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Basic aspects for choosing crops for recombinant production of therapeutic and industrial molecules

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---ABSTRACT---
The production of recombinant proteins in plant systems has been attractive for several aspects related to production scale and economic and qualitative advantages.The commercial success of some of the first recombinant products produced in transgenic plants and the advanced stage of development of many others - synthesized in different higher plant species - confirm the potential of this expression system.This review focuses on the description of the main aspects that should be taken into consideration when choosing plants as vehicles for the biosynthesis of therapeutic and industrial molecules.
KEYWORDS; *transgenic crops, plant bioreactors,proteins with exogenous function*

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I. INTRODUCTION

Vegetables are one of the most economical systems for the large-scale production of proteins of pharmacological interest, representing from 2 to 10% of the costs of eukaryotic fermentation systems, approximately 0.1% of those for mammalian cell culture and a reduction of 10 to 50 times the final cost of production in bacterial systems.

This savings are mainly due to factors such as eliminating the use of expensive fermenters; the fact that vegetables represent nature's lowest cost biomass; the scheduling capacity of production; the prior establishment of harvesting, transportation, storage and processing practices of plant material; the possibility of protein compartmentalization in organelles typical of plant cells; the presence of natural protein storage organs - such as seeds, tubers and rhizomes - and the elimination of the need for purification of recombinant protein when the storage tissue is consumed in natura.

Plant drug production systems also make it possible to modulate and scale protein production according to the demand for recombinant protein and the culture cycle - generally shorter than that of large mammals secreting protein in milk or urine.

Basically, the methods of purification and processing of recombinant proteins are similar regardless of the production system used, representing more than 85% of the total cost of production of these drugs.

The absence of pathogens common to humans and plants represents a clear source of cost reduction as well as a qualitative and biosafety advantage, since the need for accurate purification of recombinant proteins produced in plant system is quite reduced compared to those produced in plants. mammalian cells and microorganisms, potential carrier sources of toxins and various other contaminants.

In addition, plants whose leaves, fruits, tubers and seeds are consumed in natura are potential candidates to function as biofactories of human and veterinary immunization antigens, promising promising vehicles for expression of vaccine subunits.

From the qualitative point of view factors such as high conservation in plant and animal systems of the processes of synthesis, secretion and post-translational modification - protein folding, oligomerization, glycosylation in the Endoplasmic Reticulum (RE) and processing in the Golgi Complex - with more frequent differences just as to the pattern of plant glycosylation, they approximate how these proteins are synthesized and processed in plants and in the human organism.

Table 1 compares the various economic, qualitative and biosafety-related aspects among the various recombinant protein expression systems already developed.

Table 1: Comparison between the different recombinant protein production systems of pharmaceutical interest

System	**Typical expression. levels**	**Total cost**	**Production timescale (first mg of recombinant proteins)**	**Scale-production capacity**	**Product quality**	**glycosylation**	**Contamination risks**	**Cost of storage**
plant cell culture	Average	Medium	Medium	Average	High	Minor differences	low	Moderate
transgenic plants (stable nuclear trasnformation)	Low	Medium	Long	Very high	High	Minor differences	low	Expressionless (seeds) Moderate (Leaves)
Transplastomic plants	High	Medium	Long	Very high	High	Minor differences	low	Moderate
transgenic plants (transienttransformation /expression)	Very high	Low	Very short	Very high	High	Minor differences	low	Low

Adapted from Ma et al., 2003

The differences between recombinant protein expression systems in terms of glycosylation are due to the synthesis of glycan side chains occurring mainly in the Golgi apparatus. Glycan projections - catalytically added to proteins after translation - differ between animals and plants only in later stages of maturation. These peculiarities are mainly due to the type of enzymatic processing machinery employed in the final glycosylation steps. At these stages, mammalian and plant-specific glycosyltransferases are responsible for catalyzing the addition reactions of N-linked glycans to the folded polypeptide sequence.

