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REVIEW ARTICLE

Potential of Plants to Produce Recombinant Protein Products

Ky Young Park and Soo Jin Wi*

Department of Biology, Sunchon National University, Sunchon, Chonnam 57922 Korea

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Abstract Plants have great potential as photosynthetic factories to produce pharmaceutically important and commercially valuable biomedicines and industrial proteins at low cost. The U.S. Food and Drug Administration (U.S. FDA) has approved the drug Elelyso (taliglucerase alfa) produced by carrot cells for treatment of type 1 Gaucher's disease in 2012. The commercial potential of biomedicines produced by molecular farming has dramatically improved due to the success of an experimental drug called ZMapp, which has immunological activity in Ebola patients. A cocktail of three monoclonal antibodies was produced in tobacco (*Nicotiana benthamiana*) plants (Chen and Davis 2016). At present, very few drugs made by this technology have been approved by worldwide authorities such as the U.S. FDA. However, plants have been proposed as a novel paradigm for commercial production of proteins over the next decade. In recent years, leading researchers on molecular farming have given more priority to the area of animal-free therapeutic proteins such as parenteral and oral vaccines. Although plant-based platforms have considerable advantages over traditional systems such as bacterial and animal systems, there are several obstacles to commercial-scale production, especially with regards to improving the quality and quantity of plant-produced biologics and industrial materials. One of the biggest barriers to commercialization of this technology is the intense scrutiny of these new plant varieties by regulatory agencies and the public as well as the high costs associated with their regulatory approval.

Keywords: Pharmaceutical, Plant-made recombinant protein, Plant molecular farming, Public, Regulatory agency

*Corresponding author; Soo Jin Wi
Tel : +82-2-750-3617
E-mail : akrp@sunchon.ac.kr

Introduction

After plant-based expression systems emerged as miniature factories for producing recombinant proteins in the early 1990s, several plant-derived recombinant proteins have already been marketed (Fischer et al. 2013). Production of recombinant proteins in plants, which is performed by insertion of foreign genes encoding commercially important proteins into plant cells and then the manufacture of genetically modified organisms (GMO), is now one of the major applications of applied biotechnology (Federation of American Scientists, FAS; http://fas.org/biosecurity/education/dualuse-agriculture/2.-agricultural-biotechnology/usda-regulation-of-pharma-crops.html). Therefore, plants have great potential as photosynthetic factories with lower costs for production of pharmaceutically important and commercially valuable biomedicines and industrial proteins (Malabadi et al. 2016).

Large-scale application of this biotechnology can be referred to as molecular farming, and production of biomedicines and pharmaceuticals is called biopharming or molecular pharming. Based on the definition provided by the Canadian Food Inspection Agency (CFIA; http://www.collectionscanada.gc.ca/webarchives/20071119210921/http://www.inspection.gc.ca/english/plaveg/bio/mf/mf_glose.shtml), plant molecular farming involves the growth of plants in agriculture to produce pharmaceutical or industrial compounds instead of food, feed, or fiber (Twyman et al. 2003). Possibilities range from the manufacture of medical products, such as therapeutic proteins, vaccines, pharmaceuticals, diagnostic products, and other biologics, to the production of potential novel compounds such as biodegradable plastics and industrial chemicals (Fact Sheet in CFIA). Therefore, this technology can be used to expand the use of plants in agriculture to new products other than food, feed, or textile fibers.

The gene for a desired protein can be inserted into the main genome of the cell nucleus or circular genome of chloroplasts in plants. Production of biopharmaceuticals in transgenic plants may offer a cost-effective and highly scalable alternative to using engineered bacteria or mammalian cell

culture (Hood 2002). One advantage of biopharming is that plant cells possess the biochemical machinery necessary to fold complex proteins and perform post-translational modifications such as glycosylation for increasing efficacy required for full biological activity (Shaaltiel et al. 2007). Moreover, plants do not contain animal-originated viruses and other infectious pathogens that cause disease in humans. Beyond the theoretical aspect of this technology, there are now reported many cases confirming its commercial success or potential.

