Chapter 1
Introduction: Plant-Produced Protein Products

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1.1 A Short History of Recombinant Protein Production in Plants

Recombinant protein production in plants encompasses vaccines, pharmaceuticals, and industrial proteins. Within each of these categories are numerous products and host systems with applications to multiple diseases and industrial processes. This industry requires gene transfer from other organisms into plants and allows the plants to overproduce the proteins for the desired application.

Several companies and university laboratories have had programs in plant expression of proteins over the past two decades. The plant biotechnology companies that are focused on production of those proteins are listed in Table 1.1. Significant effort has gone into developing these new products using several plant systems. The choice of system depends on many factors including the type of protein, the technology utilized, the platform of the company, and the funding source (Howard and Hood 2005). Several of these companies are still functional, and others have closed but reemerged as new entities.

1.2 Advantages of Using Plants

Compared to animal and microbial systems, the advantages of using plants for protein production are numerous. For example, plants do not harbor animal pathogens, which is particularly advantageous for pharmaceuticals and vaccines

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Table 1.1 Protein production companies, crops, and product foci over the last three decades

Company	Crop	Main products	Status	Comments
ProdiGene	Maize	Pharma, vaccines, enzymes	Development	Inactive
EpiCyte	Maize/tobacco	Antibodies	Development	Out of business
SemBioSys	Safflower	Pharma, vaccines	Clinical trials	Out of business
Meristem	Maize/tobacco	Lipase, lactoferrin	Development	Out of business
Crop Tech	Tobacco	Enzymes for ERT	Licensed to Protalix and Pfizer	Out of business
Biolex	Lemna	Pharmaceuticals	Research only	Out of business-some products in development by Synthon
Medicago	Transient tobacco	Pharma, vaccines	In production	Company sold to Mitsubishi
Planet Biotechnology	Stable tobacco	Anthrax antitoxin	Successfully protects animals in trials	Planning clinical trials
Ventria	Rice	Blood proteins and therapeutics	Selling research products	Human clinical trials in process
Icon Genetics	Transient tobacco	Biotherapeutics and monoclonal antibodies	Clinical trials in process	Acquired by Nomad
Syngenta	Maize	Amylase—enolase for corn ethanol	In production	First industrial output trait deregulated
Applied Biotechnology Institute	Maize	Cellulases, HepB vaccine, brazzein	Development	Licensed ProdiGene technology
Infinite Enzymes	Maize	Cellulases	Reagent sales	Other enzymes in pipeline
BioStrategies	Transient tobacco	Pharma enzymes for ERT	Development	SBIR funded
Caliber Biotherapeutics	Transient tobacco	Vaccines and monoclonal antibodies	Preclinical trials	Production with G-Con pods
Fraunhofer MBC USA	Transient tobacco	H1N1 and malaria vaccines	Preclinical and clinical trials	Nonprofit organization
Kentucky BioProcessing (formerly LSBC)	Transient tobacco	Aprotinin, vaccines, pharmaceutical	Used in research and cell culture	In production
Mapp Biopharmaceutical	Transient tobacco	Monoclonal antibodies	Preclinical trials	Made by Kentucky BioProcessing

Protalix	Carrot cell culture	Glucocerebrosidase	In production for Gaucher's syndrome	Primarily Israeli market
ORF Genetics, Iceland	Barley	Human growth hormone, cytokines	Diagnostics, research, cosmetics	In production from seed
Dow Agrosciences	Cell culture	Newcastle disease viral vaccine	Approved	Unknown if company is selling
Inserogen	Tobacco	Vaccines and biotherapeutics	Development	Start-up

ERT enzyme replacement therapy, *LSBC* Large Scale Biology Corporation

(Ramessar et al. 2008; Sabalza et al. 2011). Pathogen-free pharmaceuticals are desirable whether delivered orally or through injections. Thus, the plant host can be a food crop, such as corn, canola, or rice, or a nonfood crop, such as tobacco.