The main consequence of these differences is the inability of plants to add terminal residues of galactose and sialic acid to nascent proteins and also to the addition of xylose-β (1 → 2) and fucose-α (1 → 3) carbohydrate groups, which are absent in plants. mammals.These differences in glycan structure may potentially alter the activity, biodistribution, and stability of recombinant plant-expressed proteins, as well as the possibility of allergenicity.However, strategies for the humanization of the recombinant protein glycosylation pattern are routinely adopted to minimize or eliminate the risks of allergenicity.

Leading commercial producer of cereal protein Prodigene Inc. (http://www.prodigene.com) used transgenic maize (Z. mays) plants to obtain the two products mentioned: avidin and β-glucuronidase proteins and It aims to further explore the potential for accumulation of other proteins, such as vaccine subunits, antibodies and recombinant enzymes.

Despite the obvious advantages, the production of recombinant proteins in plants runs into limitations common to species already used for this purpose, especially the low levels of transgene expression and accumulation of recombinant proteins and also problems associated individually with each target species, such as low postharvest protein stability and difficulties in genetic transformation and manipulation.

Some of these problems can be overcome by using different molecular strategies to maximize gene expression and accumulate recombinant proteins.

II. ALTERNATIVE TYPES OF PLANT BIORREACTORS

Alternative plant bioreactors developed so far fall into two categories, mainly as regards the type of expression of the desired recombinant protein: transient expression and stable expression.The first group includes agro-infiltrated plants and those infected with transgenic viruses. In the second are full trsnagenic plants with stable genome transformation, the suspensions of genetically modified cells and transgenic plants mentioned above.

II.I AGROINFILTRATED PLANTS

Plant bioreactors developed so far fall into two categories, mainly as regards the type of expression of the desired recombinant protein: transient expression and stable expression.

The first group includes agro-infiltrated plants and those infected with transgenic viruses. In the second are the suspensions of genetically modified cells and transgenic plants mentioned above.

Transient expression is generally used to verify the activity efficiency of the gene construct employed in genetic transformation experiments and to validate the structure and function of small amounts of recombinant protein.However, infiltration of plant leaves by vacuum or syringe using transgenic A. tumefaciens suspensions may result in the transient transformation of several cells and high levels of expression within a few days of the experiment.

Despite the short time to maintain expression levels (mainly due to the replacement of transformed cells with non-transgenic ones), some reports of the use of this method to produce large-scale recombinant proteins have already been described.Alfalfa (M. sativa) leaf infiltration, for example, could scale up to 7,500 leaves per week by researchers at Medicago Inc (http://medicago.com), resulting in the production of over 100 micrograms of protein. recombinant every week.

II.II PLANTS AGROINFILTRATED WITH VIRAL VECTORS

Another emerging technology is transient expression of recombinant proteins based on the use of plant viruses as expression mediating agents.

Advantages of this system include the short time of onset of expression, the systemic infection capacity provided by viruses, leading to the production of recombinant protein in all plant cells and the ability to use more than one vector in the same plant, with the highest reported expression levels among all the plants systems,allowimg the assembly of multimeric proteins in the same cell environment.

Plants infected with recombinant viruses have already been used to produce vaccine candidate proteins and antibodies.

II.III FULL TRANSGENIC PLANTS

The most popular vegetable bioreactors are full transgenic plants. These crops have undergone stable genetic transformation of their nuclear, mitochondrial or chloroplasmic genomes (transplastomic plants).As a consequence of the stable integration of transgenes into the genetic material of somatic cells as well as gamete producing cells, the new traits arising from transgene expression can be transmitted to the progeny.

Because transgenic plants are regenerated in vitro from previously transformed germ cells or totipotent cells, all cells of the resulting individuals carry the exogenous nucleotide sequence (transgene) and can transmit copies to their offspring on the same principles that govern heredity in non-transgenic plants.

In vitro morphogenesis is based on the totipotency of plant cells and their ability to grow these cells in the laboratory, using appropriate culture media and growth regulators to modulate plant regeneration. Two in vitro morphogenesis models and their variations are used for plant regeneration aiming at their genetic transformation: organogenesis and somatic embryogenesis.

Organogenesis stimulates the formation of aerial parts or roots in callus culture (mass of cells of continuous proliferation and more or less disordered) or other explants, from the neoformation of vegetative or floral stem buds that convert into stem axes or roots. . In this method the regenerated organs have multicellular and subepidermal origin and the regeneration occurs from meristematic cell groups of the original tissue.

