Chapter 9
Mathematical Modelling in Recombinant Plant Systems: The Challenge to Produce Heterologous Proteins Under GLP/GMP

Abstract In the last time and in the near future, the markets are demanding large recombinant proteins production capacity, due to the production levels of traditional platforms will be certainly not suffice to make available these demands (Huang and McDonald, Biotechnol Adv 30:398–409, 2012). The explore of alternative tools to assure adequate supplies for a growing demand constitutes a challenge of present times being the "bioengineered pharmaceutical plant" a promising strategy to respond to this require at affordable costs. However, as a commercial process requires high productivities and product yield at minimum cost, and, also, regulations must be follows like GMP and GLP. A mathematical model like the ones described in the present chapter allows making predictions and control of biosynthesis and yielding into the process.

Keywords Mathematical modeling • Recombinant proteins-*Nicotiana tabacum-molecular* farming-cell suspension cultures

9.1 Introduction

According to FDA/USDA (2002) "bioengineered pharmaceutical plant" is a plant manipulated by recombinant DNA technology to express a gene encoding a biological or drug product. Plants expressing transiently o constituently recombinant proteins are considered competitive systems for producing high-value recombinant proteins for medical and industrial purposes. This technology (molecular pharming® or biopharming or agropharming) has advanced greatly as a secure technology, capable of rendering valuable recombinant proteins free of toxins and animal pathogens in a relatively short time and cost-effective (Ma et al. 2005; Franconi et al. 2010; De Muynck et al. 2010; Paul and Ma. 2011; Yuan et al. 2011). This scenario opens up promising horizons to produce recombinant proteins with attractive prospects for commercial exploitation. Many proofs of principles studies just to commercialization have been obtained in the last 25 years (Twyman et al. 2003; Arcalis et al. 2013). Sigma-Aldrich commercializes plant-made recombinant *avidin* and bovine *trypsin*, Dow AgroSciences LLC produces plant-made recombinant veterinary vaccines and it has recently been approved by FDA the enzyme Taliglucerase alfa, a carrot-expressed recombinant glucocerebrosidase for the

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treatment of Gaucher disease, to be commercialized by Pfizer (FDA application No (NDA) 022458, 2012). Furthermore, Heber Biotec (Havana, Cuba) has produced the first whole antibody in tobacco plants. It was also demonstrated that most of the recombinant antibodies produced in plants maintains the functional properties as the ones produced in mammalian cell cultures (Kaiser 2008; Zimran et al. 2011; Zeitlin et al. 2011).

9.2 Industrial Production

Manufacturing bioengineered pharmaceutical plants are documented clearly in standard operating procedures (SOPs), Outlines of Production, or other records, following strict regulations (FDA and USDA Regulations 1999, 2002).

Facilities and procedures used for the manufacturing of regulated products, like bioengineered pharmaceutical plants, should be designed following good laboratory practice and good manufacturing processes (GLP and GMP resp.), conditions to ensure pure, sterile, non-toxic, in the case of vaccines assure potency, among other characteristics (Shepherd 1999; EudraLex 2011, ISO 14644-1, Fischer et al. 2012). Product characterization, the manufacturing process, and *in vitro* studies followed by animal studies, are prerequisites to initiating clinical trials. GLP are production and testing practices that helps to ensure a quality product. GLP arranges with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. Also, GLP practices involve a set of regulations designed to establish standards for the conduct and reporting of nonclinical laboratory studies and to ensure the quality and integrity of nonclinical safety data submitted to the regulatory authorities. In that way, GLP data are intended to promote the quality and validity of test data.

GMP has been legislated to pharmaceutical and medical device companies that must follow these procedures, assuring high quality products and batch-to-batch reproducibility. GMPs regulate the manufacture, control, accountability, and documentation of drug or biologic substances and products manufactured for human or veterinary use. The regulations, also, require adequate controls for the release and security of the products before they are allowed to arrive into the general marketplace.

