Asian Journal of Biology

3(3): 1-8, 2017; Article no.AJOB.34748
ISSN: 2456-7124

Plants as Expression Systems for Recombinant Proteins

Fanuel Kawaka[1*] and Alex Ngetich[2]

[1]Department of Pure and Applied Sciences, Technical University of Mombasa, P.O.Box 90420, Mombasa, Kenya.
[2]Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.

Authors' contributions

This work was carried out by both the authors. Author FK reviewed the relevant literature and drafted the manuscript while author AN reviewed and corrected the final document. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2017/34748
Editor(s):
(1) Farjana Sultana, College of Agricultural Sciences, International University of Business Agriculture and Technology (IUBAT University), Bangladesh.
Reviewers:
(1) Sérgio Emílio dos Santos Valente, Universidade Federal do Piauí, Campus Ministro Petrônio Portela Teresina-PI, Brazil.
(2) Kathleen Hefferon, Cornell University, USA.
(3) Zixian Lu, Academy of State Administration of Grain, Beijing, China.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/20409

Review Article

Received 9th June 2017
Accepted 4th July 2017
Published 8th August 2017

ABSTRACT

Plants provide a wide range of important biological molecules that are needed in research, medicine and industry. Currently, there is a growing interest in the use of plants as expression systems for the production of recombinant proteins using different host cells. The synthesis of proteins in plants is safe, economical and can be scaled up compared to the microbial and mammalian expression systems. Despite the fact that plant systems are widely recognized for producing recombinant proteins, there are certain improvements that should be addressed before the system is considered commercially viable. Such challenges include the need to identify promoters that would provide comparable yield to other systems, enhance protein stability and recovery. In this review, we describe the production of some plant based proteins, challenges and advantages of plants systems.

**Corresponding author: E-mail: fkjairo@hotmail.com;*

Keywords: Microbial; mammalian; expression systems; promoters.

1. INTRODUCTION

Plants are increasingly being considered as alternative expression systems for the production of proteins. The interest in plants is due to their ability to be easily transformed as cheap sources of protein. Currently there are several biotechnology companies that are actively developing, field testing, and patenting plant expression systems [1]. Traditionally plants have been used as sources of many biologically active molecules including proteins. These molecules are required for research, medicine and industrial applications however their extraction from natural sources is usually difficult and expensive [2]. Target recombinant proteins are usually expressed in transgenic plants with desired characteristics. Transgenic plants are genetically engineered through breeding techniques using recombinant DNA technology. Generally transgenic plants are produced in two ways; first by using *Agrobacterium* mediated transformation, particle bombardment and other standard transformation techniques. There are several *Agrobacterium* transformed plants producing recombinant proteins including *Nicotiana tabacum*, a model expression system for proteins [3]. Secondly, non transgenic plants are infected with recombinant viruses that express transgenes during their replication in the host [1].

The two commonly used host viruses are tobacco with tobacco mosaic virus and cowpeas with cowpea mosaic virus [1,4]. Despite the product yield being typically similar to those of the transformed plants, they are always higher [5]. Expression of proteins in transgenic plants involves cloning and expression of the gene of interest in plant cells, regeneration and selection of the plants, recovery and purification of the protein and finally characterization of the final product [6].

There are general procedures in obtaining and purifying desired proteins from plants [7]. Irrespective of the plant species, usually the plant source material is first ground (seeds) or homogenized (green tissue) followed by cold buffer extraction and centrifugation. However there are challenges encountered during extraction of proteins like those posed by the plant enzymes. Kusnadi et al. [6] described the problems as inefficiency of protein extraction, possible structural modification of the product due to secondary reaction with phenolics and proteolytic degradation. The quantity of phenolics varies among different plants but green plant parts contain more phenolics than seeds. The principle of plant protein purification is similar despite the source of the target protein and the choice of purification sequence does not depend on the scale and tissue from which the plant has been extracted [7]. Protein scale up process may further lead to problems such as reduced extraction, purification yields and activity loss due to foaming shear [8]. Recombinant proteins are usually isolated together with plants natural proteins to assess the accumulation levels, establish protein functionality and to confirm the amino acid sequence. Based on the type of the recombinant protein and transgenic plant, different extraction methods are always used. This review describes some plant based proteins, challenges and advantages of plants as expression systems for proteins.