The cultivation of embryos with their apical meristematic region preserved or simply from the apical or axillary meristems themselves, in the presence of cytokines in culture medium, is an efficient inducer of direct organogenesis of aerial part, capable of regenerating transgenic plants without the need for passage. tissue by intermediate phases of de-differentiation 愀such as callus, for example.

Somatic embryogenesis advocates the development of embryos from somatic cells as a result of external stimulation (contact with growth phytoregulators such as 2,4-D - 2,4 dichlorophenoxyacetic acid). This method of tissue culture leads to adventitious multiembryonic explant formation, with the originated embryos having their own vascular axes, single cell or few cell histological origin and superficial localization.

Both forms of in vitro plant regeneration allow, from the initial explants, to continuously obtain tissues and experimental material through cyclic cultures.

As a result of the integrated action between the three sets of the main techniques that constitute the process of genetic transformation of plants, it is possible to construct plasmid vectors containing genes of interest, introduce them into cells and plant explants, verify the integration of exogenous sequences into the genome of the plant. plant species concerned and to express them stably over successive generations in order to obtain transgenic plants and recombinant proteins in significant quantities.

II.IV TRANSGENIC CELL SUSPENSIONS

Plant cell cultures are usedfor the production of recombinant proteins as an alternative to transient expression systems and transgenic plants.They combine the merits of whole-plant expression systems (such as synthesis, post-translational processing and assembly mechanisms) with those of microorganism cell cultures (intrinsic protein secretion capacity).

Genetic transformation of these cells usually takes place through biobalistic introduction of exogenous genes and signal peptides are frequently used in gene constructs capable of destining nascent proteins to the secretory pathway, culminating in their extrusion to the culture supernatant.

Another possible fate of these proteins is cell retention. Both secretion and retention depend on the production strategy to be employed, the degree of purification desired, the use of signal peptides and the cell wall permeability to the proteins produced in question - proteins between 20 and 30 kDa tend to cross wall easily while larger molecules tend to be trapped.

The high degree of physical restriction of recombinant protein biosynthesis and the possibility of production under good handling practices - such as the precise control of growing conditions, the use of sterile environments, well defined culture media and buffers and the requirement of purification protocols represent the great qualitative advantages of this system, although some of them add to the final production.

Cells from tobacco leaves (N. tabacum) are the most used so far, but those from soybean (G. max), tomato (L. esculentum), rice (O. sativa) and root tobacco (N. tabacum) have been used for the secretion of more than 20 different recombinant proteins, including antibodies, interleukins, erythropoietin and vaccine antigens.

However, few recombinant proteins produced in cell suspensions presented satisfactory biosynthesis yields to make their commercial production viable, which is the main limiting factor for a greater popularization of this expression system.

III. MAIN PLANTS USED AS RECOMBINANT PROTEIN BIOREACTORS.

The choice of plant species for the production of pharmaceuticals and industrial proteins represents one of the most important criteria aiming at the success of the molecular strategy of gene expression adopted.

Genomic, biochemical, physiological and even morphological properties and peculiarities inherent to each species exert crucial influence on many factors, such as the yield of obtaining recombinant proteins, the ability to promote post-translational modifications in complex proteins, the final cost of production and protein stability at the structural level.

Table 2 illustrates the properties and peculiarities of the major plant species used as recombinant protein bioreactors for the pharmaceutical and industrial sectors.

Table 2: Main properties of plants used as bioreactors.

