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Research Paper

Soyabeans as Bioreactor For Biopharamaceuticals And Industrial Proteins: A Review

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ABSTRACT:*The production of a large number of recombinant proteins is in high demand, both for scientific evaluation and product development. Furthermore, current treatments that use recombinant proteins plays significant role on strain and health systems in developing and developed countries. In developing countries, cost is the main constraint for most of the population. The use of plants provides an additional alternative to bacterial and mammalian cell based recombinant protein production systems. In some cases, the use of plants may determine whether an effective cancer antigen, a microbicide against HIV, may be economically viable for a biopolymer to reach the market. In this case seeds are very efficient production system and have special compartments for storing proteins. These characteristics may provide the seeds to produce a very efficient system for the large-scale production of recombinant proteins at an economically viable cost. Existing tools, including synthetic biology and metabolic engineering, should expand even further the ability to manipulate seeds to produce recombinant proteins. The studies presented in this article indicate that the seeds are capable of producing recombinant proteins with various biochemical characteristics at high levels. Specific bioassays have demonstrated the functional activity of the recombinant protein produced. Plant bioreactors are attractive expression systems for the economic production of pharmaceuticals. Soybean is an excellent protein producer in the plant crop. Soybean plants are also a good source of abundant and cheap biomass and can be cultivated under controlled greenhouse conditions. There is a lot of potential to bring this is an important step in the development of genetically engineered products that are affordable and safe for medicinal, food and other uses.*

KEYWORDS: *Plant Farming; Transgenic Soybean; Glycine Max; Seed Storage Proteins; Protein Storage Vacuoles.*

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I. INTRODUCTION

Proteins are synthesized as part of the natural metabolism of all life forms and are central in many cellular processes and diverse as cell signaling and replication, reaction inducing, immune responses, and the maintenance of complex structural elements of many specialized organisms. Native and recombinant proteins are widely used as laboratory reagents in research, as sophisticated diagnostic tools in medicine, and in the food and nutrition industry. Recombinant proteins also play important roles in important areas of the agricultural and biopharmaceutical industries. Commercial production of native proteins from their natural biological sources often requires difficult and expensive extraction processes, which can decisively contribute to shortcomings in terms of cost and production scalability. Furthermore, when a protein of interest is a biopharmaceutical product, purification of the molecule from its natural source can represent a considerable risk for pathogen contamination and disease transmission. Since the development of recombinant DNA technology in the early 1970s, commercial production of recombinant proteins has traditionally relied on microbial fermentation systems and transgenic mammalian cell culture. Although these systems are highly efficient in terms of productivity, they present some disadvantages in terms of authenticity, safety and production cost. These significant limitations have driven the development of alternative production platforms over the past two decades based on the efficient scale, cost-effectiveness, high product quality and low contamination risk offered by vegetable bioreactors, such as plant cell suspensions, virus-infected plants and whole transgenic plants [1]. Plant bioreactors are potentially the most economical expression system for the large-scale production of many molecules of pharmaceutical or industrial interest. Since 1986, when the first relevant plant-produced

biopharmaceutical-human growth hormone (HGH)-was synthesized by transgenic tobacco plants, more than 100 different proteins have been successfully expressed in various transgenic crops; These crops mainly include tobacco suspension cells, cereals and legumes, oilseeds, potato tubers, leafy crops, such as lettuce and spinach, and edible fruits, such as tomatoes and bananas [2]. In 2010, approximately 30 plant-derived pharmaceuticals (PMPs), including vaccines, antibodies, nutraceuticals and therapeutic human proteins, entered the final stages of clinical trials before commercialization. In addition to three previously commercialized proteins derived from maize, bovine trypsin, avidin and β-glucuronidase, four PMPs have already been launched to the market, all of which have been used in research laboratories worldwide since 1998. [3, 4]. (Table 1) and the figure (1) below.