The commercial potential of biomedicines produced by molecular farming has dramatically risen due to the success of an experimental drug called ZMapp, which is a cocktail of three monoclonal antibodies produced in tobacco (*Nicotiana benthamiana*) plants (Chen and Davis 2016). At present, very few drugs made by this technology have been approved by worldwide authorities such as the U.S. Food and Drug Administration (U.S. FDA). In 2012, the U.S. FDA has approved sale of the drug Elelyso (taliglucerase alfa) manufactured in carrot cells by the Israeli firm Protalix Biotherapeutics for treatment of type 1 Gaucher's disease. This was the first plant-made drug approved by major regulators. However, plants have been proposed as a novel paradigm for commercial production of proteins over the next decade (Hood and Howard 2014).

In Korea, despite recent progress in plant biotechnological research at the laboratory level, the industrial infrastructure for plant molecular farming is highly vulnerable. Large numbers of GMO events from several plant species such as rice, potato, carrot, cabbage, hot pepper, apple, and soybean are under developed for expression of important traits in Korea (Lee 2010). A total of 422 cases of experimental research have been allowed by Korean authorities (2015. 12.31). However, very few events have obtained government approval for safety assessment (SA) application, and genetically modified (GM) plants developed by Korean researchers or in Korea are not yet approved for cultivation or commercialization. Only 149 events of imported GMOs have been approved for foods or food ingredients (2016. 8. 2, Ministry of Food and Drug Safety, MFDS), whereas 132 events of imported GMOs consisting of five species, soybean, corn, cotton, canola, and alfalfa, have been approved for agricultural use (Rural Development Administration, RDA).

The public perception toward GM plants is very negative in Korea, completely preventing their cultivation. At present, however, herbicide-resistant turf grass developed by Korean researchers is under preparation for field testing for SA. In addition, several GM crops, including GM rice resistant to leaf roll (folder) disease, are currently in field trials for SA application. As Korean regulatory authorities do not have enough experience to approve domestically developed GM crops, it is too difficult to gain approval for recombinant protein-producing GM plants. Therefore, manufacturers of plant-made products must consider the position of the government during the research process and overcome huddles to commercialization.

This review paper provides a brief global overview of plant molecular farming for recombinant protein production, together with strategic proposals for development of plant molecular farming in Korea.

Benefits of Plants for Production of Recombinant Proteins

Commercialization of plant-produced recombinant proteins has progressed slowly over the past two decades ever since plants were demonstrated as miniature factories for commercial protein production. This technology has remained in early stage and is not robust enough to compete on a cost basis with other existing platforms. The benefits of molecular farming based on true costs at the industrial scale are not readily known. Therefore, there is little motivation to fund technological improvements to a system that is considered as a complementary system to existing platforms (Howard and Christon 2014). However, plant production has the advantage of being an animal-free source of proteins with lower unit costs. In recent years, leading researchers on molecular farming have given more priority to the area of animal-free therapeutic proteins such as parenteral and oral vaccines.

The advantage of plant production technology was confirmed when two American health aid workers survived an Ebola outbreak after receiving the experimental Ebola drug ZMapp, which is a cocktail of three monoclonal antibodies (c13C6, c2G4, and c4G7) produced in tobacco plants. Five patients who received the experimental drug are still alive, including the two Americans. Two other patients, a Liberian physician and Spanish missionary priest, died despite taking ZMapp (Healy 2014). LeafBio, Inc., the commercial arm of Mapp Biopharmaceutical, Inc., announced the results of a clinical trial of its ZMapp™ therapy for treatment of Ebola on February 23, 2016 (Lyon 2016; Press Release, Mapp Biopharmaceutical, Inc.). The trial, conducted in Liberia, Sierra Leone, Guinea, and the United States over nearly 1 year, was concluded on January 29, 2016. As a result, ZMapp™ showed antiviral activity in humans, and the U.S. FDA has encouraged Mapp Biopharmaceutical to continue making ZMapp™ available to patients under an expanded access treatment protocol during the product's ongoing development.

