PLANT-BASED EXPRESSION SYSTEMS IN BIOPHARMACEUTICAL PRODUCTION: A COMPREHENSIVE REVIEW

Sihana A. LIKA[1], Hanife R. AHMETI[1], Qail IBRAIMI[1], Arbnore Q.NAZIFI[1], Shqipe XHAFERI[1], Flora DOKO[1]

*11*Department of Pharmacy, Faculty of Medical Sciences, NMK*

**Corresponding Author: e-mail: sihana.alika@hotmail.com*

Abstract

The development of biotechnology has given rise to a paradigm shift in the field of pharmaceutical production, which is the transgenic plant for the synthesis of bioactive substances that play a role in the production of drugs and more. It is known as molecular agriculture, which uses plant genetic technology to produce certain proteins with therapeutic functions, including monoclonal antibodies, hormones, vaccines and other biologically active components. The most important methods are additional methods of minimal cost and potential biosafety. Unlike traditional production systems (cells, bacteria or yeast), plants on a flexible and workable platform, the characteristics of most of the decentralized biological drugs. The techniques of other technologies are glucocerebroside (elements for the treatment of Gaucher disease) in transgenic carrot cells, as well as recombinant treatments against Ebola, HIV and influenza, such as plants such as Nicotiana benthamiana or corn. These results demonstrate the potential in the true nature of plants that they convey in addressing the challenges of the global health situation. However, it is not difficult to undertake, as it is important, it is important to control the products of the biosynthesis, to identify the products of the biosynthesis, to know that it is mandatory for the educational process, the capacity to record the post-protection of the proteins and the factors of the rules and ethics that are related to the GMO in the care of a specialist. The rules and precise rules are also related to the integration of this technology into pharmaceutical systems.

Keywords: transgenic plants, genetic engineering, pharmaceutical production.

Introduction

Transgenic plants are plants whose DNA has been modified using genetic engineering techniques. The aim is to introduce a new trat into the plant that does not occur naturally in the species. A transgenic plant contains a gene or genes that have been artificially inserted into it. The sequence of the inserted gene is known as a transgene and can come from an unrelated plant or from an entirely different species. The goal of introducing a combination of genes into a plant is to make it more useful and productive. This approach offers advantages such as improved longevity, higher yield, improved quality, resistance to pests, resistance to heat, cold and drought, and to a range of biotic and abiotic stresses. Transgenic plants can also be produced in such a way that they express foreign proteins of industrial and pharmaceutical value (Paul *et al*, 2011).

Plants engineered to produce vaccines or antibodies (Plantibodies) are free from human diseases, thus reducing the costs of screening for viruses and bacterial toxins. The first transgenic plants were reported in 1983. Since then, many recombinant proteins have been expressed in several agronomically important plant species, including tobacco, corn, tomatoes, potatoes, bananas, alfalfa, and canola. In general, tobacco plants have been used most, although potatoes and bananas are also being considered for human purposes (Albiri et al, 2015&Fisher et al 2015).

Genetically modified plants are created in a laboratory by altering the genetic makeup, usually by adding one or more genes to the genome of a plant. The nucleus of the plant cell is the target for the new transgenic DNA. Most genetically modified plants are created by the biolistic method (particle gun method) or by the Agrobacterium tumefaciens-mediated transformation method (Shrawat et al, 2006).

The "Gene Gun" method, also known as "Micro-Projectile Bombardment" or the "Biolistic" method, is most commonly used in species such as maize and rice. In this method, DNA is attached to small particles of gold or tungsten, which are then fired at plant tissues or single plant cells, under high pressure using a gun. The accelerated particles penetrate both the cell wall and membranes. The DNA is separated from the coated metal and integrated into the plant genome within the nucleus. This method has been successfully applied to many crops, especially monocotyledons, such as wheat or maize, for which transformation using Agrobacterium tumefaciens has been less successful. This technique is considered relatively safe and efficient. The only disadvantage of this process is that serious damage to the cell tissue can occur (Chawla et al, 2000).