Proteins are synthesized as part of the natural metabolism of all life forms and are cen- tral to the innumerous cellular processes that are as diverse as cell signalling and replication, reaction catalysis, immune responses, and the maintenance of the complex structural elements of many typical organelles. Native and recombinant proteins are widely used in research as laboratory reagents, in medicine as sophisticated diagnostic tools and in the food and nutrition industry. Recombinant proteins also play key roles in important sectors of the agriculture and the biopharmaceutical industries. The commercial production of native proteins from their natural organism sources frequently requires difficult and expensive extraction processes, which can decisively contribute to drawbacks in terms of costs and production scalability. Furthermore, when a protein of interest is a biopharmaceutical product, the purification of the molecule from its natural source may represent a considerable risk for pathogen contamination and disease transmission. Since the development of recombinant DNA technology in the early 1970s, the commercial production of recombinant proteins has traditionally relied on microbial fer- mentation systems and transgenic mammalian cell culture. Although these systems are highly efficient in terms of productivity, they present some disadvantages in terms of authenticity, safety and production costs. These important limitations have prompted the development of alternative production platforms in the past two decades based on the efficient scale-up, cost-effectiveness, high product quality, and low contamination risk presented by vegetable bioreactors, such as plant cell suspension, virus-infected plants and whole transgenic plants (Fischer et al., 2004). Plant bioreactors are potentially the most economical expression systems for the large-scale production of many molecules of pharmaceutical or industrial interest. Since 1986, when the first relevant plant-made biopharmaceutical - human growth hormone (hGH) - was synthesized by transgenic tobacco plants, more than 100 different proteins have successfully been expressed in different transgenic crops; these crops primarily in- clude tobacco suspension cells, cereal and legume seeds, oilseeds, potato tubers, leafy crops, such as lettuce and spinach, and edible fruits, such as tomatoes and bananas (Spök et al., 2008). In 2010, approximately 30 plant-made pharmaceuticals (PMPs), including vac- cines, antibodies, nutraceuticals, and therapeutic human proteins have entered the □nal stages of clinical trials prior to commercialization. Four PMPs have already been launched on the market, in addition to three previously commercialized proteins derived from maize, bovine trypsin, avidin, and β-glucuronidase, all of which have been utilized in research laboratories worldwide since 1998 (Table 1) (Ma et al., 2003; Obembe et al., 2011).**Proteins are synthesized as part of the natural metabolism of all life forms and are cen-tral to the innumerous cellular processes that are as diverse as cell signalling and replication, reaction catalysis, immune responses, and the maintenance of the complex structural elements of many typical organelles. Native and recombinant proteins are widely used in research as laboratory reagents, in medicine as sophisticated diagnostic tools and in the food and nutrition industry. Recombinant proteins also play key roles in important sectors of the agriculture and the biopharmaceutical industries. The commercial production of native proteins from their natural organism sources frequently requires difficult and expensive extraction processes, which can decisively contribute to drawbacks in terms of costs and production scalability. Furthermore, when a protein of interest is a biopharmaceutical product, the purification of the molecule from its natural source may represent a considerable risk for pathogen contamination and disease transmission. Since the development of recombinant DNA technology in the early 1970s, the commercial production of recombinant proteins has traditionally relied on microbial fer-mentation systems and transgenic mammalian cell culture. Although these systems are highly efficient in terms of productivity, they present some disadvantages in terms of authenticity, safety and production costs. These important limitations have prompted the development of alternative production platforms in the past two decades based on the efficient scale-up, cost-effectiveness, high product quality, and low contamination risk presented by vegetable bioreactors, such as plant cell suspension, virus-infected plants and whole transgenic plants (Fischer et al., 2004).Plant bioreactors are potentially the most economical expression systems for the large-scale production of many molecules of pharmaceutical or industrial interest. Since 1986, when the**

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Figure 1. Picture of Soybeans plants

Seed-based platforms are particularly interesting for the accumulation of recombinant proteins because they present a high endogenous protein content and provide a biochemical environment that is optimized for long-term storage of these molecules. Furthermore, stable accumulation of recombinant proteins within seeds can be maximized by localizing nascent polypeptides to the endoplasmic reticulum (ER) lumen or by targeting different subcellular organelles of the seed, such as the vacuole. Vacuoles are usually involved in the stable compartmentalization of large amounts of proteins that can be used as modifying compounds during seed germination and provide the seedling with nutrients.

Table 1.Plant- made pharmaceuticals (PMPs) currently approved for commercialization.