This research was funded by the U.S. Biomedical Advanced Research and Development Authority (BARDA), the Department of Defense, the Department of Health and Human Services (National Institutes of Health), and the Office of the Assistant Secretary for Preparedness and Response (Homepage of ZMapp Bio). Tobacco-produced

ZMapp has several advantages. The first benefit of ZMapp is its speed of development. In the Ebola outbreak of 2014, the U.S. government and World Health Organization (WHO) sought to speed up production of Ebola drugs, as tens of thousands of people were expected to be infected by the virus within several months. ZMapp took 4 to 6 months to produce a clinical-grade medicine compared with the 12-to-20-month process required to make biologic drugs conventionally (Healy 2014). This fast new expression system cannot be matched by production technologies based on mammalian cell cultures (Chen and Davis 2016). Therefore, novel transient expression systems with suitable vectors are feasible for production of therapeutic proteins at unprecedented speed to control potential pandemics.

Currently, the technology for plant-based recombinant protein production has been newly developed. One of key benefits offered by plants as expression hosts is their lower costs compared to mammalian systems. Regarding economic evaluation of current plant-based platforms, production costs can be substantially reduced compared with those of traditional platforms (Tusé et al 2014). There have been two techno-economic case studies representing plant-made enzymes using tobacco host plants for diverse applications; human butyrylcholinesterase produced indoors for use as a medical countermeasure and cellulase produced in the field as a cellulosic biomass. Their analyses indicate that substantial cost advantages over alternative platforms can be achieved using plant systems, but these advantages are molecule/product-specific and depend on the relative cost efficiencies of alternative sources of the same product. Plant-sourced cellulase can be produced for just under $7/kg. These costs compare favorably to minimum $10.6/kg cost of the same fungal-sourced product (all costs adjusted to 2013 U.S. dollars) (Tusé et al 2014). They also reported that butyrylcholinesterase using a transient expression plant system costs approximately $474/dose to manufacture, although its cost can be reduced to less than $200/dose in a toll-manufacturing facility. Most of the capital costs (~60%) and a significant portion of the operating costs (>70–75%) are associated with the recovery and purification steps. Considering that oral immunization is a beneficial approach in terms of costs, patient comfort, and protection of mucosal tissues, use of food-grade plants such as rice, carrot, maize, lettuce, and strawberry can lead to highly advantageous vaccines in terms of costs, easy administration, and safety (Rosales-Mendoza et al 2016).

In addition to fast production and inherent safety due to lack of adventitious animal and human source agents, industrially relevant benefits of plant systems include proper post-translational modifications. Plant-host engineering provides a method for producing proteins with unique and uniform eukaryotic protein processing, thereby providing further opportunities to develop biologics with increased efficacy of therapeutics or proper functions of industrial products relative to their conventionally produced counterparts (Chen and Davis 2016). This technology allows complex proteins to be folded and assemble efficiently due to the presence of chaperones and protein disulfide isomerases that catalyze the formation of disulfide bonds, a capability not shared by bacterial production systems (Tschofen et al 2016).

New Approaches to Challenges Facing Plant-based Recombinant Protein Production

Although plant-based platforms have considerable advantages over traditional systems such as bacterial and animal systems, there are several challenges facing commercial-scale production, especially with regards to improving quality and quantity of plant-produced biologics and industrial materials. Economic viability of a production process depends on the yields of the product. The largest challenge facing recombinant protein systems is scaling up production of the biomass to meet demand. One approach to this problem is agroinfiltration, in which *Agrobacterium tumefaciens* is injected or vacuum-infiltrated into leaves so that leaf cells take up but do not integrate T-DNA into the nuclear genome, resulting in production of milligrams of protein within a few days (Tschofen et al. 2016). However, TMV-based vectors showed extremely inefficient Agrobacterium delivery, which was estimated at about one infection per 10^8 *Agrobacterium* cells. More recent work on vectors built from different tobamoviral strains, which encode replication proteins, has been more successful (Yamanaka et al. 2000) but still not efficient. Due to this limitation, the process is essentially infiltration of whole mature plants or detached mature leaves with a highly diluted suspension of agrobacteria harboring a proviral replicon in its T-DNA (Gleba et al. 2007).

Other transient expression systems have been developed based on systemic spreading of plant RNA viruses with greater efficiency. In recent years, advances have been made in the development of second-generation (deconstructed virus) vectors (Gleba et al. 2007), which have been combined with CPMV-HT (Cowpea mosaic virus hypertranslatable) platforms (Dugdale et al. 2013). This process, which relies on *Agrobacterium* as a vector to deliver DNA copies of one or more viral RNA replicons to plant cells, has been shown to work with numerous proteins.

