Biotechnol Lett (2014) 36:2367–2379
DOI 10.1007/s10529-014-1621-3

REVIEW

# Recombinant plant-derived pharmaceutical proteins: current technical and economic bottlenecks

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Received: 29 May 2014 / Accepted: 9 July 2014 / Published online: 22 July 2014


**Abstract** Molecular pharming is a cost-effective platform for the production of recombinant proteins in plants. Although the biopharmaceutical industry still relies on a small number of standardized fermentation-based technologies for the production of recombinant proteins there is now a greater awareness of the advantages of molecular pharming particularly in niche markets. Here we discuss some of the technical, economic and regulatory barriers that constrain the clinical development and commercialization of plant-derived pharmaceutical proteins. We also discuss strategies to increase productivity and product quality/homogeneity. The advantages of whole plants should be welcomed by the industry because this will help to reduce the cost of goods and therefore expand the biopharmaceutical market into untapped sectors.

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**Keywords** Molecular pharming · Plant-made pharmaceuticals · Plant production systems · Proteins from recombinant plants · Recombinant pharmaceutical proteins

## Introduction

Molecular pharming is the use of plants to produce recombinant pharmaceutical proteins (Twyman et al. 2003, 2005; Paul and Ma 2011). This began with the initial demonstration that a functional antibody could be produced in tobacco (Hiatt et al. 1989) and led to a large number of studies in which plants were used to produce antibodies, vaccine antigens, hormones, signaling proteins, blood products and enzymes (Stoger et al. 2002a, 2005; Ma et al. 2003; Claparols et al. 2004; Streatfield 2007; Twyman et al. 2005) as well as protein polymers and structural proteins (Hood 2002; Hood et al. 1997; Fischer et al. 2003). Unlike conventional medicinal plants which are valued for their natural products, molecular pharming involves the modification of plants to produce recombinant proteins, which are either extracted and purified or used in unprocessed or minimally-processed plant tissues (Hofbauer and Stoger 2013).

Established production systems for recombinant proteins include microbes (bacteria and yeast), mammalian cell lines [particularly Chinese hamster ovary (CHO), baby hamster kidney (BHK) and myeloma

**Table 1** Comparison of major standard production platforms for recombinant proteins (bacteria, mammalian cells) with the three major platforms based on plants (cell suspension cultures, transient expression, transgenic plants)

| Platform | Intrinsic yield | Scalability | Overall productivity | Timescale | Cost upstream | Cost downstream | Main safety risks | PTMs |
|---|---|---|---|---|---|---|---|---|
| Bacteria | High* | Low | Moderate | Weeks | Low | High* | Endotoxins | None |
| Mammalian cells | High | Low | Moderate | Months | High | High | Viruses, prions | Human-like |
| Plant cells | Moderate | Low | Moderate | Weeks to months | Moderate | High | Metabolites | Flexible |
| Transient expression | High | Moderate | High | Days to weeks | Low | High | Metabolites, endotoxins** | Flexible |
| Transgenic plants | Moderate | High | High | Months to years | Low | High (low***) | Metabolites (none***) | Flexible |

* The intrinsic yield of bacterial cells is high but many complex proteins are produced as inclusion bodies that need to be resolubilized, increasing downstream production costs

** Endotoxins are bacterial contaminants, and in the transient expression system they may be present if the system is based on infiltration with Agrobacterium tumefaciens but not in platforms based on plant viruses

*** The species is important—if tobacco is used, metabolites such as nicotine must be removed which increases the processing costs, but if cereal seeds or other edible tissues are used these tissues can generally be regarded as safe and processing costs may be reduced or eliminated altogether if the product is orally administered as unprocessed or part-processed tissues such as flour paste or fruit juice (updated from Twyman et al. 2003)

cells], baculovirus expression systems in insect cells, transgenic animals producing recombinant proteins in their milk or eggs, and plants (Stoger et al. 2002a, 2005; Ma et al. 2003; Twyman et al. 2003; Fischer et al. 2004; Ferrer-Miralles et al. 2009). The main systems are compared in Table 1.