Species	**Advantages**	**Disadvantages**
model plants		
Arabidopsis	Wide availability of mutants, genetic accessibility and ease of transformation	Needless to commercial production (low biomass)
simple plants		
Physcomitrella patens (Hedw.) Bruch &Schimp., Chlamydomonas reinhardtii Lemna	Facility contingency, clonal propagation, secretion in the culture medium, regulatory compliance, homologous recombination in Physcomitrella	production scheduling difficulties
hardwoods		
Tobacco	High production, technology transformation and expression, rapid escalation, not used for human consumption and other animals	Low post-harvest protein stability, presence of alkaloids
Alfalfa and clover	High production useful as animal vaccination, clonal propagation, homogeneous addition of N-glycans (alfalfa)	Low postharvest protein stability, presence of oxalic acid
Lettuce	Edible, useful for human vaccination	Low post-harvest protein stability
Cereals		
Corn and Rice	protein stability during storage, high production, processing and handling	

	facilities	
Wheat and Barley	protein stability during storage	Low levels of production, processing and handling difficulties
Vegetables		
Soy	Saving, high biomass possibility of expression in the seeds coats High protein concentration in the seeds	Low expression levels, difficulties in processing and handling
Peas and Guandu	High protein concentration in the seeds	Low expression levels
Fruits and Tubers		
Potato and Carrot	Edible protein stability in storage tissues	Potatoes need to be cooked
Tomato	Edible, subject to restraint in greenhouses	Farming more expensive, need to be refrigerated after harvest
oilseeds		
Canola and Camelina	Platform melting the Oleosin protein budding system developed	Low production levels

Adapted from Fischer et al., 2004.

From an agronomic point of view, transgenic seed-producing plants are promising highly stable expression and compartmentalization systems of recombinant proteins in transgenic cells in these organs.

The seeds have large amounts of protein biomass and have been the preferred target of accumulation of recombinant proteins for long periods of time. Strategies used to exclusively target transgene expression to seeds and subcellular structures of seed cells involve the assembly of expression cassettes containing tissue-specific promoters and seed-typical signal peptides into plasmids used in genetic transformation experiments. .

Agronomic, economic and biosafety aspects involving seed production in these plants should be studied prior to the elaboration of the molecular strategy for obtaining recombinant drugs and industrial proteins (Table 3).

Table 3: Agronomic properties of different seed-producing plants with potential to function as recombinant protein production platforms.

Species	**Approximate seed biomass (kg / ha)**	**protein seed content (%)**	**Type of pollination**	**average production cost (US $ dollars / ha)**	**trading platform**
Cereals					
Corn	8670	10	cross pollination	75	Large Scale Biology Corp., Meristem Therapeutics, Maxygen Inc., ProdiGene Inc.
Rice	7270	8	Self-pollination	112	Ventria Biosystems Inc.
Barley	3100	13	Self-pollination	98	Ventria Biosystems Inc., ORF Genetics, Maltagen
Wheat	2700	12	Self-pollination	104	
Legumes					
Soy	2600	40	Self-pollination	163	-
Pea	2500	40	Self-pollination	128	-
Oil plants					
Canola	1500	22	cross pollination	238	SemBioSys Genetics Inc.
safflower	1500	25	cross pollination	240	SemBioSys Genetics Inc.
Camelina	1100	25	cross pollination	not sold	UniCrop Ltd

Adapted from Stoger et al., 2005.

IV. CONCLUSIONS

At first it takes a long time to optimize the expression of a recombinant protein in any biological production system, especially when various subcellular compartments such as those observed in plant cells are available.

Once the appropriate combination involving the transgene receptor plant type has been achieved, the different elements of regulation of gene expression in the expression cassette, the molecular strategy of protein subcellular addressing, and the genetic transformation and tissue culture protocols, The functioning of the plant as a correct, safe and economical biosynthesis agent of a given recombinant protein is put in check over several stages until they have a direct or indirect influence on the consumer's quality of life.

Transgenic plants have already demonstrated their crucial importance in modern agriculture and food production, in which they have legitimized, for over two decades, a considerable impact on reducing production costs and environmental damage, as well as increasing agricultural yields.

REFERENCE

[1]. Babino, A., Pritsch, O., Oppezzo, P., Du Pasquier, R., Roseto, A., Osinaga, E., and Alzari, P.M. (1997). Molecular cloning of a monoclonal anti-tumor antibody specific for the Tn antigen and expression of an active single-chain Fv fragment. Hybridoma 16, 317-324.