Development of deconstructed viral vector systems (e.g. magnICON, geminiviral, and pEAQ) has successfully addressed the challenges facing insufficient protein expression levels, consistency, and speed of biologic production in plants (Chen and Davis 2016). For example, transient expression with deconstructed viral vectors allows production of up to 5 milligrams of monoclonal antibody per gram of fresh leaf

weight within 2 weeks (Hefferon 2012). Other advances have looked at the development of inducible viral systems in which deconstructed viruses cannot spread systemically.

To produce the 10 grams of antibodies needed for a single course of ZMapp immunization, Mapp Biopharmaceutical currently requires 30 to 50 kilograms of tobacco leaves. If tobacco plants could be engineered to produce antibodies more efficiently, that volume could be reduced to about 10 kilograms (Healy 2014). In fact, there are many challenges from logistical, regulatory, and economic aspects to large-scale production and use in the case of ZMapp. When the Ebola outbreak was threatening, Department of Health and Human Services (U.S. government) promptly devoted $24.9 million over 18 months to help San Diego-based Mapp Biopharmaceutical Inc. accelerate production.

Transient expression technology with deconstructed viral vectors has considerable advantages over processes that require generation and selection of transgenic plants, which can take months to years (Chen and Davis 2016). The rapid and high-level protein production capability of transient expression technology makes it an optimal system to produce milligram and gram levels of biologics for pre-clinical studies. Despite the advantages of a transient expression system, large-scale production using a greenhouse is still limited and therefore relatively expensive. Agrobacteria is more expensive and can accommodate only limited volumes of plant materials.

Therefore, this intermediated version of deconstructed viral vectors have been further studied for the development of a scaled-up manufacturing system of biologics in stable transgenic plants, the production of which often results in low and inconsistent protein yield. Inducible expression of recombinant proteins with deconstructed viral vectors and chemical inducers can be used for yield optimization, as it allows separation of the growth and production phases in manufacturing. The process is based on inducible release of viral RNA replicons from stably integrated DNA proreplicons after ethanol treatment in transgenic plants (Werner et al. 2011). Two components, the replicon and cell-to-cell movement protein, are placed separately under the control of an inducible promoter to achieve tight control of replicon activation in uninduced transgenic plants. After induction by incubation for 24 h in ethanol vapor, transgenic *Nicotiana benthamiana* (*N. benthamiana*) plants incorporating this double-inducible system demonstrated high (over 5,000 fold) RNA amplification, followed by high absolute levels of protein (up to 4.3 mg/g of fresh biomass) (Werner et al. 2011).

The platform has the combined advantages of a transient gene expression and stable gene transformation in plants. The technology, In Plant Activation (INPACT), is based on a two-cassette system, which is stably integrated into the host nuclear genome (Dugdale et al. 2013). Expression of the gene of interest is activated by an ethanol spray, and there is negligible expression in the absence of the ethanol inducer molecule due to the unique split gene arrangement. In their experiment, *Nicotiana tabacum* cv Samsun (*N. tabacum* cv Samsun) rather than *N. benthamiana* was used due to the potential of high biomass tobacco for field production. The INPACT system is based on the replication machinery of the tobacco yellow dwarf mastrevirus and is essentially a transient gene expression from a stably transformed plant. The INPACT cassette is uniquely arranged such that the gene of interest is split and only reconstituted in the presence of TYDV-encoded Rep/RepA proteins. Rep/RepA expression is under the control of the AlcA:AlcR gene switch, which is responsive to trace levels of ethanol. The authors reported that transgenic tobacco (*N. tabacum* cv Samsun) plants containing an INPACT cassette encoding the β-glucuronidase (GUS) reporter showed negligible background expression but accumulated very high GUS levels (up to 10% total soluble protein) throughout the plant within 3 days of 1% ethanol application. The INPACT gene expression platform is scalable, not host-limited, and has been used to express both therapeutic and industrial proteins. Thus, deconstructed viral vectors offer a set of versatile tools that can rapidly evaluate biologic candidates and then transition them to large-scale commercial manufacturing (Chen and Davis 2014). These improved vectors with higher efficiency can be used with several other plant species, although not as efficiently as in *N. benthamiana* (Gleba et al. 2007).