9.3 Industrial Production in Plant Platforms

Plants offer numerous advantages for the production of biopharmaceutical proteins including the capacity to express complex heterologous proteins with posttranslational modifications as glycan patterns and proline hydroxylation among others (Vézina et al. 2009; Eskelin et al. 2009; Gomord et al. 2010; Ahmad et al. 2010;

Xu et al. 2011; Fischer et al. 2012; Strasser 2012). Also, the plant platform has demonstrated to be a secure technology, capable of rendering valuable recombinant proteins free of toxins like retrovirus, prions, mycotoxines and animal pathogens (inherent safety reflecting the inability of human pathogens to replicate in plants) in a relatively short time (Ma et al. 2005; Franconi et al. 2010; De Muynck et al. 2010; Paul and Ma 2011; Yuan et al 2011). Moreover, transient expression has the advantage of a rapid scaled up, therefore offering a rapid response to emerging pandemics or bioterrorism response or the possibility to develop personalized (patient-specific) vaccines (Giritch et al. 2006; Daniell et al. 2009; D'Aoust et al. 2010; Rybicki 2010; Twyman et al. 2012). McCormick et al. (2012) described a plant viral expression system to produce personalized recombinant idiotype vaccines following the concept of a tailor- made programmed. The vaccines against follicular B cell lymphoma were derived from each patient's tumor and it was reported in a phase I clinical trials. Transient tobacco-viral expression system was used for rapid production of the needed amount of idiotype vaccine.

Additionally, other advantages are the low cost of upstream production and the potential for large-scale cultivation (Molina et al. 2004; Escribano and Perez-Filgueira 2009; Thomas et al. 2011; Fischer et al. 2013). The industrial recombinant protein production is achieved by two main platforms: transient expression in plants and fermentation-based platforms with stable expression or transgenic expression in cell suspension cultures.

9.4 Transient Expression Platform

In the last years, bioprocess with binary or viral vectors have also been interested for the rapid and systemic infection and the large amounts of product obtained to achieve an industrial scale. Agro-infiltration, or transient expression system, is a biotechnology method to transiently express a gene during a short period of time without genetic modification (epigenetic modification) in a protoplast, cell, tissue, organ or plant infected. Binary or viral vectors harboring a sequence of the recombinant protein could be introduced in the nucleus of plant target cells by Agrobacterium sp. infection or biobalistic among other technologies (Gleba et al. 2007; Lico et al. 2008; Sainsbury and Lomonossoff 2008; Paul and Ma 2011). The epigenetic expression of the recombinant protein is obtained few days post infection (usually 4–7 days with binary vector or 10–14 days from viral vectors), in significant quantities. After this period of time, the heterologous expression down due to silencing RNAs that encoded to the recombinant protein. Double-stranded RNA is formed between heterologous RNA and complementary endogenous small interfering RNA (siRNA or miRNA). The double strand structure obtained (dsRNA) can trigger posttranscriptional gene silencing (PTGS). The dsRNA activated was cleaved into 22–25 nt RNAs which act as guides to target homologous mRNA sequences for their destruction (Mishra and Mukherjee 2007; Chau and Lee 2007).

For that reason, during transient expression assays never occurred an integration of the transgene into the host genome due to the completely destruction of foreign RNA expression. It is important to point out that transient expression is not considered to be an alteration of genomic plant material and, in consequence, the plant is not considered like GMO (Genetically Modified Organism). Also, cell, tissue, organ or plants transiently transformed do not exhibit macroscopic responses to *A. tumefaciens* infections or bombardment by the gene gun.

The agroinfiltration assays are commonly carried out using *N. tabacum* (tobacco) plants grown in pots and incubated in room chambers. The main reason of use tobacco plants is due to their leaves that are easy to infiltrate and manipulate. Furthermore, tobacco plants are recommended due to the protection against food-chain contamination. Other species agroinfiltrated are tomato and lettuce (Wroblewski et al. 2005).

Also, a variety of strategies were emerging in order to increase yield and achieve an efficient transient platform production during the last decade. One of these emerging technologies is "Magnifection" described as scalable process that can be done on an industrial scale (Gleba et al. 2005). This method involves an efficient vector design carrying T-DNAs encoding RNA replicons combined with and efficient systemic DNA delivery of the viral expression platform by mixing different Agrobacterium lines harbouring fractions of the viral machinery and agrobacteria delivery. In addition, speed, expression level, and yield of a plant RNA virus were drastically increased by altering the codon usage of the virus and the inclusion of typical eukaryotic introns. These modifications were able to the efficiency of delivery, resulting in a reduction in the amount of required infectious agrobacterium (Marillonnet et al. 2005).

In 2006, Bayer and Icon Genetics adopted this methodology and have developed a new production process combining these technologies and transient transformation platform in tobacco plants. Finally, in 2009, Bayer Innovation GmbH present the whole process (http://www.youtube.com/watch?v=fVOBEk5DVZc) following the concept of tailor made pharma.