2. SOME RECOMBINANT PROTEINS EXPRESSED IN PLANTS

2.1 Phytase

Recombinant proteins such as phytase from *Aspergillus niger* have successfully been expressed in transgenic plants at higher levels. Phytases are enzymes that release phosphates from phytates, a major form of phosphorus (P) in animal feeds. Production of phytase from microbial sources is associated with high production and processing costs [9]. Therefore transgenic plants offer alternative expression system for phytase for commercial use. Phytase genes from microbial sources have been expressed in many plants including tobacco, canola, soybean, wheat, rice, alfalfa, sesame, *Arabidopsis*, maize, *Trifolium repens*, *Medicago truncatula* and potato [10-12]. The technique involves fusing the fungal phytase gene (*phy*A) to a plant endoplasmic reticulum and placing it under the control of the constitutive 35S cauliflower mosaic virus (CaMV) promoter in a binary transformation vector [9]. The gene is inserted into the tobacco genome by *Agrobacterium*-mediated transformation [13]. Several studies have demonstrated that transgenic plants contain sufficient phytase activity that could replace microbial phytases [14,15]. The plants could reduce downstream processing and formulation costs involved in the commercial production of phytases and serve as bioreactors.

2.2 Hirudin

Hirudin is an anticoagulant protein that was initially isolated from the salivary gland of leech, *Hirudo medicinalis* and is used for treating thrombosis [16]. Despite hirudin being produced mostly by recombinant bacteria and yeast, plants such as oilseed rape, tobacco, and Ethiopian mustard have been engineered to produce the same protein [17]. The fusion of hirudin genes with oleosin genes has reduced the cost of protein purification making commercial production easier and cheaper [18]. The plant oleosin-hirudin fusion protein is expressed and accumulated in the seeds and separated from other seed proteins by flotation and centrifugation [1]. The method ensures that hirudin becomes active only after harvesting thus limiting environmental exposure [1]. It is therefore evident that the fusion of the oleosin and hirudin has a potential for commercial production of the protein in transgenic plants.

2.3 Enkephalin

Enkephalin is one of the earliest transgenic peptides in plants with potent painkilling effects. The peptide is released by neurons in the central nervous system and cells in the adrenal medulla. The method of expression involves insertion of DNA coding sequence into the gene coding for a seed storage protein called 2S albumin [19,20]. The 2S albumins are amongst the smallest seed storage proteins with conserved and variable sequences derived from group of structurally related genes [21]. The strategy employed to produce Enkephalin involves substituting part of the variable sequence with DNA sequence coding for the five amino acid neurohormone. The DNA construct is then flanked on both sides by codons coding for the tryptic cleavage site. The expression of the altered 2S albumin gene results in the production of a hybrid storage protein containing Enkephalin sequence [22]. The sequence subsequently released from the altered protein tryptic cleavage and purified by High Performance Liquid Chromatography (HPLC).

2.4 Thaumatin

Thaumatin is a sweet-tasting protein that is widely used in the food industry. The peptide is isolated from the fruit of the African katemfe (*Thaumatococcus daniellii* Bennett) [23]. Besides the sweet taste, thaumatin enhances certain flavours while masking others, binding specifically with taste receptors [24]. The antifungal activity and sweet taste of the protein makes it a good candidate for use in genetic engineering for producing disease tolerant crops with modified tastes. The protein has naturally been extracted from plant fruits for commercial production of sweeteners and flavor enhancers. Attempts for commercial production of thaumatin in microorganisms has resulted in low product yields reducing the economic feasibility for large-scale production [23]. Thaumatin gene has successfully been expressed in potato hairy roots and cucumber using an *Agrobacterium rhizogenes*-based approach [25]. Thaumatin expression in the plants was confirmed at protein level and sensory evaluations [26].

2.5 Monellin

Monellin is a sweet protein consisting of two noncovalently bound polypeptide chains, A and B [27,28]. The protein is extracted and purified from the West African plant Dioscoreophyllum cumminsii and sweeter than sugar with a slow onset of sweetness and a lingering after taste [27,29]. The A and B chains have a secondary structure containing 44 and 50 amino acids, respectively [30,31]. Native Monellin protein is pH and temperature dependent with an optimal taste ranging between pH (2 and 9) [32]. Higher temperatures above 50°C dentures the protein and causes the separation of the units leading to the loss of sweetness [33]. The instability challenges of the protein have been addressed through downstream processes [27]. Further, thermal stability and quality of sweetness of the sweet tasting protein has been increased through site-directed gene mutagenesis and protein modification [34]. The technique involved obtaining mutants by alanine substitution of amino acids at different sites in the Monellin protein [35]. Results showed that the thermal stability and sweetness of the natural Monellin protein can be improved by gene mutation technique [36]. These findings demonstrate the potential of commercial production of Monellin as an alternative source of sugar.