Plants provide significant advantages over microbial and mammalian production systems because they offer safe and inexpensive production that can be scaled to agricultural levels. Scalability is important because the demand for low-cost/high-volume pharmaceutical proteins is increasing beyond current fermenter-based production capacity, and only plants can provide the economy of scale necessary to meet this demand (Kunka et al. 2005). Plants can also fold and assemble complex eukaryotic proteins and are able to carry out many of the essential post-translational modifications required for optimal activity. Plants do not support human-tropic pathogens and, unlike bacteria, do not produce endotoxins (Ma et al. 2003; Stoger et al. 2005). Unlike the fixed capacity of microbes and insect/mammalian cells in fermenters, plants can be cultivated to meet different demands, including the rapid scale-up required to produce vaccines against emerging pandemic diseases, and the slower but ultimately greater scale-up required to meet the sustained demand for 'commodity' biopharmaceuticals such as microbicide components (Fischer et al. 2013). Plants can be grown in soil or synthetic substrates using only water, fertilizers and light, and thus do not require large upfront investments in fermenter-based systems (Knäblein 2005).

The early development of molecular pharming faced technical bottlenecks such as low yields and structural heterogeneity, which are now being tackled head-on and even turned into advantages. Many different strategies have been developed to increase protein yields as discussed below, and product heterogeneity has been addressed by tailoring the glycan structures in plants, even allowing the production of tailored protein glycoforms that cannot be generated in mammalian cells (Aviezer et al. 2009a, b). These advances have also helped to overcome economic barriers, such as the absence of a cogent regulatory framework governing the manufacture biopharmaceutical products, and the lack of support for translational research and clinical development. The industry and regulators are beginning to acknowledge the potential of plants now that corresponding good manufacturing practice (GMP) guidelines have been developed (Fischer et al. 2012). Several products have completed clinical trials and the first plant-derived pharmaceutical protein was recently approved for human use (Fischer et al. 2012).

## Overcoming the remaining technical constraints

After a short period of interest, molecular pharming was largely dismissed by the biopharmaceutical industry, mainly because the yield of recombinant proteins in plants was several orders of magnitude below what could be achieved in mammalian cells. Many strategies have been developed to increase yields, focusing on two major principles that affect economic viability: the amount of product that accumulates per unit biomass (since the costs of upstream production relate to the amount of biomass produced), and the quality of the final product in terms of functionality and homogeneity (since the costs of downstream processing relate to the amount of final drug substance produced per unit volume of the feed stream).

### Product yield

The product yield in molecular pharming is regarded by the industry in terms of the high titers achieved in mammalian cells growing in fermenters, which is measured in grams of product per liter of fermentation culture over a standard fermentation run of 1–2 weeks (Twyman et al. 2013). The overall yield is therefore the intrinsic yield (grams of product per liter of fermentation culture) multiplied by the volume of the fermenter, and if the product has a high demand then it may also be necessary to determine how many fermentation runs can be completed in a given campaign period in order to establish the productivity of the system. Similarly in molecular pharming, the productivity is the intrinsic yield (grams of product per unit of plant biomass) multiplied by the amount of biomass produced and, if necessary, the number of 'harvests' that can be completed in a given campaign period. Because plants are more scalable than fermenter systems, the biomass yield tends to be much higher even though the intrinsic yield is lower (Twyman et al. 2013). The intrinsic yield depends on the rate of protein synthesis balanced against the rate of protein degradation, and if synthesis is more rapid than turnover then the protein accumulates. The intrinsic yield therefore reflects genetic, epigenetic, biochemical and environmental factors that influence protein synthesis and turnover, and the manipulation of these factors singly or in combination can thus help to increase the yield of pharmaceutical proteins in plants.

### Genetic factors

At the genetic level (DNA sequence), product yield is influenced by the efficiency of transcription (the amount of mRNA produced), the rate of mRNA turnover, the efficiency of protein synthesis and any genetic factors that affect protein stability (such as the presence of sequences that control protein trafficking). The choice of promoter is critical because this ensures strong transcription, thus boosting the amount of mRNA produced, and the choice of constitutive, organ-specific or inducible promoters should be made on a case-by-case basis, e.g. seed-specific expression is often useful for the expression of proteins that interfere with vegetative growth, but is particularly valuable for the production of oral vaccines in cereals because the protein must accumulate in edible organs (Peremarti et al. 2010). Other sequences can be included in the expression cassette to enhance mRNA stability and improve the efficiency of translation, e.g. introns (Mitsuhara et al. 1996; Parra et al. 2011), the Kozak consensus sequence (Sharma et al. 2008; Kawaguchi and Bailey-Serres 2002) and leader sequences from *Alfalfa mosaic virus* (AMV) RNA-3, *Tobacco mosaic virus* (TMV) or endogenous gene sequences such as the 5′ untranslated region from the *Petunia hybrida* chalcone synthase gene, which act as translational enhancers (Mitsuhara et al. 1996; Lu et al. 2008; Sharma et al. 2008).