Post-translational modifications during and after protein synthesis include glycosylation, γ-carboxylation, β-hydroxylation, amidation, proline hydroxylation, and sulfation (Wang et al. 2014). Glycosylation has received the most attention due to differences in N-glycan and O-glycan structures between plants and mammals. Post-translational modifications of proteins in plants are different from those in animal/human cells. Glycosylation can affect protein structure, biological activity, and stability when the protein is injected as a drug (Strasser et al. 2014). In particular, plant cells have different N-glycosylation machinery that results in the addition of plant-specific sugars (β1,2-xylose and core α 1,3-fucose residues), which might be immunogenic in humans. In contrast, plants do not synthesize animal-specific sugars such as β1,4-galactose residues or sialic acid (Gleba et al. 2007).

Precise control of glycosylation has recently allowed production of plant-derived glycoproteins with human-like or human-compatible glycans to improve efficacy or longevity or to simplify downstream processing using either RNA interference (Cox et al. 2006) or by expressing a human or chimeric β1,4-galactosyltransferase (Bakker et al. 2006). It is therefore possible to make host plants expressing recombinant proteins that are essentially devoid of β1,2-xylose and core

α1,3-fucose residues more human-like and safer. Such engineered expression hosts could be used both for transient or transgenic expression systems to express glycosylated proteins. In the near future, post-translational modification can be improved in plants by using new genome engineering techniques such as zinc finger and the recent CRISPR/Cas9 system (Gleba et al. 2007; Tschofen et al. 2016). Even though glycosylation is less relevant for non-pharmaceutical proteins, other forms of modification are necessary for some products to assemble properly; for example, proline hydroxylation is required for assembly of collagen (Faye et al. 2005).

To make ZMapp, infected tobacco plants in a transient expression system are grown in windowless hydroponic warehouses under artificial light. Within 7 to 10 days after virus infiltration, the virus kills the plant, turning it yellow and causing it to wilt. After the dying plants are harvested and ground into a green sludge, the mixture of recombinant antibody proteins is purified (Healy 2014).

An enclosed glasshouse facility was constructed to grow transgenic tobacco plants. The cost of biomass produced in such facilities will always exceed that of open-field crops due to high startup costs, labor, and running costs. There are several key advantages to growing plants in an enclosed glasshouse, such as eliminating both potential gene flow through escape of pollen and release of product into the environment and facilitating compliance with environmental regulations (Paul and Ma 2011). Close control of environmental factors using an enclosed facility can be used to optimize plant-made protein yield and maintain quality of protein products, which may improve batch-to-batch consistency after downstream processing. These factors are core factors of Current Good Manufacturing Practice (cGMP), an international standard for production of therapeutics.

Even though initial laboratory-scale experiments are suitable for the purification of gram levels of recombinant proteins from a few kg of biomass in transient or transgenic plants, pilot-scale experiments are larger than laboratory-scale but smaller than industrial-scale. Therefore, more specialized equipment can be provided for pilot-scale production in order to further characterization of biological activities and safety analysis. However, many researchers and manufacturers face difficulties in pilot-scale production during development, as it is too difficult to get a permit for therapeutic proteins (Sparrow et al. 2014).

Regulatory Challenges

Despite resolving the technical challenges of commercial-scale production, there are still many challenges in garnering acceptance from large pharmaceutical companies and obtaining U.S. FDA approval for plant-produced recombinant biologics, which are essential steps to fulfilling the marketed potential of this technology (Chen and Davis 2016). One of the biggest barriers to the commercialization of this technology is the intense scrutiny of these new plant varieties from regulatory agencies and the public as well as the costs associated with regulatory approval (Sparrow et al. 2013).

In the case of ZMapp, a key roadblock is the novelty of the ZMapp production process. Therefore, the U.S. FDA has to draft quality-control guidelines that can govern large-scale production of plant-produced antibodies and for safety assessment of pre-marketed and post-marketed biologics. Government officials, company scientists, and independent experts are exploring ways to ramp up production and development of regulations to ensure the drug can be made in large quantities (Healy et al. 2014).