The FAST technique (Fast Agro-mediated Seedling Transformation) was developed in order to express a wide variety of constructs driven by different promoters in Arabidopsis cotyledons (Li et al. 2009). This method has an especially high potential, ideal for future automated high-throughput analysis (Kolukisaoglu and Thurow 2010). Giritch et al. (2006) using a plant viral vector obtained high expression of heterologous proteins with potential to scale up the process (see below).

ProficiaTM is described as an efficient method to response quickly to emerging diseases (http://www.medicago.com/English/Technologies/Why-Proficia/default.aspx). This platform is described with the high advantage to produce vaccines or therapeutics antibodies in "unmatched speed" obtaining an end product like a vaccine in less than 3 weeks.

Other commercially approaches are extensively reviewed by Paul and Ma (2011).

9.5 Fermentation-Based Platforms

Fermentation-based platforms are carried out in bioreactor batch cell cultures with industrial capacity resulted in high cell densities and production levels. The recombinant or transgenic plants are obtained by introduction a DNA segment (T-DNA), carrying the recombinant protein, into the nucleus of infected plant cells using *Agrobacterium tumefaciens* as biological delivery system or direct transfer methods like biobalistic, microinjection among others (Alvarez and Marconi 2011). Using either indirect or direct methods, it is possible to introduce foreign DNA into any regenerable plant cell type. Using Agrobacterium technology, the T-DNA plasmid containing the heterologous DNA sequence is hazardous inserted in the plant genome, into a chromosome, by illegitimate recombination or non-homologous end-joining (Somers and Makarevich 2004). The stable transformation using Agrobacterium technologies integrate one copy of T-DNA sequence into the cell genome using a T-vector carrying: the recombinant protein, the selectable and the marker genes. After 48 hs post-infection, the recombinant explants must be isolated from the large excess of untransformed cell population using the selectable marker (like antibiotics, herbicides, among others) allowing the recombinant cells proliferation. Cells that do not have the T-DNA integrated into their genome will die, obtaining uniform (nonchimeric) transformants from vegetatively propagated cells. Cloning transformed cells (calli) are induced from recombinant explants grown in a defined media containing growth regulators.

Transformed cells could be grown as microorganism or animal cell culture, easy to scale up. Plant cell bioreactors are obtained in large scale as a safe, convenient and economical production system for recombination proteins in shorter production cycles. This clean technology, growing transgenic material in a confined environment, has been widely accepted by the public perception due to their biosafety implications.

Geneware® expression technology (www.kbpllc.com) developed by Kentucky BioProcessing, LLC (KBP; Owensboro, KY), is specialized on the expression, extraction, and purification of recombinant proteins from plants at commercial scale level. Sigma-Aldrich Fine Chemicals (SAFC; St. Louis, MI) named in the introduction, produce in plant-based platforms therapeutic protein extraction and purification, bio-conjugates, excipients and adjuvants. Medicago Inc., (Quebec City, Canada) manufacturing capability for plant manipulation, product recovery and purification.

Other companies producing plant-based pharmaceuticals are Protalix (Carmiel, Israel) that utilizes a novel bioreactor for plant cell culture system, ProCellExTM, based on disposable sterile plastic bags; Biolex Therapeutical (Pittsboro, NC) based on the aquatic plant *Lemna minor* (www.biolex.com/lexsystem.htm). PharmaPlanta has a pilot scale facility to produce recombinant subunit vaccines, mAbs and other therapeutic proteins, among other products (Huang and McDonald 2012).

9.6 The Main Problem

Commercialization plant-based expression systems were characterized by the lack of a support for large scale facilities manufacturing these products conform to current Good Laboratory and Manufacturing Practice guidelines. The main obstacles to solve are the lack of reproducibility between batches and the variable expression levels within the batch culture. The variations observed in growth and production is a consequence of plant physiology *per se* with genetic and metabolic regulatory controls characteristic of plant kingdom. In transient expression system, the strict temperature control during the growth of plants at the room chamber and the defined post-infiltration harvest-time and the age of leaves to agroinfiltrate are critical for the reproducibility of the results (Buyel 2013). In batch cultures, the plant cells tend to aggregate, are prone to rapid sedimentation, and are vulnerable to high shear sensitivity. For that reason, the manner in which the bioreactor is operated is critical for the reproducibility of the results (Huang and McDonald 2012; Marconi et al. 2014).