### Epigenetic factors

Whereas genetic factors depend on the DNA sequence, epigenetic factors are those which affect gene expression but are not encoded in the corresponding gene. In transgenic plants, the gene encoding the pharmaceutical protein of interest is integrated into the host genome and is therefore influenced by numerous epigenetic factors, including the position, structure and complexity of the transgene locus (Kohli et al. 2003, 2006). These factors are difficult to control because the integration mechanism is not sequence dependent, and the general approach is to produce large numbers of transformants and screen them for plants with high and stable transgene expression levels (Naqvi et al. 2010). This screening process often

identifies plants with transgenes integrated at permissive sites, whereas others may experience a position effect that shuts off transgene expression, reflecting the influence of genomic DNA surrounding the site of transgene integration (Wilson et al. 1990). Transgenes can be integrated at a silencing locus (position-dependent silencing) or influenced by nearby regulatory sequences, such as enhancers (Topping et al. 1991). Another factor that influences transgene stability is the structure of the locus, including the number of transgene copies, how they are arranged and whether or not they are intact, all of which influence the likelihood of physical interactions and further recombination within the locus (physical instability) as well as the induction of silencing through DNA methylation and/or the production of aberrant RNA species (Heinrichs 2010).

High-copy-number transgenic loci are, in some cases, prone to instability and silencing but there are many other cases showing a correlation between copy number and expression level, suggesting the silencing may not depend on the copy number but on another triggering factor such as a hairpin sequence (Datta et al. 2003; Maqbool and Christou, 1999; Maqbool et al. 2001; Tu et al. 2000; Ye et al. 2001). Many promoters contain inverted repeat motifs, so it may be beneficial to use a range of promoters, and if multiple promoters with the same properties are unavailable, to explore other strategies such as synthetic or hybrid promoter constructs (Peremarti et al. 2010). However, the use of the same promoter to control up to five different transgenes has been reported with no evidence of silencing (Naqvi et al. 2009). Transgenes can be protected from silencing by buffering, which involves flanking the transgene with matrix attachment regions (MARs) such as the tobacco *rb7* sequence that blocks position effects by establishing an independent chromatin domain (Allen et al. 1993, 1996; Vain et al. 1999; Halweg et al. 2005).

Transient expression systems, often based on viruses, the *Agrobacterium tumefaciens* T-DNA system or combinations thereof, involve the introduction of expression constructs into non-transgenic plants, allowing strong expression from the episomal construct for a few days before it is degraded (Paul et al. 2013). Because the transgene does not integrate, there are no epigenetic effects. However, transient expression systems are sensitive to minor environmental variations which have a much less significant impact on transgenic plants (Stevens et al. 2000; Buyel and Fischer 2012). The ability to manufacture large quantities of protein in a short time and to rapidly scale up the manufacturing process makes transient expression technologies ideally suited to meet the surge in capacity required to manufacture vaccines for emerging infectious diseases (Paul et al. 2013).