Because several of the plant hosts are food plants, oral delivery of the proteins for therapeutic purposes is possible. Oral delivery has been demonstrated for potato (Tacket et al. 1998), corn (Lamphear et al. 2004; Hayden et al. 2012), and banana (Mason et al. 2002). In each case, the integrity of the protein must be ensured through the formulation process, e.g., extrusion or cooking. If raw, the plant host must be edible without processing, such as a fruit or vegetable. In contrast to injected pharmaceuticals, the cold chain may not be required to transport these orally delivered products to the target population, which is particularly useful when serving developing countries. This is a distinct advantage for plant systems—high product stability at ambient temperatures.

Direct addition of the proteins in their host tissue may be possible without the need for purification. This can be an advantage for pharmaceuticals as well as industrial proteins and enzymes. The less processing required for a formulation, the more cost-effective the manufacturing. Thus, direct addition of the plant part containing the enzyme of interest saves money on production and increases the margin for the producer. Direct addition would be particularly useful for industrial enzymes that accumulate in dry seed, such as corn, where stability is ensured in the seed until such time as it is used (Howard et al. 2011).

An additional advantage is when current agricultural crops are used as plant hosts; their production and processing are well established and usually inexpensive. As an example, corn requires few inputs other than nitrogen if grown in the corn belt. Dry mill processing is very well established on a volume basis, and every fraction of the whole or milled corn has a market. If value can be added to one of the lower value coproducts, for example, by putting a high-value protein in the germ (Hood et al. 2007), then an advantage is gained in increasing the value of this coproduct of the corn-to-ethanol industry.

Scaling up production of proteins from crops is also advantageous over animal or microbial systems. For crops, scale-up involves planting and harvesting more acres and does not require additional capital investment in physical infrastructure. The only capital investment involves planting and harvesting equipment, which, although somewhat expensive, does not require the level of investment required for scaling up microbial or animal systems. Thus, high-volume production can be achieved relatively easily.

1.3 Issues for Commercialization

Intellectual property for the specific gene and its expression in a plant host is only one part of the legal landscape for commercializing products using the plant production platform. Plant-enabling methods have been developed over many years with many companies and university laboratories participating in the

platform. Thus, a plethora of patents surround the technology and are often barriers to entry for commercialization of products from genetically engineered plants. During the development of potential products, it is critical to be aware of the technology pieces that are utilized to ensure freedom to operate on the pieces. Licenses for technology can sometimes burden the developer with high royalty fees, pushing the products' costs to a price greater than they are worth.

1.3.1 Regulatory Issues and Public Acceptance

1.3.1.1 Europe

The European Food Safety Authority (EFSA) is a European Union (EU) agency mandated to evaluate the risks of all transgenic crops based on scientific evidence. This evidence is evaluated by a panel of experts, and testing is carried out at an EU reference laboratory. As such, EFSA is best placed to advise individual Member States and the EU as a whole on safety issues (Sabalza et al. 2011). EU legislation for the approval of GE crops (Directive 2008/27/EC and Regulation EC 1829/2003) is the most onerous and restrictive in the world. Regulatory compliance for a new crop with first-generation simple agronomic traits can cost up to €11 million (~US$15 million) and requires a dedicated legal team working for many years (Kalaitzandonakes et al. 2007).

The EU regulatory approach is precautionary, process-based, and includes mandatory labeling and traceability requirements (Ramessar et al. 2008). The approach has been described in detail in a recent review (Sparrow et al. 2013). Briefly, EU legislation is adopted through a system of interactions between the three main EU institutions: the European Parliament, the Council of the European Union, and the European Commission (Sparrow et al. 2013). The EFSA published guidance notes in 2009 on the risk assessment of genetically modified plants used for nonfood or non-feed purposes (EFSAPanel 2009) including molecular pharming applications. The European Medicines Agency (EMEA) that oversees the assessment of biopharmaceuticals and vaccines published guidance notes in 2006 on the "quality of biological active substances produced by stable transgene expression in higher plants" (EMEA 2008), which looks at such issues.

More recently a further requirement was imposed on all transgenic plants, including those for molecular pharming applications. The European Commission mandated a compulsory 90-day animal feeding trial and, to make matters even more complicated, is considering extending that to a 2-year trial based on the now-discredited article by Seralini et al. (Seralini et al. 2012; Arjó et al. 2013). The scientific community as well as regulators themselves questioned the validity of such whole food-based animal trials (Kuiper et al. 2013).