The relationship between relative addition rates of nutrients and relative growth rate varies as an unpredictable function in cells and plants. It is due to plants altering periods of assimilation of nutrients with starving stages. The consequence is a pulse on/off between the high nutrient concentration with high availability and relative growth rate, with starving periods where the growth is arrested (Poorter and Lewis 1986; Cannel and Thornlay 2000). During the assimilation period a relative growth rate could be described as:

$$\boxed{\frac{1}{W}\frac{dW}{dt} = \beta\left(N - N_{min}\right)}$$

Where W is weight, β is the slope of the response and N is the nitrogen into the plant and N_{min} is the nitrogen at minimal concentration when growth is arrested. The N is integral to the leaf proteins, being of photosynthetic machinery (Wright et al. 2004).

Taking into account these variations, in growth, and as a consequence, in production parameters, mathematical models could be applied to predict the growth of plant cells being useful tools for the industry. Many predictive mathematical models describing the dynamics of biomass growth, product biosynthesis rates and nutrient substrate consumption are derived from microbial cultures. Also, the complexity of models varies enormously, being affected by the abilities, available data and the objectives of the modelers. However, it is expected that a mathematical model can be used to predict or represent or to describe the behavior of a system using mathematical language. Also, models must facilitate process optimization, including of increasing product quality and productivity and reducing manufacturing cost, risk, and time. In the present chapter, two models are present for transient and stably plant and cell cultures.

9.7 Mathematical Modeling for Transient Platform

Johannes F. Buyel (2013) presented an interesting Thesis where a mathematical modeling is developed for plant transient expression systems. The protein model to be express was a monoclonal antibody 2G12 described as specific for the HIV gp120 glycoprotein. Also, a marker protein, DsRED was used as tracer in the experiments. The set of experiments was done using transient expression in *Nicotiana tabacum* var. Petit Havana SR1 by infiltration of leaves with *Agrobacterium tumefaciens* harboring the recombinant plasmid.

The infiltrated leaf was subdivided into four positions parallel to the main vein as a matrix (Fig. 9.1). This variable was indicated by the suffix *p*. Also, the plant leaves were identified by age since the number 1 assigned for the oldest to the apical leaf (number 8). The suffix to denote the individual leaf into de plant was *k* (same figure).

The quantity and distribution of the recombinant protein could be processed like a mathematical matrix. The initial concept is described by this equation:

$$\boxed{C[P] = \frac{m[P]}{V}}$$

Where, C[P] is the recombinant protein concentration and m[P] is the mass of the recombinant protein in a known extract volume (V).

The matrix could be described as:

$$\boxed{V_{k,p} = m_{t,k} \cdot \eta_k \cdot \Psi_{k,p} \cdot \varepsilon}$$

Where the extract volume (V) for the position *p* in a *k* leaf has a mass average of m in this leaf *k*, η modified by effective biomass ratio for each *k* leaf (calculated by the intercostal field biomass (g) per biomass extracted (g)) and the position correction Ψ for each leaf *k* (biomass of position *p* per biomass extracted in g). Finally, ε is the

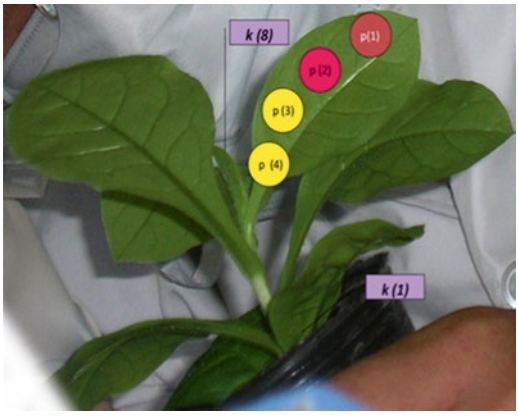

Fig. 9.1 *N. tabacum* leaves to be used to transiently express a protein

extract ratio calculated by the volume of solids-free extract (mL) per biomass extracted (g).

The final recombinant protein yield is obtained from a linear second-order polynomial fit:

$$\mathfrak{f}(V_{k,p}, \beta_k) = \beta_{k,1} + \beta_{k,2}\, V_{k,p} + \beta_{k,3}\, (V_{k,p})^2; \beta_k = (\beta_{k,1}, \beta_{k,2}, \beta_{k,3})$$

The study includes a quantitative impact of many parameters, determined and modeled using a design of experimental approach. The post-infiltration incubation temperature, plant and leaf age and incubation time with Agrobacterium were found to be major factors influencing protein yields and growth variation.