## Biochemical factors

The stability of a protein is determined both by its intrinsic properties and its surroundings, which means many different biochemical factors can affect protein accumulation. Subcellular localization therefore plays an important role in determining the yield of a protein because the biochemical milieu (e.g. the surrounding pH and salt concentration, the presence of proteases, oxidizing and reducing agents, chaperones and protective metabolites) can result in stable accumulation or degradation. Comparative targeting experiments have shown that the secretory pathway is generally more suitable for the folding and assembly of complex multimeric proteins than the cytosol, and it therefore promotes accumulation (Zimmermann et al. 1998; Schillberg et al. 1999). A common strategy in molecular pharming is therefore to target recombinant proteins to the ER by adding a signal peptide so they are co-translationally imported across the ER membrane, and folded within the lumen with the help of protein disulfide isomerases and molecular chaperones. They may also acquire *N*-linked glycans and assemble into multimers if appropriate (Gomord et al. 2010). The principal routes for protein secretion are from the ER to the Golgi complex and from there to vacuoles (e.g. PSVs) or full secretion to the apoplast (Vitale and Denecke 1999; Jurgens 2004). Although most recombinant proteins are more stable in the apoplast than the cytosol, stability may be even greater in the ER lumen and ER-derived protein storage organelles. Recombinant proteins can be retained in the ER using an H/KDEL *C*-terminal tetrapeptide tag in addition to the signal peptide (Conrad and Fiedler 1998), often increasing the accumulation of recombinant proteins in transgenic plants by one or two orders of magnitude (Wandelt et al. 1992). This strategy has been used to boost the accumulation of antibodies, vaccine antigens and many other recombinant proteins in transgenic plants (Arakawa et al. 1998; Stoger et al. 2000; Vaquero et al. 2002; Ko et al. 2003). Protein

stability is often highest in storage organelles derived from the endoplasmic reticulum (ER), which is why seeds, particularly cereal seeds with their specialized ER-derived protein bodies, are an ideal vehicle for the stable accumulation of recombinant proteins (Peters and Stoger 2011; Sabalza et al. 2013; Wakasa and Takaiwa 2013). The endosperm tissue provides a suitable environment for protein accumulation because there is little protease activity, little water, and additional protection within specialized storage compartments such as protein bodies (PBs) and protein storage vacuoles (PSVs). For example, antibodies accumulate in seeds and remain stable for several years with no loss of activity when stored at ambient temperatures (Stoger et al. 2005; Ramessar et al. 2008a, b, c; Rademacher et al. 2008).

Further strategies have been developed to increase protein stability by expressing recombinant proteins as fusions with stabilizing sequences, such as the γ-zein coding region, elastin-like polypeptides (ELPs) and fungal hydrophobins, which protect the proteins from degradation (Conley et al. 2011). Protein stability can be also enhanced by reducing the impact of cellular proteases. For example, this can be achieved by coexpressing a protease inhibitor such as sPI-II (Kim et al. 2002), co-expressing a protective binding protein such as an antibody with its corresponding antigen (Stoger et al. 2002a, b) or the development of specialized plant lines lacking protease activity, although the success of the latter strategy will require a more detailed characterization of the complex family of proteases expressed in different plant cells (Schillberg et al. 2013).

## Environmental factors

The environment can also affect recombinant protein yields because light, heat, water availability and nutrition influence plant growth and productivity, since these are dependent on primary metabolism including energy generation/carbohydrate production and protein synthesis. Nitrogen plays a key role in protein metabolism because it is directly associated with amino acid and protein synthesis, making it particularly important in the context of molecular pharming (Twyman et al. 2013). For pharmaceutical production the current trend is to grow plants under closed and controlled conditions where growth parameters and protein yield are monitored as part of the quality control regime (Fischer et al. 2012).

## Downstream processing

Downstream processing is currently a bottleneck not only for molecular pharming in plants but also for conventional production systems. The burden can be alleviated by maximizing production yields and taking advantage of innovative purification strategies to improve product recovery (Paul and Ma 2011). Several strategies have been developed to reduce protein purification costs in plants, such as the oleosin platform that allows recombinant proteins to be isolated from the lipid fraction of seeds followed by endoprotease cleavage (Boothe et al. 2010; Nykiforuk et al. 2011). A similar concept is exploited when proteins are expressed as fusions with an integral membrane-spanning domain derived from the human T cell receptor, allowing protein extraction in a small volume using appropriate buffers and detergents (Schillberg et al. 2000). Other separation methods have been devised based on cross-flow filtration, which rely neither on fusions nor on chromatographic separations (Aspelund and Glatz 2010). The ELP-fusion strategy discussed above not only improves the stability of recombinant proteins expressed in plants, but also facilitates an inexpensive purification method based on reversible temperature-dependent precipitation, known as inverse transition cycling (Conley et al. 2011).