The other method used to develop genetically modified plants is the "Agrobacterium" method. It involves the use of soil-dwelling bacteria known as Agrobacterium tumefaciens. It has the ability to infect plant cells with a portion of its DNA. The portion of DNA that infects a plant is integrated into a plant chromosome via a tumor-causing plasmid (Ti plasmid). The Ti plasmid can control the plant's cellular machinery and use it to make many copies of its own bacterial DNA. The Ti plasmid is a large circular piece of DNA that replicates independently of the bacterial chromosome. This plasmid is important because it contains DNA transfer regions (tDNA), where a researcher can insert a gene, which can be transferred into a plant cell through a process known as “flower dipping”. A flower dipping involves immersing flowering plants in a solution of Agrobacterium carrying the gene of interest, followed by transgenic seeds, which are collected directly from the plant. This process is advantageous because it is a natural method of transfer and is therefore considered a more acceptable technique. In addition, Agrobacterium is able to transfer large fragments of DNA very efficiently. One of the major limitations of Agrobacterium is that not all important food crops can be infected by these bacteria. This method works particularly well for dicotyledonous plants such as potatoes, tomatoes and tobacco plants (Meagher 2000&Koornef et al, 2010).

Methodology

For the preparation of this paper, various scientific sources published in international peer-reviewed journals, academic books and relevant reports in the field of pharmaceutical biotechnology were used. The literature search was conducted through electronic databases such as PubMed, Scopus and Google Scholar. Keywords such as “transgenic plants”, “plant molecular farming”, “biopharmaceuticals”, and “recombinant proteins” were used. The sources were selected based on scientific relevance, methodological quality and their contribution to understanding current developments, applications and challenges related to the use of transgenic plants in the production of biopharmaceuticals.

Results and discussion

Transgenic plants contain inserted genes that are derived from other species. The inserted genes can come from species within the same kingdom (plant to plant) or between kingdoms (bacteria to plant). In many cases, the inserted DNA must be modified in order to be expressed correctly and efficiently in the host organism. Transgenic plants are used to express proteins, such as the cry toxins from Bacillus thuringiensis, herbicide resistance genes, and antigens for vaccinations.

Cisgenic plants consist of the use of genes, found within the same species or in a closely related species, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to cross by conventional means (such

as potatoes). These plants in the cisgenic category should not require the same level of regulatory control as other genetically modified organisms (Flack et al, 1999).
GM technology has been used to produce a wide range of crops. As the global population continues to expand, food security remains a major concern. Genetically modified foods offer significant benefits by improving crop yields, reducing transportation costs, and increasing nutrient content. Developments, resulting in commercially produced varieties in countries such as the US and Canada, have focused on conferring resistance to insects, pests, or viruses, and on producing tolerance to specific herbicides. While these traits have had benefits for farmers, the benefits for consumers have been less apparent beyond these. In limited cases, a reduced price due to reduced cost and increased ease of production. Several genetically modified crops for malnutrition are expected to be available for cultivation in the next five to ten years (Davies, 2007).

Transient expression platform

It usually takes six months to a year, or more, to produce transgenic plants. Several generations are required to create plants that are homozygous for the transgene. Most transformation technologies also result in the random insertion of genes into the plant genome. This random insertion, together with the need to identify regulatory elements (promoters) that enable high levels of expression of foreign genes, often results in low production of the recombinant protein, even in stably transformed plants.
The time required to use a standard PMF approach is inadequate for dealing with sudden viral epidemics, such as the Ebola, SARS (Severe Acute Respiratory Syndrome) or MERS-CoV outbreaks. Transient expression systems can be used as an alternative to produce recombinant proteins within three to five days.
Several viral vectors have been developed for small- to medium-scale production of proteins via PMF. For example, Huang Z et al. (2010) developed a highly efficient DNA replication system based on bean yellow mosaic virus (BeYDV), incorporating multiple replication cassettes into a single vector. They were able to produce 0.5 mg of an antibody per gram of leaf (fresh weight) in tobacco leaves within four days of infiltration.
Using a similar methodology, Mapp Biopharmaceutical Inc. transiently expressed humanized antibodies, MB-003 and ZMab, in tobacco leaves. Later, these two antibodies were combined and named ZMapp. The use of these antibodies as a pharmaceutical drug was successful in curing 100% of rhesus monkeys infected with Ebola (Qiu et al, 2014).