Product	Trade name	Crop	Original organism	Commercial purpose	Company
Aprotinin	AproliZean	Maize	Cow	Research	ProdiGene
Aprotinin	Apronexin	Tobacco	Cow	Research	Kentucky Bio Processing, LLC by Large Scale Biology
Avidin	Recombinant Avidin	Maize	Chicken	Research and diagnose reagent	ProdiGene
Beta-glucuronidase	NA	Maize	Bacteria	Research and diagnose reagent	ProdiGene
Trysin	TrypZean	Maize	Cow	Research	ProdiGene
Lactoferrin	NA	Rice	Human	Research	Ventria Bioscience
Lysozyme	NA	Rice	Human	Research	Ventria Bioscience

Various cereals (mainly maize, rice and barley) and cereal legumes (pea and common bean) have been discovered as hosts of various biopharmaceuticals. Among them, soybeans have the highest seed protein content, mostly due to the abundance of soybean protein storage vacuoles (PSVs), which are seed-specific organelles for protein accumulation that act as the end points of the plant secretory pathway [5]. Soybean plants also represent a good source of abundant and cheap biomass, especially if they are cultivated under controlled

greenhouse conditions. Under containment, the plant cycle can be manipulated and the final seed yield can be maximized for large-scale protein production in a restricted area. The use of dicotyledonous seed-specific promoters with strong transcriptional activities is critical for the design of a successful strategy for recombinant protein expression and accumulation in soybean seeds. Furthermore, recent studies have shown that fusion of the PSV signal peptide upstream of the protein coding sequence is a promising approach to avoid post-translational degradation and increase target protein yield [6,7].

Stirred tank bioreactors are the standard in the Biotechnol- ogy industry and have been used for over 40 years [9]. The aim of this paper was to design and implement a controlled and monitored bioreactor for the purpose of bacteria growth (E. coli). The E. coli is used to produce important antibiotics and cancer fighting drugs, as well as DNA repair via two enzymes in the E. coli, which are DNA polymerase 1 and DNA ligase, that help repair frequently damaged DNA. An approach has been designed and established afterwards to build a bioreactor from square one. The work was divided into two sections

II. SOYBEAN TRANSFORMATION

When choosing a plant-based bioreactor system, it is crucial to establish an effective genetic transformation method to obtain stable transgenic lines expressing the molecule of interest [8] published the first two effective methods for soybean transformation (utilizing Agrobacterium and biolistic, respectively), much effort by several groups has been made to improve the system. Currently, there are several choices of methods for introducing foreign DNA into soybean plants by Agrobacterium or biolistic [9].

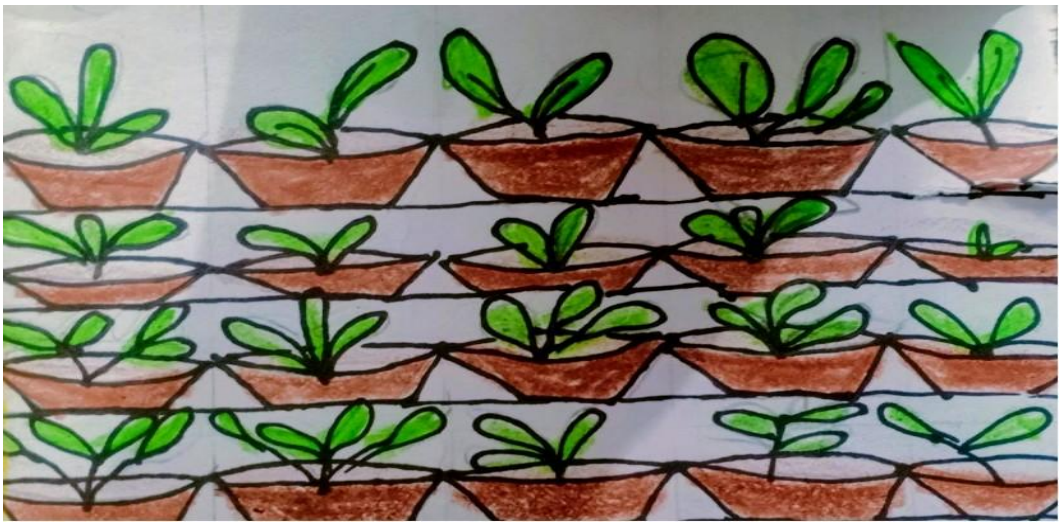

Fig 3. Transgenic soybean plants cultivated in a greenhouse express different biopharmaceuticals, such as hGH, hFIX and the CV-N. High leaf and seed biomass were obtained by submitting the plants to a daily photoperiod of 23 h. The average grain yield achieved under these conditions was 1000 seeds per plant, approximately 10 times higher than in open-field cultivation and Stirred tank bioreactors are the standard in the Biotechnology industry and have been used for over 40 years. The aim of this paper was to design and implement a controlled and monitored bioreactor for the purpose of bacteria growth (E. coli). The E. coli is used to produce important antibiotics and cancer fighting drugs, as well as DNA repair via two enzymes in the E. coli, which are DNA polymerase 1 and DNA ligase, that help repair frequently damaged DNA.