## Plant glycans

The secretory pathway is conserved between plants and mammals, which means that plants carry out protein folding and post-translational modification in much the same way as mammals allowing the production of mammal-like recombinant proteins in plants (Gomord et al. 2010). However, whereas plants can synthesize *N*-glycan core structures identical to those in mammals, the terminal residues differ, mainly because plant complex *N*-glycans lack β(1,4)galactose (and sialic acid) and core α(1,6)fucose. Instead, they carry β(1,2)xylose and core α(1,3)fucose, which are not found in mammals (Gomord and Faye 2004; Faye et al. 2005). Plant glycans are immunogenic in several mammals although their role in human allergies has not yet been clarified (Garcia-Casado et al. 1996; Van Ree et al. 2000; Bardor

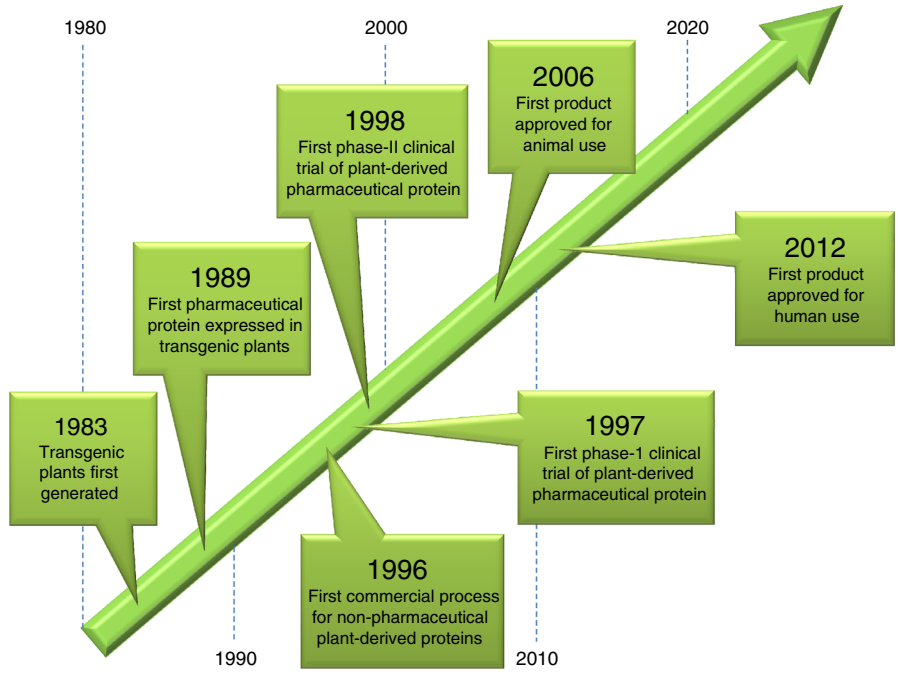


**Fig. 1** Major landmarks in the commercial development of molecular pharming, revised from Fischer et al. 2013)

et al. 2003). They do not appear to be immunogenic in mice and only after multiple exposures in rats (Gomord et al. 2005; Faye et al. 2005).

New strategies have been developed to remove plant glycans and humanize the *N*-glycan and *O*-glycan profiles of recombinant human glycoproteins produced in plants (Yang et al. 2012; Strasser 2013). Thus far, such efforts have focused on the targeted expression of therapeutic proteins, e.g. the use of ER-retention signals such as KDEL to prevent proteins being transported through the Golgi complex, where plant-specific modifications are carried out. More recently, plants have also been engineered to abolish the genes encoding enzymes that carry out plant-specific modifications and to introduce the enzymatic machinery needed to humanize the glycan profiles of recombinant proteins (Gomord et al. 2010; Yang et al. 2012; Castilho et al. 2013). For some recombinant products plant-specific glycosylation is even desirable. A good example is recombinant human glucocerebrosidase produced in carrot cells, the first plant-derived biopharmaceutical which has received approval for human use (Morrow 2012). The glycoprotein is targeted to accumulate in vacuolar compartments to take advantage of plant-specific paucimannosidic structures for improved uptake (Shaaltiel et al. 2007).

## Commercialization of molecular pharming: inertia in the biopharmaceutical industry

The first commercial pharmaceutical protein derived from plants was approved in 2012, i.e. more than 20 years after human pharmaceutical proteins had been expressed in tobacco and potato (Hiatt et al. 1989; Fischer et al. 2012, 2013). Despite the advantages of molecular pharming discussed above, the major biopharmaceutical players have been slow to adopt the technology (Fig. 1), reflecting their continuing reliance on fermentation infrastructure, the long-term absence of a universal regulatory framework, and the low yields reported in early molecular pharming experiments (Ma et al. 2003).