Bioreactor platforms - plant cell culture system

Currently, bioreactors based on plant cell culture represent more potential than traditional plant molecular farming (PMF) platforms that use whole plants for drug production. Similar to bioreactors used for microbial or animal cells, plant cells are cultured in closed and sterilized vessels, free from human pathogens or soil contamination. This approach helps eliminate biosafety concerns associated with unintended pollen dispersal and cross-fertilization.
Cultivated plant cells require only simple nutrients, which makes the operating cost much lower compared to microbial or animal systems. The purification and processing of recombinant proteins is also simpler, due to the absence of complex plant fibers and numerous secondary metabolites – significantly reducing the overall cost of production (Fox, 2012). To date, over 20 recombinant proteins have been produced using plant cell culture systems. Tobacco varieties such as BY-2 and NT-1 are among the most widely used PMF as bioreactor cell lines. The recombinant protein can be programmed to be secreted directly into the culture medium, to simplify the purification process. However, the pore size of the plant cell wall may prevent the

secretion of some foreign proteins, depending on their size and spatial structure. In addition, proteolytic activity in cultured cells can cause a decrease in antibody yield (Spok et al.2008).

Moss culture

Currently, moss, a non-seed plant, is being studied as a promising candidate for the production of pharmaceutical proteins in bioreactors. The ability of moss cells to photosynthesize in culture significantly reduces the cost of nutrients required for cultivation.
Similar to yeast and plant cell cultures in suspension, recombinant proteins in moss cells can be engineered to be secreted into the culture medium, which greatly facilitates the process of purification and processing of the produced proteins.
Through genetic manipulation, moss cells can produce humanized forms of glycosylated proteins, such as the Lewis Y-specific monoclonal antibody MB314, reducing common concerns associated with plant-specific glycosylation, which can negatively impact drug functionality in humans (Huang et al, 2012).
The moss culture Physcomitrella patens (P. patens) is the most widely used plant material in this technology for protein production in bioreactors. A German biopharmaceutical company, Greenovation Biotech GmbH, has developed a P. patens-based platform, called Bryo-Technology, for large-scale, high-quality production of recombinant proteins. Examples of products from this platform include:
Moss-GAA for Pompe disease,
Moss-GBA for Gaucher disease,
and Moss-AGAL for Fabry disease.
Of these, Moss-AGAL has completed preclinical trials and is in the process of entering phase I clinical trials (Orrellana et al, 2015).

Algal bioreactors

Microalgae cultures have been used for years for both biofuel production and the production of foreign proteins. Microalgae have a very simple structure and can be unicellular, colonial, or filamentous. They are able to produce large amounts of biomass within a very short period of time, due to their very short life cycle.
The purification and processing process of recombinant proteins in algae is similar to that used for yeast and bacteria, and is therefore less expensive compared to systems using whole plants (Mathieu-Rivet et al, 2014).
However, recombinant proteins produced by algae do not undergo several post-translational modifications, which may negatively affect their use for the production of glycoproteins. For example, algae may not be able to produce human forms of glycosylated proteins, due to the lack of specific enzymes required for these modifications.
Despite this, a variety of recombinant therapeutic and diagnostic proteins, including vaccines, enzymes, and antibodies, have been successfully produced in algal system (Gong Y et al, 2011).
An optimized microalgae production system has been developed by the American company PhycoBiologics, which uses specialized bioreactors for this purpose. In this system, it has been possible to obtain quantities of recombinant proteins that constitute up to 20% of the total soluble proteins, which makes the algal platform a very promising opportunity for the commercial production of pharmaceutical drugs (Teng C et al, 2002).
Seed-based platform for recombinant protein production
One of the main challenges in plant-based pharmaceutical drug production (PMF) is the stability of recombinant proteins during storage and purification of harvested material.

Currently, most pharmaceutical proteins are synthesized in green parts of plants to maximize biomass, but proteins in leaves are susceptible to rapid proteolytic degradation after harvest. Also, long-term storage of leaf material is a major challenge, as proteins lose their activity over time (Lu Z, et al, 2012).

In this context, the seed-based protein production platform is becoming a very promising alternative. If foreign proteins are intended to be produced in seeds, they have higher stability and can be stored for long periods (up to 3 years) at ambient temperature without significant loss of activity. Furthermore, seeds have lower levels of native proteins, secondary metabolites, and phenolic compounds that can complicate purification, making the process simpler and more economical (Ou J et al, 2014).