9.8 Mathematical Modeling for Fermentation-Based Platforms

The recombinant full antibody r14D9 was used as a protein model to be expressed in *Nicotiana tabacum* cv. Xhanti NN cell suspension cultures (Petruccelli et al. 2006; López et al. 2010). For the bioreactor production, cell suspension expressing the antibody r14D9 with the retention signal KDEL at endoplasmic reticulum were scaled up from a 225 mL Erlenmeyer flask to 2L-bioreactor. Growing cell suspension cultures in a batch bioreactor is often complicated due to the cell aggregation process that leads to the formation of zones where the cell growth is limited. The growth could be restricted by oxygen and nutrients demands, increase the heterogeneity of culture and concentration of toxic substances, among others. Also, plants cells have a critical inoculation density below which growth is arrested. The consequence will be the accumulation of non-dividing cells in a short period of time, and finally aborting the experiments.

Typical slope of the growth kinetics and production of recombinant protein have high standard error with high dispersion of scores around the means (Fig. 9.2). This curves could be characterized with an early exponential period with low recombinant protein production, a gradual slowdown as dividing cell reaches its maximum and a subsequent stationary face (same figure). The recombinant protein production shows more unpredictable features. However, the recombinant protein production curve suggests the production rate follows the dividing or proliferating cells concentration.

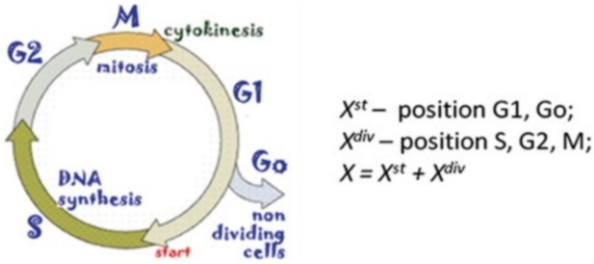

Fig. 9.2 Schematic representation of plant cell cycle

As a result, the cell population rapidly differentiates into dividing (growing and dividing cells) and non-dividing (stable) cells and each population growth with a proper kinetics. Thus, in order to calculate the dynamics of cell growth it is necessary to take into account the difference in energy substrate metabolism of stable and proliferating cells in such zones and use both structured and unstructured models that were previously successfully applied for microbial batch processes (Klykov and Kurakov 2012, 2013).

The mathematical model is based on the theory of energy-limited growth that allows precise evaluation of a cell population age structure into the bioreactor.

The model is based on treating the biomass as two main groups: dividing and non-dividing cells (using a combination of statistical data and qualitative causal assumptions) (Fig. 9.2). Therefore, a phytofermentation process is a "mixture" of cells of different ages being this population characterized by the parameter R:

$$R = \frac{X^{st}}{X}$$

Where X^{st} is the non-dividing cell concentration at the synchronized degree R, and X is the total biomass. The age structure of the cell population varies, thus characterizing these variations is fundamental for the structured model.

On the other hand, the unstructured model is based on the decrease of the absolute and the specific growth rates of the biomass, as these parameters are directly related to the biomass concentration at the growth limitation phase when oxygen is limited (GIP: growth inhibition phase):

$$A = \frac{m}{a}$$

In this model, the energy substrate consumption rate for viability maintenance (m) is specified by the oxygen and trophic coefficient (a) that is assumed as a constant during GIP. The population is synchronized to non-proliferating cells, which consume oxygen only for primary metabolism.

Equation of the structured model for biomass in the GIP is:

$$\frac{d^n X^{div}}{d\left(X^{st}\right)^n} = \frac{K(-1)^{(n-1)}\, n!}{A^2\left(X^{st}\right)^{(n+1)}} - C$$

Where n – is an integer that specifies the order of the derivative of this function; X^{div} – is the quantity of dividing cells; X^{st} – is the quantity of non-dividing (stable) cells; k – is the overall growth rate multiplied by the rate of accumulation of stable (or non-dividing) cells; A – is the ratio of energy required to maintain viability to energy required for biomass accumulation and/or maintaining the rate of accumulation of stable (non-dividing) cells. In addition, if $n = 1$ then $C = 1$, if $n \geq 2$ then $C = 0$.