Molecular pharming initially offered a range of diverse and overlapping platforms with different advantages and limitations that could be selected to match the requirements of different recombinant proteins. However this diversity has made it difficult to establish molecular pharming as a single, competitive platform (Fischer et al. 2012). The pharmaceutical industry favors a small number of standardized platforms whose performance has been incrementally improved over several decades, e.g. the bacterium *Escherichia coli*, the yeasts *Saccharomyces cerevisiae and Pichia pastoris*, and selected mammalian cell

lines such as CHO cells (Fischer et al. 2013). The recent renaissance in molecular pharming reflects a similar focus on specific platforms, including plant cell suspension cultures, transient expression in tobacco, and transgenic tobacco and cereals (Fischer et al. 2013).

Although the breakthrough into commercial pharmaceutical products only occurred in 2012, molecular farming (i.e. for non-pharmaceutical proteins) was already well-established. This small industry sector was initiated by a small number of companies in the 1990s, the prominent example being Prodigene Inc. (College Station, USA) who successfully launched several plant-derived recombinant proteins for non-pharmaceutical use, e.g. technical reagents, such as avidin and β-glucuronidase, produced in maize seeds (Hood 2002). These industry pioneers succeeded because they considered the whole production process from an economic perspective, including the often-ignored but vital downstream processing steps, and this led to the realization that molecular farming was economically viable even when the natural source of a protein is abundant and a market already established (e.g. egg whites for avidin and *Escherichia coli* for β-glucuronidase). It may seem strange that pharmaceutical products took more than 15 years to achieve the same success but the reason for this is clear. Unlike technical reagents, such as β-glucuronidase, pharmaceuticals must be manufactured under strict regulatory guidelines that comply with the principles of GMP, and until the 2000s the regulations were fragmented and contradictors (see next section). In 2006, a veterinary subunit vaccine against Newcastle disease in poultry was produced in tobacco cell suspension cultures by Dow AgroSciences (Indianapolis, USA). It was approved for commercialization by the US authorities, which was an important breakthrough in terms of proof-of-principle and regulatory acceptance (Dow AgroSciences 2006) but it was not marketed, perhaps due to the low product yields that never exceeded 8 μg/ml culture medium (Cardineau 2008). The commercial breakthrough for products intended for human use required several events to coincide, namely support for translational research, the emergence of niche markets ideal for plants and the development of new regulatory processes.

The importance of translational research is best exemplified by the 2009 DARPA initiative to develop strategies for the large-scale manufacture of influenza vaccines. The challenge was met by several organizations, companies and consortia developing transient expression in tobacco and alfalfa as a way to scale up production rapidly to meet urgent demands. For example, the Fraunhofer USA Center for Molecular Biotechnology (Delaware) focused on its 'launch-vector' system, and has produced up to 200 mg hemagglutinin antigen per kg fresh leaves within weeks of receiving novel sequencing information. Similar projects were launched by Kentucky Bioprocessing (Owensboro) using its Geneware expression technology, by the GreenVax consortium (Texas A&M University and G-Con), and by Medicago USA (North Carolina) which has benefited recently from significant investment by the Japanese pharmaceutical company, Mitsubishi Tanabe Pharma Corp. These organizations have invested heavily in GMP manufacturing facilities to provide the infrastructure required for the production of clinical-grade products, leading to successful phase-1 clinical trials (Fischer et al. 2013).

Even so, the only plant-derived biopharmaceutical product currently approved for human use is a recombinant form of human glucocerebrosidase (known as prGCD, taliglucerase alfa or Elelyso) indicated for the treatment of Gaucher's disease, produced in carrot cells by Protalix BioTherapeutics, Israel (Shaaltiel et al. 2007; Morrow 2012). The development of production platforms based on plant cells was encouraged because the existing regulatory framework focused on mammalian cells (see below), but this approach abandons the advantage that whole plants offer in terms of scalability. The same limitations apply to other 'bioreactor' systems such as the LEX system based on the aquatic plant *Lemna minor*, which was recently acquired by the Dutch pharmaceutical company, Synthon, in 2012 (Paul et al. 2013). However, these platforms remain advantageous in niche markets because they can exploit unique aspects of plants, such as the control of glycosylation. In the case of Elelyso, the protein is targeted to the cell vacuole where the complex type *N*-glycans are trimmed to the paucimannose form, exposing terminal mannose residues that interact favorably with macrophages when administered to humans (Shaaltiel et al. 2007). In contrast, the commercial recombinant human glucocerebrosidase produced in mammalian cells (imiglucerase or Cerezyme) has terminal sialic acid residues that must be trimmed off in vitro before formulation, increasing production costs and making it among the most expensive