The seed-based platform has been successfully developed in several plant species, such as Arabidopsis, tobacco, rice, and maize. For example, in Arabidopsis, the use of a seed-specific regulatory sequence for common bean (fasciatus) has enabled the achievement of high levels of recombinant proteins—up to 36.5% of the total soluble protein in the seeds. In transgenic rice, human lysozyme was achieved to constitute 1% of the seed weight, while in tobacco seeds, single-chain variable fragments (scFV) reached up to 25% of the total protein when linked to elastin-like polypeptides (Huang N. 2004).

Another major advantage of the seed-based platform is the possibility of targeting proteins to different compartments within the cell, such as the endoplasmic reticulum (ER), which contains very few proteases, reducing degradation and increasing protein yield. For this purpose, the KDEL signal is used, which helps to retain proteins in the ER (Scheller J et al, 2006).

Biopharmaceutical Manufacturing: Proteins, Antibodies, and Vaccines

The market for recombinant protein biopharmaceuticals is large and growing rapidly, with almost all major pharmaceutical companies reporting an increasing share of their revenues from these products rather than small molecule drugs (Goodman M, 2009). Typically, these pharmaceuticals are manufactured using mammalian or bacterial cell-based systems, which are complex to operate and inherently susceptible to contamination with human pathogens. Manufacturing facilities must comply with current good manufacturing practices (cGMP), they require large capital expenditures, and they involve considerable financial risk. Plant biotechnology has the potential to overcome some of these limitations, but several hurdles remain before this new industry can compete effectively in the pharmaceutical sector. Interest in the potential of plants as co-biofactors for recombinant proteins of pharmaceutical importance was first stimulated by the report of a tobacco line engineered to synthesize a functional murine monoclonal antibody (mAb) (Hiatt, A et al, 1989).

Range of plant production platforms

Technological developments in this field have led to an expansion of the number of well-developed systems available for the production of recombinant pharmaceuticals in plants. Many plant species are now amenable to genetic manipulation (Sourrouille C et al, 2008).

Plant-made pharmaceuticals (PMPs) from plant tissues

The first recombinant protein products from plant biotechnology to reach the market were not pharmaceuticals, but enzymes and diagnostic reagents. The production and purification of egg white avidin and bacterial beta-glucuronidase for commercial purposes were first reported in 1998 (TwymanR et al 2003 &Hood et al, 1999) and both were commercialized at one point through a collaboration between the former biotechnology company Prodigene (College Station, TX, USA) and Sigma-Aldrich (St. Louis, MO, USA). However, despite active research and development efforts and venture capital, new non-pharmaceutical products entering the market have remained rare. Both of these precursor products were expressed in a transgenic food crop (maize) in an open field environment, in order to take advantage of the high protein

yields of this crop and the scalability and economics of conventional agronomic practices (Evangelista et al, 1998) The use of food crops for recombinant protein production has been associated with negative sentiment among consumer groups and the food industry, leading to the adoption of a "zero tolerance" policy by the US Food and Drug Administration (FDA) for the release of GMP transgenes and contamination of food crops in the current draft GMP guidance documents for industry. The high-profile loss of content experienced by Prodigene has led the United States Department of Agriculture/Animal and Plant Health Inspection Service to impose additional requirements for all future field releases of GMP crops in the US. These factors have led to a notable shift from open-field transgenic food crops to fully enclosed non-food culture systems for the production of PMPs. Unfortunately, this has left the field off the established path to commercialization defined by these non-pharmaceutical precursors (FDA).

Three broad classes of drugs have received particular attention from the PMP community.

The first class is therapeutic antibodies and antibody-derived proteins. The production of antibody PMPs is now supported by a set of well-developed technologies that can offer excellent yields and unique technical advantages. Indeed, plants remain the only viable production platform for the production of SIgA molecules. Antibodies produced in plants have been approved for topical use in the EU (CaroRXTM, Planet Biotechnology) and are progressing through clinical trials for systemic use in the US (NHL scFvs). Transgenic tobacco, either in an open field or closed greenhouse environment, offers a cost-effective production route that is currently used in the production of antibodies for topical use (e.g., CaroRXTM and mAb 2G12), as well as an antibody used in the downstream processing of a hepatitis B vaccine. The involvement of the EU Pharma-Planta consortium in bringing mAb 2G12 to the brink of a phase I clinical trial has put clear regulatory guidelines in place for the production of PMPs in greenhouses from whole plant biomas (Pujol M et al, 2005). The second class are subunit vaccine antigens. Noninvasive immunization by oral administration is an attractive route of immunization, and plant expression hosts have been associated with oral administration since the inception of the technology. Several edible "vaccines" have progressed to phase I clinical trials, although important issues regarding dose standardization and food chain contamination, as well as the efficacy of oral vaccination, remain to be further investigated. Oral vaccine antigens produced by plants may find greatest relevance as booster components of heterologous prime-boost strategies, as has been demonstrated for measles and hepatitis B vaccines (Thanvala Y et al, 2005). The rapid production of vaccine antigens in transiently transfected leaves of N. benthamiana offers a further potential advantage of plant-based production of vaccine antigens. This approach is illustrated by the seasonal and pandemic influenza vaccines currently in Medicago-sponsored clinical trials (Webster D et al 2002).