Once authorization has been received, farmers must ensure that they comply with the conditions laid down by the authorities in their Member State and/or local region, often finding that illegal national or regional bans on GM agriculture have

been imposed. Farmers must abide by the coexistence measures that have been implemented in each Member State or region, and the complexity of these regulations and their strict implementation often means that it is impossible to comply. The four major obstacles to GM agriculture in the EU post-authorization are:

1. Public field registers showing the location of commercially grown GM crops are compulsory in almost all Member States and tend to discourage farmers from adopting GM agriculture because of the threat of vandalism by activists.
2. Six Member States use a "safeguard clause" nominally based on environmental or health concerns, to implement national cultivation bans for approved GM crops (Austria, France, Germany, Greece, Luxemburg, and Hungary).
3. Stringent coexistence measures have been implemented in Belgium, the Czech Republic, Germany, Hungary, Portugal, Romania, and Slovakia, which make it impossible to grow GM crops without risking litigation from the surrounding farms.
4. The negative publicity surrounding GM agriculture in Europe, which means farmers are ostracized and intimidated directly or indirectly.

The public in Europe has adopted a predominantly anti-GM stance, which is fueled by politicians and media eager to exploit public sentiment. This vicious cycle also shows no sign of going away any time soon (Farre et al. 2011). As discussed above the rules governing the commercial cultivation of GM crops in Europe are obstructive and arbitrary, making it virtually impossible for a farmer to make an independent decision to adopt the technology on his/her land even if the crop in question has been approved (Ramessar et al. 2010).

Across Europe the political viewpoint of cultivating GM crops is far from harmonious, with a number of Member States banning such cultivation (http://www.greenbiotech.org) (Ramessar et al. 2008, 2009, 2010; Sabalza et al. 2011). Given the state of play surrounding the cultivation of agricultural GM crops, it is unlikely that we will see a pharmaceutical crop grown commercially in Europe any time soon (Masip et al. 2013; Sparrow et al. 2013).

1.3.1.2 United States

Regulations in the USA for transgenic plants are set by the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). The regulatory framework is complex and expensive with a lack of standardization for data collection and analysis (Hood et al. 2012). The framework is somewhat coordinated in that each agency is responsible for specific types of approvals—USDA for plant pests, FDA for food and feed issues, and EPA for pesticides, although sometimes the lines overlap or are blurred. A recent review describes the legislation and several case studies that apply the standards as they currently stand in the USA (Sparrow et al. 2013). To facilitate the process, particularly for small and specialty crop developers, a basic road map

should be created from which a specific regulatory path can be planned and implemented (Hood et al. 2012).

Public acceptance in the USA is much less of an issue than in Europe. Although anti-GMO groups are active in the USA, their impact has waned over the years. The success of genetically engineered crops has been good, showing higher yields and fewer pesticide or herbicide inputs. The vast majority of corn and soybeans in the USA are produced from GE crops and occur in many processed foods. Thus, even though some resistance occurs against GE plants in the press, the basic fact is that most citizens are consuming GE foods on a daily basis without incident. Indeed, each of the crops was subjected to a vast array of safety studies that were reviewed not only by the USDA APHIS but also by the FDA to ensure human safety. Miller (2011, 2012) published some editorial opinion pieces recently on the status of GE crops worldwide and received a great deal of criticism. However, the facts are correct and supported by such groups as the Grocery Manufacturers Association (http://www.gmaonline.org/news-events/newsroom/gma-commends-ama-action-in-support-of-continued-use-of-genetically-engineer/).

1.4 The Case Studies

Several reviews of plant-produced proteins have been written over the last several years (Fischer et al. 2004; Stoger et al. 2005; Streatfield 2007; Daniell et al. 2009; Egelkrout et al. 2012). Each of these reviews describes issues concerning expression, different product categories, and advantages of different plant systems. Plant biotechnology and gene transfer have been practiced as a technology since the early 1980s, and the vast majority of products commercialized have been input traits that assist with production, e.g., insect and herbicide resistance (Castle et al. 2006; Fraley 2009).