current biopharmaceuticals on a per gram basis (Hollak et al. 2010). Protalix BioTherapeutics is currently developing a portfolio of other biopharmaceuticals using its carrot cell ProCellEx system including a version of Elelyso suitable for oral delivery (http://www.protalix.com/development-pipeline/overview-development-pipeline.asp).

Transgenic plants have several advantages over other platforms, including scalability to agricultural production levels and the stability offered by protein expression in seeds. Although a longer development phase is required to establish productive lines and generate seed banks, the payoff is that transgenic plants are suitable for low-margin or commodity products where the demand and economy of scale rule out the use of fermenters (Paul et al. 2013). For example, the demand for antibody-based HIV microbicides could be 0.5 g per person treated per year (Shattock and Moore 2003). As some ten million women could potentially receive such a treatment, demand could be in the order of several tonnes per year. This would be impossible to achieve using conventional fermenters due to the limited scalability and cost (Ramessar et al. 2008c; Sabalza et al. 2012). Furthermore, the largest market segment for HIV prophylaxis is resource-limited countries that can ill afford the development costs.

Seeds are promising manufacturing platforms for developing countries because they are natural protein storage organs with an appropriate biochemical environment to achieve stable protein accumulation with no substantial loss of activity (Sabalza et al. 2013). This stability means that seeds containing recombinant proteins can be stored and distributed in countries where a cold chain is unreliable or unavailable; hence seeds are likely to be the most suitable production system for deployment in developing countries (Twyman et al. 2005). Edible seeds also have GRAS status (generally regarded as safe for human consumption) making them particularly suitable for the development of oral vaccines that can be administered as flakes or flour with minimal purification (Twyman et al. 2005; Peters and Stoger 2011). Downstream processing costs can be reduced by using seed extracts rather than purified seed-derived proteins. This would be suitable for topical products, such as griffithsin, cyanovirin-N and HIV-neutralizing antibodies, which could be used for passive immunization to prevent the spread of HIV. For such applications, the presence additional plant proteins and metabolites would not present a significant risk because of the routine contact and consumption of such compounds when preparing and eating food (O'Keefe et al. 2009; Ramessar et al. 2008a, c; Sexton et al. 2009; Sabalza et al. 2012, 2013). The industry is overlooking these specific advantages of whole plants and this is contributing to the slow development of a regulatory process, which in turn creates a disincentive to take up the technology (Sharma and Sharma 2009; Lico et al. 2012).

## A global regulatory framework for molecular pharming

Initially, regulatory guidance for the production of recombinant pharmaceutical proteins in plants existed only as draft legislation and, in the EU, this was based on the existing regulations for mammalian cells. This legislation was therefore inappropriate for applications involving whole plants. More recent guidelines have been drafted that better reflect the idiosyncrasies of plant systems, although there remain major differences between the systems adopted in the USA and the EU (Ramessar et al. 2008a; Fischer et al. 2012, 2013).

In the USA, the licensing of most drugs and diagnostics is overseen by the Food and Drugs Administration (FDA), whereas veterinary vaccines are regulated separately by the US Department of Agriculture (USDA) Center for Veterinary Biologics. Draft legislation, prepared jointly by the USDA and FDA for the production of pharmaceutical proteins in plants, considered a wide range of platforms and accepted that some flexibility would be required for the implementation of GMP guidelines (FDA, USDA 2002). In the EU, the European Medicines Agency (EMA) oversees medicines and the European Food Safety Authority (EFSA) considers the non-food uses of plants (Spök et al. 2008). Molecular pharming is therefore subject to overlapping regulations and must meet the requirements under Directive 2008/27/EC (governing the release of transgenic plants into the field), Regulation 1829/2003/EC (governing food and feed products) and EMA guidelines on the manufacture of medicinal products. As stated above, the draft EU guidelines were hastily adapted from the existing guidelines regulating mammalian cells and did not include any of the biologically-relevant aspects of plants, e.g. the production of seeds, instead relying on