The equation of a structured model for substrate (metabolites) in growth inhibition phase is:

$$\boxed{d(P \text{ or } -S)/d\tau = k^{div}{}_{P,S}X^{div} + k^{st}{}_{P,S}X^{st}}$$

P and S symbols are the valuable metabolite and the substrate, respectively, $k^{div}{}_{P,S}$ and $k^{st}{}_{P,S}$ coefficients are constants synthesis (utilization) of dividing and non-dividing cells. In this model, it is assumed that metabolites are synthesized only by proliferating (dividing) cells. Non-proliferating (stable) cells, as a rule, destroy these products. Therefore, signs of the constants for metabolite synthesis and degradation are opposite. The same should be stated for substrates utilized for cell construction.

The equation for the unstructured model in growth inhibition phase (GIP) is:

$$\boxed{X = X_p - \left(X_p - X_{lim}\right) * \exp^{\left(-A*(\tau - \tau\,lim)\right)}}$$

Where X is the amount of biomass calculated using an unstructured model; X_p – the maximum estimated amount of biomass; X_{Lim} – the amount of biomass at the start of the limitation of cell growth; τ and τ_{lim} are the terms of the estimated time of cultivation and cultivation duration from start until the beginning of the limitation of cell growth.

These equations describe all the known diversity of the processes with S-like growth curves and changes in the concentrations of substances in closed systems, which is an entirely new and previously unknown fact. It is known that a physical law means a generalization of a numerical relationship between the objects of the real physical world that is running under specified conditions for the class of the objects and does not follow from any of the previously discovered laws. There is no reason not to admit the two described equations for the GIP as laws for GIP. The data obtained were used for the selection of techniques to increase the protein expression by genetically modified microorganisms (Klykov et al. 2011).

The proposed modeling approach for the cultivation of plant cells using a structured model reflects the real dynamics of changes in the age structure of cell populations in the batch culture. Isolating growing cells based on physiological age into two groups – dividing and stable cells (non-dividing), allows applying a structured model for scaling up from the bench to a phytofermenter. Processing data, obtained during cultivation and according to the structured model, gives the opportunity to deeper understand the dynamics of changes occurring in the cell population, accurately predict both the dynamics of biomass growth and biosynthesis of a target protein or metabolite and significantly reduce the cost of improving cultivation of cells on an industrial scale.

9.9 Conclusions

Development of transgenic plants is a technology used to basic studies like functional genomics research and, also, in modern plant breeding and in the last years as a platform for recombinant proteins production. There are many protocols to obtain transient or transgenic plants expressing heterologous proteins. In both systems, heterologous protein production like transient platforms or fermentation-based platforms proved to be a suitable process to obtain large- scale productions. However, a commercial process requires high productivities and product yield at minimum cost (Scragg 1992), and, also, regulations must be follows like GMP and GLP. A mathematical model like the ones described before allows making predictions and control of biosynthesis and yielding into the process.

References

Ahmad A, Pereira EO, Conley AJ, Richman AS, Menassa R (2010) Green biofactories: recombinant protein production in plants. Recent Pat Biotechnol 4:260–262

Alvarez MA, Marconi PL (2011) Genetic transformation for metabolic engineering of tropane alkaloids. In: Alvarez MA (ed) Genetic transformation, vol 15. Intech Publisher, Croatia, pp 291–304

Arcalis E, Stadlmann J, Rademacher T, Marcel S, Sack M, Altmann F, Stoger E (2013) Plant species and organ influence the structure and subcellular localization of recombinant glycoproteins. Plant Mol Biol 83:105–117

Buyel T (2013) Manufacturing biopharmaceutical proteins by transient expression in *Nicotiana tabacum* (L.). PhD thesis, Institute for Molecular Biotechnology at the RWTH Aachen University

Cannel MGR, Thornlay JHM (2000) Modelling the components of plant respiration: some guiding principles. Ann Bot 85:45–54

Chau BL, Lee K (2007) Function and anatomy of plant siRNA pools derived from hairpin transgenes. Plant Methods 3:13–37

D'Aoust MA, Couture MM, Charland N, Trépanier S, Landry N, Ors F et al (2010) The production of hemagglutinin-based virus-like particles in plants: a rapid, efficient and safe response to pandemic influenza. Plant Biotechnol J 8:607–619

Daniell H, Singh ND, Mason H, Streatfield SJ (2009) Plant-made vaccine antigens and biopharmaceuticals. Trends Plant Sc 14:669–679

De Muynck B, Navarre C, Boutry M (2010) Production of antibodies in plants: status after twenty years. J Plant Biotechnol 8:529–563

Escribano JM, Perez-Filgueira DM (2009) Strategies for improving vaccine antigens expression in transgenic plants: fusion to carrier sequences. Methods Mol Biol 483:275–287

