, Stoger E, Schillberg S, Christou P, Twyman RM (2004) Plant-based production of biopharmaceuticals. *Curr Opin Plant Biol* 7:152–158
- Giddings G, Allison G, Brooks D, Carter A (2000) Transgenic plants as factories for biopharmaceuticals. *Nat Biotechnol* 18:1151–1155
- GM Science Review (2003) First report: an open review of the science relevant to GM crops and food based on the interests and concerns of the public
- Gomord V, Sourrouille C, Fitchette AC, Bardor M, Pagny S, Lerouge P, Faye L (2004) Production and glycosylation of plant-made pharmaceuticals: the antibodies as a challenge. *Plant Biotechnol J* 2:83–100
- Halsberger AG (2003) Codex guidelines for GM food include the analysis of unintended effects. *Nat Biotechnol* 21:739–741

- Hellwig S, Drossard J, Twyman RM, Fisher R (2004) Plant cell cultures for the production of recombinant proteins. *Nat Biotechnol* 22:1415–1422
- Hoag H (2003) Tougher rules aim to prevent gene flow into crops. *Nature* 422:103
- Hood EE, Woodward SL, Horn ME (2002) Monoclonal antibody manufacturing in transgenic plants—myths and realities. *Curr Opin Biotechnol* 13:630–635
- Horn ME, Woodward SL, Howard JA (2004) Plant molecular farming: systems and products. *Plant Cell Rep* 22:711–720
- Kapusta J, Modelska A, Figlerowicz M, Pniewski T, Letellier M, Lisowa O, Yusibov V, Koprowski H, Plucienniczak A, Legocki AB (1999) A plant-derived edible vaccine against hepatitis B virus. *FASEB J* 13:1796–1799
- Kormarmytsky S, Borisjuk NV, Borisjuk LG, Alam MZ, Raskin I (2000) Production of recombinant proteins in tobacco guttation fluid. *Plant Physiol* 124:927–933
- Kuiper HA, Kleter GA, Noteborn HP, Kok EJ (2001) Assessment of the food safety issues related to genetically modified foods. *Plant J* 27:503–528
- Luna SV, Figueroa J, Baltazar M B, Gomez RL, Townsend R, Schoper JB (2001) Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science* 41:1551–1557
- Ma JKC, Drake PMW, Christou P (2003) The production of recombinant pharmaceuticals in plants. *Nat Rev Genet* 4: 794–805
- Ma JKC, Barros E, Bock R, Christou P, Dale PJ, Dix PJ, Fischer R, Irwin J, Mahoney R, Pezzotti M, Schillberg S, Sparrow P, Stoger E, Twyman RM (2005a) Molecular farming for new drugs and vaccines. *Current perspectives on the production of pharmaceuticals in transgenic plants*. *EMBO Rep* 6:593–599
- Ma JKC, Chikwamba R, Sparrow P, Fischer R, Mahoney R, Twyman RM (2005b) Plant-derived pharmaceuticals—The road forward. *Trends Plant Sci* 10:580–585
- Mäkinen K, Nuutila AM (2004) Barley seed as a production host for industrially important proteins. *AgBioTechNet* 6:1–8
- Marquet-Blouin E, Bouche FB, Steinmetz A, Muller CP (2003) Neutralising immunogenicity of transgenic carrot (*Daucus carota* L.) derived measles virus haemagglutinin. *Plant Mol Biol* 51:459–469
- Mascia PN, Flavell RB (2004) Safe and acceptable strategies for producing foreign molecules in plants. *Curr Opin Plant Biol* 7:189–195
- McGarvey PB, Hammond J, Dienelt MM, Hooper DC, Fu ZF, Dietzschold B, Koprowski H, Michaels FH (1995) Expression of the rabies virus glycoprotein in transgenic tomatoes. *Biotechnology* 13:1484–1487
- Mor TS, Gomez-Lim MA, Palmer KE (1998) Perspective: edible vaccines – a concept coming of age. *Trends Microbiol* 6:449–453
- National Research Council (2001) Genetically modified pest-protected plants: science and regulation. National Academy Press, Washington, DC.
- Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK (1996) Identification of a Brazil-nut allergen in transgenic soybeans. *New Eng J Med* 334(11):688–692
- Petersen RKD, Arntzen CJ (2004) On risk and plant-based biopharmaceuticals. *Trends Biotechnol* 22: 64–66
- Porceddu A, Falorni A, Ferradini N, Cosentino A, Calcinaro F, Faleri C, Cresti M, Lorenzetti F, Brunetti P, Pezzotti M (1999) Transgenic plants expressing human glutamic acid decarboxylase (GAD 65), a major autoantigen in insulin-dependent diabetes mellitus. *Mol Breeding* 5:553–560
- Pujol M, Ramirez NI, Ayala M, Gavilondo JV, Valdes R, Rodriguez M, Brito J, Padilla S, Gomez L, Reyes B, Peral R, Perez M, Marcelo JL, Mila L, Sanchez RR, Paez R, Cremata JA, Enriquez G, Mendoza O, Ortega M, Borroto C (2005) An integral approach towards a practical application for a plant-made monoclonal antibody in vaccine purification. *Vaccine* 23:1833–1837
- Ramirez N, Rodriguez M, Ayala M, Cremata J, Pérez M, Martínez A, Linares M, Hevia Y, Páez R, Valdés R, Gavilondo JV, Selman-Housein G (2003) Expression and characterisation of an anti-(hepatitis B surface antigen) glycosylated antibody in transgenic tobacco (*Nicotiana tabacum*) plants and its use in the immunopurification of its target antigen. *Biotechnol Appl Biochem* 38:223–230
- Ritala A, Nuutila AM, aikasalo R, Kauppinen V, Tammisola J (2002) Measuring gene flow in the cultivation of transgenic barley. *Crop Sci* 42:278–285
- Sala F, Rigano MM, Barbante A, Basso B, Walmsely AM, Castiglioni S (2003) Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. *Vaccine* 21:803–808
- Sandhu JS, Rasnyanski SF, Domier LL, Korban SS, Osadjan MD, Buetow DE (2000) Oral immunisation of mice with transgenic tomato fruit expressing respiratory syncytial virus-F protein induces a systemic immune response. *Trans Res* 9:127–135
- SemBioSys (2003) Oil bodies and associated proteins as affinity matrices. US patent 6509453
- Smyth S, Phillips PWB (2002) Production differentiation alternatives: Identity preservation, segregation and traceability. *AgBioForum* 5:30–42
- Stoger E, Ma JK-C, Fischer R, Christou P (2005) Sowing the seeds of success: pharmaceutical proteins from plants. *Curr Opin Biotechnol* 16:167–173
- Stoger E, Sack M, Fischer R, Christou P (2002) Plantibodies: applications, advantages and bottlenecks. *Curr Opin Biotechnol* 13:161–166
- Streatfield SJ, Howard JA (2003) Plant-based vaccines. *Int J Parasitol* 33:479–493
- Tackaberry ES, Prior F, Bell M, Tocchi M, Porter S, Mehic J, Ganz PR, Sardana R, Altosaar I, Dudani A (2003) Increased yield of heterologous viral glycoprotein in the seeds of homozygous tobacco plants cultivated underground. *Genome* 46:521–526
- Tekoah Y, Ko K, Koprowski H, Harvey DJ, Wormald MR, Dwek RA, Rudd PM (2004) Controlled glycosylation of therapeutic antibodies in plants. *Arch Biochem Biophys* 426:266–278
- The Royal Society of Canada (2001) Elements of precaution: recommendations for the regulation of food biotechnology in Canada.

- Twyman RM, Schillberg S, Fischer R (2005) The transgenic plant market in the pharmaceutical industry. *Expert Opin Emerg Drugs* 10:185–218
- Twyman RM, Stoger E, Schillberg S, Christou P, Fischer R (2003) Molecular farming in plants: host systems and expression technology. *Trends Biotechnol* 21:570–577
- Valdés R, Gómez L, Padilla S, Brito J, Reyes B, Álvarez T, Mendoza O, Herrera O, Ferro W, Pujol M, Leal V, Linares M, Hevia Y, Garcí Mila L, García O, Sánchez R, Acosta A, Geada D, Paez R Vega JL, Borroto C (2003) Large-scale purification of an antibody directed against hepatitis B surface antigen from transgenic tobacco plants. *Biochem Biophys Res Commun* 308:94–100
- Walmsley AM, Arntzen CJ (2003) Plant cell factories and mucosal vaccines. *Curr Opin Biotechnol* 14:145–150
- Walmsley AM, Alvarez ML, Jin Y, Kirk DD, Lee SM, Pinkhasov J, Rigano MM, Arntzen CJ, Mason HS (2003) Expression of the B subunit of *Escherichia coli* heat labile enterotoxin as a fusion protein in transgenic tomato. *Plant Cell Rep* 21:1020–1026
- Wang LJ, Ni DA, Chen YN, Lee ZM (2001) The expression of *Mycobacterium tuberculosis* MPT64 protein in transgenic carrots. *Acta Bot Sin* 43:132–137
- Yang D, Guo F, Huang N, Watkins S (2003) Expression and localisation of human lysozyme in the endosperm of transgenic rice. *Planta* 216:597–603
- Yusibov V, Hooper DC, Spitsin S, Fleysh N, Kean RB, Mikheeva T, Deka D, Karasev A, Cox S, Randall J, Koprowski H (2002) Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine* 20:3155–3164
- Zeitlin L, Olmsted SS, Moench TR, Co MS, Martinell BJ, Paradkar VM, Russell DR, Queen C, Cone RA, Whaley KJ (1998) A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes. *Nat Biotechnol* 16:1361–1364