concepts only relevant to cell cultures, such as cell banking and batch-to batch consistency based on clonal identity (CPMP 2002; Spök et al. 2008). The flexibility of the FDA/USDA guidelines was overlooked and only stable transgenic plants were considered as a legitimate platform, effectively ruling out the development of commercial processes based on transient expression in the EU (CPMP 2009; Tremblay et al. 2010). Furthermore, all biopharmaceutical products intended for phase-1 trials must now be manufactured using a GMP compliant process so, whereas in the past, it was possible to take plant-derived pharmaceutical products into clinical development without a GMP process, it is now imperative that GMP is considered at the earliest stages of process development (Fischer et al. 2012).

The many advantages provided by molecular pharming will not be realized unless the general barriers to the adoption of genetically engineered crops are overcome, and these are political rather than technical (Farre et al. 2011). Regulations differ from country to country, particularly within the EU (Ramessar et al. 2008a, b; Ramessar et al. 2010; Sabalza et al. 2011; Masip et al. 2013). The benefits of molecular pharming would be promoted by the adoption of a rational, science-based and globally harmonious regulatory framework that removes trade barriers and embraces risk/benefit analysis rather than the current precautionary approach focusing on the elimination of all risks (Ramessar et al. 2008a, b).

## Conclusions and perspectives

After more than two decades, molecular pharming has made significant progress with the recent approval of recombinant human glucocerebrosidase produced in carrot cells for the treatment of Gaucher's disease and the successful production of clinical-grade proteins in diverse plant-based production platforms. Technical barriers have been overcome by addressing factors that affect transgene expression, protein synthesis and accumulation at the genetic, epigenetic, biochemical and environmental levels, making plants more competitive as production platforms. However, the introduction of any new technology into a conservative market such as the pharmaceutical industry is challenging. Molecular pharming is unlikely to displace traditional platforms by direct competition and does not seek to do so, but it can offer an effective and economical alternative manufacturing approach in niche markets by offering rapid development and production, unparalleled scalability, unique quality attributes such as tailored glycan profiles, and oral or topical applications of minimally-processed plant tissues thus reducing downstream costs. In the developing world, molecular pharming provides a unique economic opportunity that exceeds the capabilities of conventional manufacturing processes because plants can be used for the production of high-volume/low-margin pharmaceuticals that cannot be produced economically in fermenter systems. The production of pharmaceutical proteins in whole plants, particularly in seeds, offers the most cost-efficient platform of molecular pharming in the developing world because there is no need for expensive infrastructure or highly-trained personnel, and the economy of scale keeps the cost of goods low even when demand is high because expanding production only requires more land. Therefore, in the next 5–10 years we believe there will be a change in perspective as developing countries begin to take up niche products that can only be produced at the appropriate cost/benefit ratio using plants. This will herald the beginning of a new era of plant biotechnology in which plants are more widely accepted as not only an agricultural platform but also a technological platform for the production of commodity pharmaceuticals.

Plant cells represent the first steps towards industry acceptance of molecular pharming because the technical and regulatory principles are similar to established platforms based on microbial and mammalian cells, despite the unique metabolic and biochemical properties of plants. This is a small step towards the widespread use of whole plants, which will require specific regulations that also accommodate the unique biological properties of plants, such as seed banking. In this context, the current regulatory framework applied to genetically engineered plants in the EU is another barrier to overcome before molecular pharming can be adopted for the commercial or humanitarian production of biopharmaceuticals on a large scale. The regulations must be streamlined and harmonized into an effective and rational set of guidelines to maximize the potential of plants as a source of inexpensive, life-saving pharmaceuticals while still achieving safety and adequate oversight. We believe that in the next 5 years, the number of approved products originating

from genetically engineered plant cells will increase dramatically, making the use of plant cells (and later whole plants) for pharmaceutical production a mainstream approach. Once this hurdle has been overcome, plants will eventually take their place among the established platform technologies and will become the first port of call for certain pharmaceutical products, particularly those required rapidly in large quantities, and those required on an agricultural scale.

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