In this volume, the focus is on products from plants that either have been commercialized or that are near commercialization. We have chosen protein products that illustrate the promise of the system, for example, highly purified proteins without concerns over animal pathogen contaminants and directly delivered proteins—orally delivered vaccines or minimally processed industrial products. The promise of plant-made recombinant proteins was first realized in 1997 with the introduction of avidin and β-glucuronidase. Recently, pharmaceuticals (PMP) and vaccines as well as industrial proteins (PMIP) have just recently been consummated with the introduction of Syngenta's Enogen corn that contains amylase for the starch to ethanol application (Pollack 2011) and Protalix and Pfizer's glucocerebrosidase for enzyme replacement therapy (Aviezer et al. 2009; Ratner 2010). The products described in these chapters do not represent all the work that has been done in transgenic plants but do represent several that have been moved into or near commercialization.

Hood and Howard describe development of avidin in corn seed, originally transformed in as a potential candidate for insect resistance. Although the insect resistance trait was not commercialized, avidin was subsequently purified from seed and sold. This product was a key achievement for the plant manufacturing industry as the first protein sold from transgenic plants (Hood et al. 1997) (Sigma Chemical Co. A8706) and set the stage for this platform (Chap. 2). Although the avidin market is small, its importance cannot be overstated since it was the demonstration product for the technology. The main application of this protein is as a research reagent that allowed quick market entry.

Other types of products such as vaccines and pharmaceuticals were also in development concurrently but had much longer timelines for market entry. Fischer et al. (Chap. 3) describe multiple therapeutics that include antibodies for several applications manufactured in plant production systems. These therapeutics are produced by a number of different platform technologies, and the issues for their commercialization are discussed in the context of these new products.

Krishnan and Woodard (Chap. 4) describe the development of recombinant trypsin from the maize seed production system. This product is sold under the trade name TrypZean™ and is currently used for research and for processing of therapeutic proteins. One of the largest applications of trypsin is the maturation of recombinant insulin, and the plant-derived protein could be a great improvement in this process since it is animal product-free and would not pose threats to the drug's use.

Aprotinin is manufactured in the transient tobacco system using an engineered tobacco mosaic virus vector (Chap. 5). It has major applications in surgery as a preventative for perioperative blood loss. The plant-made aprotinin is currently not approved for human use but has applications as a protease inhibitor in cell culture.

Vaccines are particularly well suited for plant production because of broad application and current need for a cold chain. Vaccines against a number of viruses have been developed using plant expression systems. Pandemic flus can threaten world health quickly and catastrophically. In order to address the need for rapid development of vaccines against urgent threats, Medicago Inc. established a platform technology that addressed surge capacity, speed, adaptability, and affordable cost per dose. The company developed a vaccine against the H1N1 flu virus in a transient tobacco expression system (Chap. 6) and showed efficacy in Phase I and Phase II clinical trials. Further development of the vaccine will be performed by Mitsubishi who recently acquired Medicago.

Malarial vaccines are extremely useful in tropical climates where mosquitoes are abundant. Streatfield et al. (Chap. 7) discuss the transient tobacco transformation system for the production of such a vaccine against the malarial parasite that is spread by the mosquito vector. Subunit vaccines using individual proteins have been difficult to develop because of the difficulty in expressing the individual antigens. The plant system has been particularly useful in this regard.

Transmissible gastroenteritis virus (TGEV) is a common pathogen of swine and is particularly dangerous to newborn piglets. Rajan (Chap. 8) describes the development of a subunit vaccine in corn seed that shows efficacy against the disease, particularly when delivered orally either through feed or colostrum from the sows. Although this highly efficacious and easily administered vaccine is available, it has not been adopted by the swine industry.

Many species and strains of rabies virus are known, posing a threat to human health worldwide, but particularly in developing countries. Loza-Rubio and Rojas-Anaya (Chap. 9) discuss the issues surrounding the development of a rabies vaccine based on the G-protein expressed in either corn seed or carrot roots. Both sources of the protein provided protection against the rabies virus in superinfected animals. These results are promising for the future of inoculation of wild animal populations to lower the load of infective viruses.