III. BIOREACTOR TECHNOLOGY

As bioreactor technology advances into a much more prolific technology, researchers aim to enhance the power behind single bioreactor systems. The motive of this paper is to be able to build a bioreactor where the bacterial optimal growth can be achieved and controlled by simple controller design. This provides proper production of the desired bacteria used to treat infections and diseases by producing biopharmaceutical ingredients. Different bioreactor designs have raised more interest and attention in recent years due to their versatility and potential for applications in diverse fields. The notable applications are included in tissue engineering, biotechnology and genetics. An approach has been designed and established afterwards to build a bioreactor from square one. The work was divided into two sections colony, therefore counting the colonies yields the number of bacteria present. In this project the culture bacteria in LB media was clear but after leaving the bioreactor on for 16hours in the designed and controlled environment, the media changed from clear into turbid solution. The turbidity or the cloudiness in the solution showed high absorbance. Through all the

controlled parameters, the optimal criteria for bacterial growth have been achieved and proved by the absorbance tested by the spectrophotometry [18].

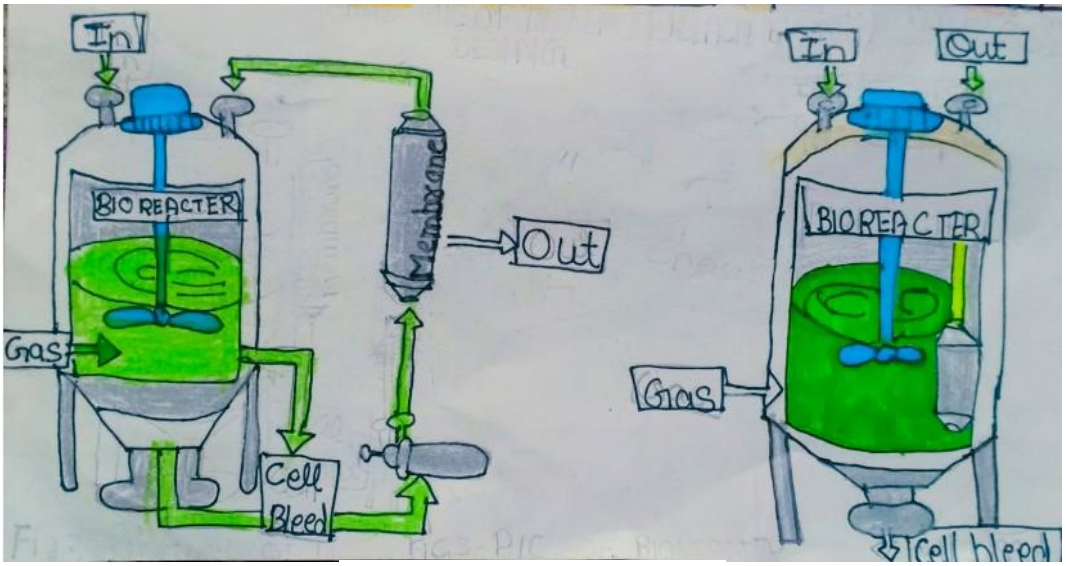

Fig 4. Picture of bioreactor design

Expression and Characterisation of Recombinant Molecules in Transgenic Soybean-Seeds are organs specialised in accumulating proteins, and they may provide a potential economically viable platform for the large-scale production and storage of many molecules for pharmaceutical and other productive sectors. Soybean has a high seed protein content and represents an excellent source of abundant and cheap biomass. Under greenhouse conditions and a daily photoperiod of 23 h of light, the soybean plant's vegetative growth can be significantly extended by inducing more than a tenfold increase in seed production when compared with plants cultivated under field conditions [17]. Some factors involved in the production of different recombinant proteins in soybean seeds are discussed in this review. These include transgenic system, regulatory sequences and the use of Mass Spectrometry as a new tool for molecular characterisation of seed produced recombinant proteins. The important intrinsic characteristics and possibility of genetically engineering soybean seeds, using current advances in recombinant DNA technology including metabolic engineering and synthetic biology, should form the foundation for large-scale and more precise genome modification, making this crop an important candidate as bioreactor for production of recombinant molecules [10,13].