The third class of PMPs are therapeutic enzymes. At least initially, PMPs in this class are likely to be "biosimilars" such as insulin (SBS-100, SemBioSys Genetics), glucocerebroside (UP-LYSO, Protalix), and interferon-alpha (LocteronR, Biolex Thera-peutics. The use of PMP technologies could allow the commercialization of biosimilar versions of drugs with existing patent protection in other manufacturing systems. However, given the extensive manufacturing capacity in the generics sector, GMPs must also rely on the advantages of a plant production platform to compete effectively (Thanavala Y et al, 2005)

Conclusions

In conclusion, it can be said that transgenic plants represent one of the most important developments in modern biotechnology, with a profound impact on agriculture, the food industry and medicine. They offer great potential for improving crop quality, increasing

resistance to biotic and abiotic stress, as well as for the production of substances of high pharmaceutical value (such as vaccines and therapeutic enzymes).

However, despite the obvious benefits, the use of transgenic plants remains a controversial topic, due to environmental, ethical and socio-economic concerns. Issues such as the impact on biodiversity, the development of resistance in insects and weeds, as well as the monopoly of corporations on modified seeds, require special attention and a careful approach from stakeholders.

To maximize the benefits and minimize the risks, an integrated approach is necessary, where scientific research, public policy and consumer education go hand in hand. In addition, advances in new genetic modification technologies, such as CRISPR/Cas9, offer opportunities to overcome some of the ethical and technical limitations of traditional transgenic methods.

In the future, the role of transgenic plants is expected to deepen not only in terms of more sustainable food production, but also in addressing global challenges such as climate change, food shortages and the development of new herbal medicines. Therefore, this field remains not only scientifically challenging, but also of strategic importance for the future of humanity.

Transgenic biotechnology presents a wide range of opportunities, ranging from improving the quality and quantity of food to preventing and treating diseases, but there are some potential risks, so some issues that need to be taken into account are:

Consumer safety and health. There are concerns about the production of recombinant proteins that may cause allergic reactions, unpredictable levels of toxicity or long-term effects in humans. Organizations such as the Royal Society and the National Academy of Sciences advise on a transparent and rigorous system of evaluations for potential allergens and toxins. The concept of "substantial equivalence" is used to assess whether a transgenic product is biologically the same as its natural counterpart.

Risk of genetic contamination and loss of biodiversity. Controlling the outcrossing of transgenic genes into wild plants or other seeds remains an ecological and ethical challenge. Exposure to herbicide resistance genes could lead to the development of "superweeds" that threaten natural ecosystems.

Unexpected allergies to foreign proteins. There is a history of concerns when genes from allergenic sources are introduced, such as genes from Brazil nuts into soybeans, which were more sensitive to people allergic to nuts.

Intellectual property control and access for developing countries. Patents on transgenic crops lead to economic dependence; farmers cannot save seeds and must buy them every year. This phenomenon raises ethical dilemmas about the fairness of access and benefit-sharing at the global level.

Patient/consumer autonomy and information. The decision to use transgenic drugs should be based on informed consent. The need for transparency in labeling is critical. Some countries require labeling for "bioengineered" products, supported by standards such as the FFDCA and the concept of substantial equivalence.

Ethical manipulation of genetic material. Some philosophical approaches consider genetic modifications to be unethical interventions in nature, challenging the principles of "respect for life" and the limits of natural equity.

Socio-economic impacts and global justice. Transgenic technology has the potential to improve food and health security, but without ethical intervention it could exacerbate social inequalities. The global need to address food security for all coincides with the values of inequality of access.

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