Newcastle disease virus is highly infective in avian species and can devastate poultry production in many countries. Gomez-Lim (Chap. 10) describes the development of plant-based vaccines against this virus using the corn/sorghum seed system for oral delivery or the tobacco system for injectable delivery. The ease of delivery of oral products would seem the preferred route and various issues to be overcome for this application are discussed.

Although several injectable vaccines for hepatitis B virus (HepB) are available, infection with this virus remains a world health problem. Hayden discusses the development and feeding trials of a plant-made oral vaccine from corn grain (Chap. 11). Oral vaccines have many advantages in that they have higher rates of dose compliance among susceptible populations. Using formulations of corn germ derived from transgenic plants expressing the S antigen, successful production of mucosal protective antibodies was achieved in mice.

Hood and Requesens (Chap. 12) describe the development of the industrial enzymes endo- and exo-cellulase in maize grain. These enzymes have applications in research, pulp processing, and biomass conversion. Early markets have been addressed with these products, and production lines have been established.

Finally, the sweet protein brazzein has been produced in maize grain. Fake and Howard (Chap. 13) describe the applications of this protein in various food-related industries and the effort to interest food companies in its use. Because the protein is a natural sweetener from an African fruit, it would be a logical substitution for such artificial sweeteners as acesulfame potassium or aspartame, particularly also because brazzein is about 1,000 times sweeter than sugar.

In the final chapter, the future of the plant-based production industry is discussed. Prospects are promising, but the major commercialization barrier is still overcoming the regulatory hurdles. Drs. Howard and Hood are pleased to present these case studies of plant-made proteins as a tribute to our colleague, Dr. Michael Horn.

References

Arjó G, Portero M, Piñol C, Viñas J, Matias-Guiu X, Capell T, Bartholomaeus A, Parrott W, Christou P (2013) Plurality of opinion, scientific discourse and pseudoscience: an in depth analysis of the Séralini et al. study claiming that Roundup™ Ready corn or the herbicide Roundup™ cause cancer in rats. Transgenic Res 22:255–267. http://parrottlab.uga.edu/parrottlab/Publications/Arjo-et-al-TRAG-2013.pdf

Aviezer D, Brill-Almon E, Shaaltiel Y, Hashmueli S, Bartfeld D, Mizrachi S, Liberman Y, Freeman A, Zimran A, Galun E (2009) A plant-derived recombinant human glucocerebrosidase enzyme—a preclinical and phase I investigation. PLoS One 4:e4792

Castle LA, Wu G, McElroy D (2006) Agricultural input traits: past, present and future. Curr Opin Biotechnol 17:105–112

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

EFSAPanel (2009) Scientific opinion on guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes. EFSA J 1164:1–42, E.F.S. Authority, ed (Parma, Italy: EFSA)

Egelkrout E, Rajan V, Howard JA (2012) Overproduction of recombinant proteins in plants. Plant Sci 184:83–101

EMEA (2008) Guideline on the quality of biological active substances produced by stable transgene expression in higher plants. In: E.M. Agency (ed) EMEA/CHMP/BWP/48316/2006. EMEA, London, pp 1–11

Farre G, Twyman RM, Zhu C, Capell T, Christou P (2011) Nutritionally enhanced crops and food security: scientific achievements versus political expediency. Curr Opin Biotechnol 22:245–251

Fischer R, Stoger E, Schillberg S, Christou P, Twyman RM (2004) Plant-based production of biopharmaceuticals. Curr Opin Plant Biol 7:152–158

Fraley RT (2009) Molecular genetic approaches to maize improvement–an introduction. In: Kriz AL, Larkins BA (eds) Molecular genetic approaches to maize improvement. Springer, Berlin, pp 3–6

Hayden CA, Streatfield SJ, Lamphear BJ, Fake GM, Keener TK, Walker JH, Clements JD, Turner DD, Tizard IR, Howard JA (2012) Bioencapsulation of the hepatitis B surface antigen and its use as an effective oral immunogen. Vaccine 30:2937–2942