Expression of functional recombinant human growth hormone in transgenic soybean Seeds-We produced human growth hormone (hGH), a protein that stimulates growth and cell reproduction, in genetically engineered soybean seeds. Utilising the alpha prime (α') subunit of β-conglycinin tissue-specific promoter from soybean and the α-Coixin signal peptide from Coix lacryma-jobi, we obtained transgenic soybean lines that expressed the mature form of hGH in their seeds. Expression levels of bioactive hGH up to 2.9% of the total soluble seed protein content (corresponding to approximately 9g kg (-1)) were measured in mature dry soybean seeds. The results of ultrastructural immunocytochemistry assays indicated that the recombinant hGH in seed cotyledonary cells was efficiently directed to protein storage vacuoles. [11, 12] Specific bioassays demonstrated that the hGH expressed in the soybean seeds was fully active. The recombinant hGH protein sequence was confirmed by mass spectrometry characterisation. These results demonstrate that the utilisation of tissue-specific regulatory sequences is an attractive and viable option for achieving high-yield production of recombinant proteins in stable transgenic soybean seeds [16].

Metabolic Engineering of Isoflavones-Isoflavones are ecophysiologically active secondary metabolites derived from the phenylpropanoid pathway. They were mostly found in leguminous plants, especially in the pea family. Isoflavones play a key role in plant-environment interactions and act as phytoalexins also having an array of health benefits to the humans. According to epidemiological studies, a high intake of isoflavones-rich diets linked to a lower risk of hormone-related cancers, osteoporosis, menopausal symptoms, and cardiovascular diseases. These characteristics lead to the significant advancement in the studies on genetic and metabolic engineering of isoflavones in plants. As a result, a number of structural and regulatory genes involved in isoflavone biosynthesis in plants have been identified and characterized. Subsequently, they were engineered in various crop plants for the increased production of isoflavones. Furthermore, with the advent of high-throughput technologies, the regulation of isoflavone biosynthesis gains attention to increase or decrease the level of

isoflavones in the crop plants. In the review, we begin with the role of isoflavones in plants, environment, and its benefits in human health. Besides, the main theme is to discuss the updated research progress in metabolic engineering of isoflavones in other plants species and regulation of production of isoflavones in soybeans [14].

Functional genomics of soybean for improvement of productivity in adverse Conditions-Global soybean production is frequently impacted by various stresses, including both abiotic and biotic stresses. To develop soybean plants with enhanced tolerance to different stressors, functional genomics of soybean and a comprehensive understanding of available biotechnological resources and approaches are essential. In this review, we will discuss recent advances in soybean functional genomics which provide unprecedented opportunities to understand global patterns of gene expression, gene regulatory networks, various physiological, biochemical, and metabolic pathways as well as their association with the development of specific phenotypes. Soybean functional genomics, therefore, will ultimately enable us to develop new soybean varieties with improved productivity under adverse conditions by genetic engineering [15].

IV. CONCLUSION

Soybean is an attractive platform as a protein bioreactor for the industrial scale-up of recombinant proteins. It is the major global vegetable protein commodity widely used as animal feed and as a component of processed food. Producing proteins in soybean has the further advantage that soy oil is a global commodity coproduct with sufficient value to subsidize the costs of protein production. While the production of proteins in soybean is based on standard biotechnology approaches that have been developed for other plant species, soybean possesses some possibly unique intrinsic advantages that separate it from other plant protein bioreactor platforms, enhancing the capacity of soybean to stably accumulate foreign proteins. There is high demand for the production of a large number of recombinant proteins both for scientific evaluation and product development. In some cases, the utilization of plants might determine whether an effective cancer antigen, a microbicide against HIV. The current tools, including synthetic biology and metabolic engineering, should expand even more the capacity to manipulate seeds for the production of recombinant proteins [19].

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