Public concerns over the potential health and environmental risks associated with transgenic plants used as molecular farming sources focus on very high concentrations of recombinant proteins. Basically, molecular farming plants are subject to review using a similar evaluation standard as GM crops. The existing guidelines on environmental risk assessment of GM plants for food/feed and non-food/feed uses are considered to be adequate.

The public worries about the effects of biologics on the morphology and physiology of host plants, possible physiological responses in humans and animals caused by plant biologically active products, economic risks to farmers and the food industry as a result of co-mingling and contamination of molecular farming plants with the food/feed chain, possible vertical transgene flow and spread by pollen, seed or fruit dispersal, unintended effects on non-target organisms, particularly birds, insects, and soil microorganisms, and horizontal gene transfer by asexual means (Jouzani and Tohidfar 2013). Therefore, in addition to the risk assessment framework of GM plants used as food/feed or processing, molecular farming plants with recombinant protein products raise new questions and concerns that trigger a need for further specific biosafety considerations due to the nature of recombinant genes (Sparrow et al. 2013).

Risk of co-mingling and contamination of transgenic plants with recombinant genes can be reduced by using as target plants for molecular farming with other agriculturally important crops, non-food/feed crops, production cell suspension cultures in bioreactors, or strict physical agronomic confinement and containment. Biosafety strategies to prevent transgene pollution include use of closed isolated physical containment facilities (greenhouses, glasshouses, hydroponics, and plant cell suspension cultures), biological containment (self-pollinating species, chloroplast transformation, cytoplasmic male-sterile transgenic plants, sexually incompatible crop with wild relatives, non-germinating seeds or non-sprouting tubers/bulbs, engineered parthenocarpy and apomixes), and transgene excision (Jouzani and Tohidfar 2013).

After regulatory approval (ELELYSO to treat type 1 Gaucher's disease from Israel-based Protalix BioTherapeutics) by the U.S. FDA in 2012, commercialization of medicinal products produced in the field have not been approved in either Europe or the U.S. Although plants are used as production vehicles for cheap production of therapeutics in containment platforms, it has become more desirable for plants to be cultivated on a larger scale. However, approval of ELELYSO™ by the U.S. FDA has opened a clear regulatory pathway for plant-made biologics, especially those derived from cultured plant cells. This regulatory pathway can be applied to the approval of recombinant proteins from whole plants. A predictable review process in this approval case can encourage large pharmaceutical companies to give attention to plant-made biologics (Chen and Davis 2016). Pfizer entered into an agreement to license

Table 1. Classes of plant-made pharmaceutical and industrial proteins

Category	Product	Platform	Company/Research team	Development stage
Pharmaceutical proteins for human and livestock				
Vaccine	H5N1, H1N1	*N. benthamiana*	Shoji *et al.*	Development
	H5, Influenza Vaccine	Tobacco (whole plant)	Medicago	Phase II&III (approved for emergency use)
	NHL Vaccine (HSV/HIV)		Icon Genetics	Phase I&II
	CaroRx (Anti-caries)	Tobacco (whole plant)	Plant Biotechnology	Phase I&II (Discontinued, 2016. 2. 17) Approved as medical device
	Anti-west virus mAb Hu-E16	*N. benthamiana*	MacroGenics, NIH	Phase II
	HIV antibody	Tobacco	Fraunhofer IME	Phase I
	HA vaccine	Tobacco	Fraunhofer CMB	Phase I
	NoroVAXX		VAXX/Arizona State University	Phase I
	ZMapp	Tobacco transient plant	MAPP	Emergency use Phase I reported
Edible Vaccine	Canine interferon α	Strawberry fruits	NAIST	Commercialization (Veterinary pharmaceutical/oral)
	Factor VIII antigens	Tobacco leaf Chloroplasts		Development
	Cry 1 and Cry2	Rice grains		Development
	IgA (Rotavirus)	Tomato fruit Matrix Fruit		Development
	ACE2 and Ang	Tobacco leaf		Development
	Designer IgA (Enterotoxigenic *E. coli* infection)	Arabidopsis seeds		Development
	HIV-1 p24 (Immunodeficiency syndrome)	*Arabidopsis thaliana* and carrot		Development
	Exendin-4 (Diabetes type 2)	Tobacco leaf		Development
	Exendin-4 (fused with transferrin) (Diabetes type2)	*N. benthamiana* leaf		Development
	PRRSV (envelope glycoprotein Porcine reproductive& respiratory syndrome virus)	Banana leaf		Development
	Typ II collagen (CII256-271& APL6) Rheumatoid arthritis	Rice seed		Development
	Protective antigen (Anthrax vaccine)	Tobacco and brassica leaf		Development
	Alpha subunit of soybean - conglycinin Hypercholesterolemia	Rice seed		Development