Hood E, Witcher D, Maddock S, Meyer T, Baszczynski C et al (1997) Commercial production of avidin from transgenic maize: characterization of transformant, production, processing, extracting, and purification. Mol Breed 3:291–306

Hood EE, Love R, Lane J, Bray J, Clough R, Pappu K, Drees C, Hood KR, Yoon S, Ahmad A, Howard JA (2007) Subcellular targeting is a key condition for high-level accumulation of cellulase protein in transgenic maize seed. Plant Biotechnol J 5:709–719

Hood EE, Vicuna-Requesens D, Eversole KA (2012) Regulatory issues of biotechnologically improved plants. In: Altman A, Hasagawa PM (eds) Plant biotechnology and agriculture; prospects for the 21st century. Academic, London, pp 541–550

Howard JA, Hood E (2005) Bioindustrial and biopharmaceutical products produced in plants. Adv Agron 85:91–124

Howard J, Nikolov Z, Hood E (2011) Enzyme production systems for biomass conversion. In: Hood E, Nelson P, Powell R (eds) Plant biomass conversion. Wiley-Blackwell, Ames, IA, pp 227–253

Kalaitzandonakes N, Alston JM, Bradford KJ (2007) Compliance costs for regulatory approval of new biotech crops. Nat Biotechnol 25:509–511

Kuiper HA, Kok EJ, Davies HV (2013) New EU legislation for risk assessment of GM food: no scientific justification for mandatory animal feeding trials. Plant Biotechnol J 11:781–784

Lamphear BJ, Jilka JM, Kesl L, Welter M, Howard JA, Streatfield SJ (2004) A corn-based delivery system for animal vaccines: an oral transmissible gastroenteritis virus vaccine boosts lactogenic immunity in swine. Vaccine 22:2420–2424
Masip G, Sabalza M, Pérez-Massot E, Banakar R, Cebrian D, Twyman RM, Capell T, Albajes R, Christou P (2013) Paradoxical EU agricultural policies on genetically engineered crops. Trends Plant Sci 18:312–324
Mason HS, Warzecha H, Mor T, Arntzen CJ (2002) Edible plant vaccines: applications for prophylactic and therapeutic molecular medicine. Trends Mol Med 8:324–329
Miller H, Concko G (2011) The rush to condemn genetically modified crops. http://www.hoover.org/research/rush-condemn-genetically-modified-crops-0
Miller H (2012) NPRs bias against genetic engineering. http://www.forbes.com/sites/henrymiller/2012/02/01/nprs-bias-against-genetic-engineering/
Pollack A (2011) US approves corn modified for ethanol. The New York Times (February 11, B1)
Ramessar K, Capell T, Twyman RM, Quemada H, Christou P (2008) Trace and traceability—a call for regulatory harmony. Nat Biotechnol 26:975–978
Ramessar K, Capell T, Twyman RM, Quemada H, Christou P (2009) Calling the tunes on transgenic crops: the case for regulatory harmony. Mol Breed 23:99–112
Ramessar K, Capell T, Twyman RM, Christou P (2010) Going to ridiculous lengths—European coexistence regulations for GM crops. Nat Biotechnol 28:133
Ratner M (2010) Pfizer stakes a claim in plant cell-made biopharmaceuticals. Nat Biotechnol 28:107–108
Sabalza M, Miralpeix B, Twyman RM, Capell T, Christou P (2011) EU legitimizes GM crop exclusion zones. Nat Biotechnol 29:315–317
Séralini G-E, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois JS (2012) Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem Toxicol 50:4221–4231
Sparrow P, Broer I, Hood EE, Eversole K, Hartung F, Schiemann J (2013) Risk assessment and regulation of molecular farming-a comparison between Europe and US. Curr Pharm Des 19:5513–5530
Stoger E, Ma JKC, Fischer R, Christou P (2005) Sowing the seeds of success: pharmaceutical proteins from plants. Curr Opin Biotechnol 16:167–173
Streatfield S (2007) Approaches to achieve high-level heterologous protein production in plants. Plant Biotechnol J 5:2–15
Tacket CO, Mason HS, Losonsky G, Clements JD, Levine MM, Arntzen CJ (1998) Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nat Med 4:607–609