Eskelin K, Ritala A, Suntio T, Blumer S, Holkeri H, Wahlstro EH, Baez J, Ma K, Nuutila AM (2009) Production of a recombinant full-length collagen type Ia-1 and of a 45-kDa collagen type I a-1 fragment in barley seeds. Plant Biotechnol J 7:657–672

EudraLex (2011) Volume 4: The rules governing medicinal products in the European Union. Part I: Medicinal products for human and veterinary use. EU guidelines for good manufacturing practice. EU Publisher, Brussels

FDA (1999) Draft guidance for industry: cooperative manufacturing arrangements for licensed biologics. US FDA, Rockville

FDA/USDA (2002) Draft guidance. Drugs, biologics, and medical devices derived from bioengineered plants for use in humans and animals. FDA, Rockville MD. http://www.fda.gov/

downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM055424.pdf
Fischer R, Schillberg S, Hellwing S, Twyman RM, Drossard J (2012) GMP issues for recombinant plant-derived pharmaceuticals proteins. Biotechnol Adv 30:434–439
Fischer R, Schillberg S, Buyel JF, Twyman RM (2013) Commercial aspects of pharmaceutical protein production in plants. Curr Pharm Des 19:5471–5477
Franconi R, Demurtas OC, Massa S (2010) Plant-derived vaccines and others therapeutics produced in contained systems. Expert Rev Vaccines 9:877–892
Giritch A, Marillonet S, Engler C, van Eldik G, Botterman J, Klimiuk V et al (2006) Rapid high-yield expression of full-size IgG antibodies in plants coinfected with noncompeting viral vectors. Proc Natl Acad Sci USA 103:4701–14706
Gleba Y, Klimyuk V, Marillonnet S (2005) Magnifection – a new platform for expressing recombinant vaccines in plants. Vaccine 23:2042–2048
Gleba Y, Klimyuk V, Marillonnet S (2007) Viral vectors for the expression of proteins in plants. Curr Opin Biotechnol 18:134–141
Gomord G, Fitchette AC, Menu-Bouaouiche L, Saint-Jore-Dupas C, Plasson C, Michaud D et al (2010) Plant-specific glycosylation patterns in the context of therapeutic protein production. Plant Biotechnol J 8:564–587
Huang T-K, McDonald KA (2012) Bioreactor systems for in vitro production of foreign proteins using plant cell cultures. Biotechnol Adv 30:398–409
Kaiser J (2008) Is the drought over for pharming? Science 320:473–475
Klykov SP, Kurakov VV (2012) New kinetic structured model for cell cultivation in a chemostat. BioProcess Int 10:36–43
Klykov SP, Kurakov VV (2013) Analysis of bacterial biomass growth and metabolic accumulation. BioProcess Tech 11:36–43
Klykov SP, Kurakov VV, Vilkov VB, Demidyuk IV, Gromov TY, Skladnev DA (2011) A cell population structuring model to estimate recombinant strain growth in a closed system for subsequent search of the mode to increase protein accumulation during protealysin producer cultivation. Biofabrication 3:045006
Kolukisaoglu Ü, Thurow K (2010) Future and frontiers of automated screening in plant sciences. Plant Sci 178:476–484
Li JF, Park E, von Arnim A, Nebenfuhr A (2009) The FAST technique: a simplified Agrobacterium-based transformation method for transient gene expression analysis in seedlings of Arabidopsis and other plant species. Plant Methods 5:6
Lico C, Chen Q, Santi L (2008) Viral vectors for production of recombinant proteins in plants. J Cell Physiol 216:366–377
López J, Lencina F, Petruccelli S, Marconi PL, Alvarez MA (2010) Influence of the KDEL signal, DMSO and mannitol on the production of the recombinant antibody 14D9 by long-term *Nicotiana tabacum* cell suspension culture. Plant Cell Tissue Organ Cult 103:307–314
Ma J, Barros K-C, Bock E, Dale RCP, Dix PJ, Fischer R, Irwin J et al (2005) Current perspectives on the production of pharmaceuticals in transgenic plants. EMBO Rep 6:593–599
Marconi PL, Alvarez MA, Klykov SP, Kurakov VV (2014) Mathematical model for production of recombinant antibody 14D9 by *Nicotiana tabacum* cell suspension batch culture. BioProcess Int 12:42–49
Marillonnet S, Thoeringer C, Kandzia R, Klimyuk V, Gleba Y (2005) Systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants. Nat Biotechnol 23:718–723
McCormik AA, Reddy S, Reinl SJ, Cameron TI, Czerwinkski DK, Vodjani F et al (2012) Plant-produced idiotype vaccines for the treatment of non-Hodgkin's lymphoma: safety and immunogenicity in a phase I clinical studies. Proc Natl Acad Sci USA 105:10131–10136
Mishra NS, Mukherjee SK (2007) A peep into the plant miRNA world. Open Plant J 1:1–9
Molina A, Hervas-Stubbs S, Daniell H, Mingo-Castel AM, Veramendi J (2004) High-yield expression of a viral peptide animal vaccine in transgenic tobacco chloroplasts. Plant Biotechnol J 2:141–153