Table 1. Continued

Category	Product	Platform	Company/Research team	Development stage
Therapeutic enzyme	Elelyso (taliglucerase)	Carrot cell suspension	Protalix	FDA cleared
	PRX-102 (β-galactosidase)	BY2 tobacco cell culture	Protalix	Phase II&III
	PRX-12 (oral glucocerebrosidase)	BY2 tobacco cell culture	Protalix	Phase III
Therapeutic protein	Cell culture products (albumin, lysozyme, transferrin, lactoferrin)	Rice (seed)	InVitria	Commercialization
	VEN100 (lactoferrin) VEN200 (albumin)	Rice (seed)	Ventria Bioscience	Phase II
	Interferon Alpha (Hetatitis C)	Duckweed (whole plant)	Synthon	Phase II
	Insulin	Safflower	SembioSys Genetics	Phase III completed
	Locteron™		Synthon	Phase II&III[b]
	Albumin	Potato		Commercialization
	Cytokine, interlukin-12	Potato hairy root		Commercialization
	Human Epidermal growth factor	Barley seed	ORF, SifCosmetics	Commercialization
	Human growth hormone	Barley seed	ORF,	Commercialization
Research enzyme	Aprotinin	Tobacco leaf transient	KRP	Commercialization
	Growth factors, cytokines, antibodies	Rice cell suspension	NBM (Korea)	Commercialization
		Industrial proteins		
Industrial enzyme	Laccase, Trypsin, Avidin	Corn seed	ProdiGene/Sigma Aldrich	Commercialization
	Cellobiohydrolase I	Corn seeds	Infinite enzymes Sigma, Aldrich	Commercialization
	α-Amylase	Corn seeds	Syngenta	Commercialization
Research reagent	Growth factors, cytokines, thioredoxin, TIMP-2	Tobacco leaves, transient	Agrenvec	Commercialization
	Collagen	Transgenic Tobacco	CollPlant	Commercialization
	Tissue culture reagent	Tobacco leaves, transient	NexGen	Commercialization
Feed Additive	Phytase	Corn seeds	Origin Agritech	Commercialization
Biopolymer	Dragline silk (Spider silk)	Corn	Syngenta	Commercialization
Biofuel	polymer-degrading enzymes	Corn	Agrivida	Development
	cellulase, hemicellulase, ligninase	Corn	Edenspace Systems Corporation	Development
	exo-1,4-β-glucanase	Corn	Infinite Enzymes	Commercialization

the worldwide rights for ELELYSO™. Other pharmaceutical companies have begun to show interest in this technology through buyouts and partnerships (Paul et al. 2015).

In Korea, a significant proportion of the public has a negative view of GM plants, and experimental field trials for very few GM events have only been recently allowed in ChunBook Province. Therefore, Korea has strict evaluation criteria for this technology and strict compliance measures to avoid potential risks to the environment or human and animal health. Under these circumstances, the public's opinion on cultivation of biologic-produced plants or consumption of plant-produced products has not been explored. However, public acceptance of biologic-produced plants may be higher than that of GM plants since people do not eat them directly.

In Europe, the public has a negative view of GM plants, similar to Korea and Japan, and GM crops are not allowed

to undergo experimental field trials at all. The European Union (EU) is considered to have the toughest and most stringent legislation on GM products and commodities in the world. Although the commercialization of medicinal or industrial products produced has yet to emerge in Europe, the EU funded molecular farming projects from 2004-2011 (www.pharma-planta.net) and gained regulatory approval for a recombinant protein product to enter phase 1 clinical trials in humans. The regulatory burden in Europe for biologic-produced GM plants such as GM crops is very costly and not appropriate for most plant-made pharmaceuticals (PMP) and plant-made industrial (PMI) applications (Sparrow et al. 2013). The authors propose that amendments to the EU Directive 2001/18/EC are necessary to fully acknowledge emerging technologies for production of PMP/PMI. Therefore, broader and more balanced legislative oversight is needed in Europe. Despite similar or even stricter conditions in Korea, there have been no experimental field trials using PMP/PMI-producing plants. Korea needs similar regulatory administration as Europe for biologic-produced plants.