[2]. Bakker, H., Bardor, M., Molthoff, J.W., Gomord, V., Elbers, I., Stevens, L.H., Jordi, W., Lommen, A., Faye, L., Lerouge, P., and Bosch, D. (2001). Galactose-extended glycans of antibodies produced by transgenic plants. Proceedings of the National Academy of Sciences 98, 2899-2904.

[3]. Barwale, U.B., Kerns, H.R., and Widholm, J.M. (1986). Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. Planta 167, 473-481.

[4]. Blixt, O., Allin, K., Pereira, L., Datta, A., and Paulson, J.C. (2002). Efficient Chemoenzymatic Synthesis of O-Linked Sialyl Oligosaccharides. J. Am. Chem. Soc. 124, 5739-5746.

[5]. Brasileiro, A.C.M. (1998). Introdução à Transformação Genética de Plantas. In Manual de Transformação Genética de Plantas, E. Cenargen, ed (Brasilia: Embrapa Cenargen), pp. 13-16.

[6]. Chargelegue, D., Vine, N.D., van Dolleweerd, C.J., Drake, P.M.W., and Ma, J.K.C. (2000). A murine monoclonal antibody produced in transgenic plants with plant-specific glycans is not immunogenic in mice. Transgenic Research 9, 187-194.

[7]. Commandeur, U., Twyman, R.M., and R.Fischer. (2003). The biosafety of molecular farming in plants. AgBiotechNet 110, 1-9.

[8]. Fernandez-San-Millan, A., Mingo-Castel, A., Miller, M., and Daniell, H. (2003). A chloroplast transgenic approach to hyper-express and purify Human Serum Albumin, a protein highly susceptible to proteolytic degradation. Plant Biotechnology Journal 1, 71-79.

[9]. Fischer, R., Stoger, E., Schillberg, S., Christou, P., and Twyman, R.M. (2004). Plant-based production of biopharmaceuticals. Current Opinion in Plant Biology 7, 152-158.

[10]. Giddings, G. (2001). Transgenic plants as protein factories. Current Opinion in Biotechnology 12, 450-454.

[11]. Gils, M., Kandzia, R., Marillonnet, S., Klimyuk, V., and Gleba, Y. (2005). High-yield production of authentic human growth hormone using a plant virus-based expression system. Plant Biotechnology Journal 3, 613-620.

[12]. Hellwig, S., Drossard, J., Twyman, R.M., and Fischer, R. (2004). Plant cell cultures for the production of recombinant proteins. Nat Biotech 22, 1415-1422.

[13]. Jaeger, G.D., Scheffer, S., Jacobs, A., Zambre, M., Zobell, O., Goossens, A., Depicker, A., and Angenon, G. (2002). Boosting heterologous protein production in transgenic dicotyledonous seeds using Phaseolus vulgaris regulatory sequences. Nat Biotech 20, 1265-1268.

[14]. Kusnadi, A.R., Zivko, L., and Nikolov, J.A.H. (1997). Production of recombinant proteins in transgenic plants: Practical considerations. Biotechnology and Bioengineering 56, 473-484.

[15]. Larrick, J.W., and Thomas, D.W. (2001). Producing proteins in transgenic plants and animals. Current Opinion in Biotechnology 12, 411-418.

[16]. Ma, J.K.C., Drake, P.M.W., and Christou, P. (2003). The production of recombinant pharmaceutical proteins in plants. Nat Rev Genet 4, 794-805.

[17]. Schillberg, S., Emans, N., and Fischer, R. (2002). Antibody molecular farming in plants and plant cells. Phytochemistry Reviews 1, 45-54.

[18]. Stoger, E., Ma, J.K.C., Fischer, R., and Christou, P. (2005). Sowing the seeds of success: pharmaceutical proteins from plants. Current Opinion in Biotechnology 16, 167-173.

[19]. Stoger, E., Sack, M., Perrin, Y., Vaquero, C., Torres, E., Twyman, R.M., Christou, P., and Fischer, R. (2002). Practical considerations for pharmaceutical antibody production in different crop systems. Molecular Breeding 9, 149-158.

[20]. Twyman, R.M., Stoger, E., Schillberg, S., Christou, P., and Fischer, R. (2003). Molecular farming in plants: host systems and expression technology. Trends in Biotechnology 21, 570-578.

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