Paul C, Ma J (2011) Plant-made pharmaceuticals: leading products and production platforms. Biotechnol Appl Biochem 58:58–67

Petruccelli S, Otegui MS, Lareu F, Trandinhthanhlien O, Fitchette A-C, Circosta A, Rumbo M, Bardor M, Carcamo R, Gomord V, Beachy RN (2006) A KDEL-tagged monoclonal antibody is efficiently retained in the endoplasmic reticulum in leaves, but is both partially secreted and sorted to protein storage vacuoles in seeds. J Plant Biotechnol 4:511–527

Poorter H, Lewis C (1986) Testing differences in relative growth rate: a method avoiding curve fitting and pairing. Physiol Plant 67:223–226

Rybicki EP (2010) Plant-made vaccines for humans and animals. Plant Biotechnol J 8:620–637

Sainsbury F, Lomonossoff G (2008) Extremely high-level and rapid transient protein production in plants without the use of viral replication. Plant Physiol 148:1212–1218

Scragg AH (1992) Chapter 4: Bioreactor for the mass cultivation of cell plants. In: Fowler MW, Waren GS (eds) Plant Biotechnology. Pergamon Press, Oxford

Shepherd AJ (1999) Chapter 19: Good laboratory practice in the research and development laboratory. In: Meager A (ed) Gene therapy technologies, applications and regulations. Wiley, Chichester, pp 375–381

Somers DA, Makarevich I (2004) Transgene integration in plants: poking or patching holes in promiscuous genomes? Curr Opin Biotechnol 15:126–131

Strasser R (2012) Challenges in O-glycan engineering in plants. Front Plant Sci 3:218

Thomas DR, Penney CA, Majumder A, Walmsley AM (2011) Evolution of plant-made pharmaceuticals. Int J Mol Sci 12:3220–3236. Molecular farming in plants: host systems and expression technology. Trends Biotechnol 21:570–578

Twyman RM, Stoger E, Schillberg S, Christou P, Fischer R (2003) Molecular farming in plants: host systems and expression technology. Trends Biotechnol 21:570–578

Vezina LP, Faye L, Lerouge P, D'Aoust MA, Marquet-Blouin E, Burel C, Lavoie PO, Bardor M, Gomord V (2009) Transient co-expression for fast and high-yield production of antibodies with human-like N-glycans in plants. Plant Biotechnol J 7:442–455

Wright IJ, Reich PB, Westoby M, Ackerly D, Zdravko B, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T et al (2004) The world wild leaf economic spectrum. Nature 428:821–827

Wroblewski T, Tomczak A, Michelmore R (2005) Optimization of Agrobacterium mediated transient assays of gene expression in lettuce, tomato and Arabidopsis. Plant Biotechnol J 3:259–273

Xu J, Ge X, Dolan MC (2011) Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures. Biotechnol Adv 29:278–299

Yuan D, Bassie L, Sabalza M, Miralpeix B, Dashevskaya S, Farre G, Rivera S et al (2011) The potential impact of plant biotechnology on the Millennium Development Goals. Plant Cell Rep 30:249–265

Zeitlin L, Pettitt J, Scully C, Bohorova N, Kim D, Pauly M, Hiatt A, Ngo L, Steinkellner H, Whaley KJ, Olinger GG (2011) Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. Proc Natl Acad Sci USA 108:20690–20694

Zimran A, Brill-Almon E, Chertkoff R, Petakov M, Blanco-Favela F, Terreros Muñoz E et al (2011) Pivotal trial with plant cell–expressed recombinant glucocerebrosidase, taliglucerase alfa, a novel enzyme replacement therapy for Gaucher disease. Blood 118:5567–5773