Therefore, to facilitate commercialization of plant-produced biologics, it is necessary to develop different scientific and regulatory biosafety strategies from those for GM plants (Jouzani and Tohidfar 2013). Further development and widespread success of this technology will be strongly influenced by the level of regulation and restrictions applied to molecular farming plants and their products. In order to directly benefit from this technology, the world will have to be proactive in the regulatory process. These circumstances for scientists and manufacturers of plant-produced recombinant proteins will improve public acceptance.

Classes of Plant-made Recombinant Proteins

The effect of ZMapp on the Ebola virus has highlighted not only the potential advantages of plant-produced recombinant proteins but also the limited capacity and lack of defined regulatory pathways for development of plant-derived pharmaceutical proteins (Sack et al. 2015). Only several manufacturing facilities have been approved for the production of GMP-grade proteins in leafy crops such as tobacco, whereas some other facilities can manufacture GMP-grade proteins expressed in rice seeds for manufacturing platforms of recombinant proteins (Xu et al. 2012; Broz et al. 2013). Since there are a limited number of GMP-compliant production facilities, a relatively small number of plant-derived pharmaceutical products such as human serum albumin (Farran et al. 2002) and influenza vaccine (Shoji et al. 2011) are currently on the market or undergoing clinical development (Table 1). However, several other companies have established commercial platforms for the production of non-pharmaceutical products to generate revenue without the lengthy and costly regulatory procedures required for clinical studies (Abiri et al. 2015). These products range from veterinary pharmaceuticals, technical enzymes, and research reagents to media ingredients and cosmetic products (Table 1; Fig. 1). It has also been reported that transgenic silkworm lines, in which Spider (*Araneus ventricosus*) dragline silk (MaSp-2) protein is expressed as a part of fibroin fiber of silk, show high-toughness (Sylvester et al. 2011). Veterinary pharmaceuticals have received attention to reduce use of antibiotics in livestock production and thus the emergence of antibiotic-resistant and potentially zoonotic pathogens (Sack et al. 2015; Topp et al. 2016). The production of recombinant vaccines by plants may help to reduce the burden of veterinary diseases, which cause major economic losses and in some cases affect human health (MacDonald et al. 2015). These circumstances have encouraged the development of cost-effective, efficient, and scalable production and delivery platforms for veterinary pharmaceuticals (Rybicki et al. 2012; MacDonald et al. 2015). Although the motivation is great, a knowledge gap exists between the ability to create and evaluate plant-based products in the laboratory and the ability to make these products ready for commercialization, including business planning, financing, and regulatory approval (MacDonald et al. 2015). Plant-based production systems are more suited to satisfy these demands than microbial and animal platforms.

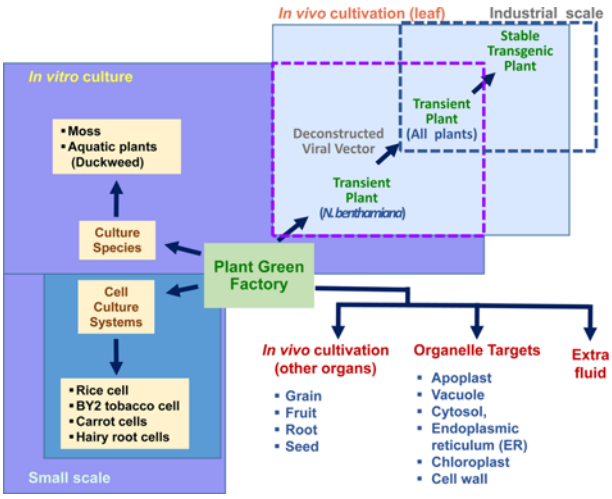

Fig. 1. Plantforms of plant-produced recombinant proteins. Purple area indicates the platform for small-scale experiment or pilot-scale production. Sky blue area indicates the platform for large-scale industry production

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Author's Contribution

WSJ and PKY collected information and wrote the manuscript.

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