2.6 Papain

Papain commonly called the vegetable pepsin is a cysteine protease isolated from the latex of the green fruit and leaves of *Carica papaya* [20]. The protein consists of a single polypeptide chain with three disulfide bridges and a sulfhydryl group that is necessary for its activity [37]. The enzyme is commercially used as a meat

tenderizer due to its ability to dissolve tissues without damaging the cells [38]. During the tenderization process, the proteolytic activity of papain is directed at collagen, the major structural protein in animals, about a third of all vertebrate protein. The collagen present in the connective tissue and blood vessels is responsible for the toughness of the meat and therefore hydrolysis of the peptide bonds in collagen is achieved either by boiling or papain activity [20]. Proteolytic activity or boiling degrades the tough insoluble collagenous fibrils yielding soluble polypeptides of lower molecular weight. The enzyme is injected directly into the blood stream of animals before slaughter to achieve even distribution. Due to the thermal stability of papain, it is able to retain its proteolytic activity during the initial stages of cooking. Industrially, papain is used in clarifying beer, clarifying preservation of spices, contact lens cleaners, detergents, pet food palatability, digestive aids, blood stain remover, blood coagulant and cosmetics [20,39]. Other uses include aiding protein digestion in chronic dyspepsia, gastric fermentation, gastritis, removal of necrotic tissue, preparation of tyrosine derivatives for the treatment of Parkinsonism, preparation of tetanus vaccines, skin cleansing agents, acne treatment [40]. The demand for more stable, highly active and specific enzymes such as papain is growing rapidly [41,42].

Currently, different plant species are being exploited to determine their potential in expressing therapeutic proteins of interest. Examples include the production of human erythropoietin and transferring in *Nicotiana benthamiana*, *a*-L-iduronidase in *Arabidopsis thaliana* and *Brassica napus* and anti-HSV glycoprotein B in soybean [43,44]. The expression of a recombinant human glucocerebrosidase (GCD) in transgenic carrot cells grown in suspension culture has been described to be capable of producing glycoproteins [45]. GCD in carrot cells is structurally and highly homologous to the commercial Cerezyme displaying comparable enzymatic activity and uptake into macrophages [46].

There are several other commercial proteins that are produced in plants primarily to enhance their agronomic performance. As indicated in Table 1, these plants are able to express and accumulate proteins of potential industrial applications that were originally produced by other systems [6]. However there are many factors to be considered when selecting transgenic plant system for production of proteins. The critical factors include the level of protein accumulation, germplasm, handling, processing of transgenic plant material, and protein purification can influence quality, quantity, purification and the cost of the final product.

Table 1. Industrial recombinant proteins expressed in transgenic plants

Recombinant protein	Initial source	Host
a-Amylase	*Bacillus licheniformis*	Tobacco
Aprotinin	Bovine	Corn
Avidin	Chicken	Corn
Chymosin	Calf	Tobacco, Potato
Cyclodextrin glucanotransferase	*Klebsiella pneumoniae*	Potato
Erythropoietin	Human	Tobacco
Glucoamylase	*Aspergillus niger*	Potato
β Glucuronidase	*Escherichia coli*	Corn
Growth hormone	Trout	Tobacco
Heat-labile enterotoxin B	*Escherichia coli*	Tobacco, potato
Hepatitis B surface antigen	Hepatitis B virus	Tobacco
γ - and k-chains hybridoma	Mouse	Tobacco
b-Interferon	Human	Tobacco
Levansucrase	*B. subtilis, Streptococcus mutans*	Tobacco, potato
Lysozyme	Chicken	Tobacco
Malarial epitopes	*Plasmodium*	Tobacco
Phytase	*Aspergillus niger*	Tobacco
Xylanase	*Clostridium thermocellum*	Tobacco
Serum albumin	Human	Human
Ricin	Castor bean	Castor bean

3. ADVANTAGES OF PLANT SYSTEMS

Several studies have demonstrated that recombinant proteins from plants are superior in terms of storage and distribution compared to animals and animal cell cultures [47,48]. The expression of proteins in plants is considered better than other systems like microbes due to their safety, cost, and protein complexity and require less time [49]. Due to practical, economic and safety benefits compared with conventional systems, the use of plants for large scale synthesis of proteins is gaining wider acceptance in the world [47,50,51].

There are minimum risks of contamination with pathogenic microbes since no known incidences plant diseases have been found to be pathogenic to humans [47]. Cultivation of plants is less complex compared to microbes or mammalian cell cultivation that is extremely expensive and requires good laboratories with competent technical and scientific expertise. Plant proteins can be expressed in the edible parts such as roots or leaves that are consumed raw thus eliminating the need for purification [52]. Plants are more economical in protein production compared to mammalian cell culture and microbial fermentation depending on the protein of interest, product yield and crop used [53].

Proteins of interest can be expressed in various targeted cells or tissues like seeds or tubers where they are more stable, easy to transport with no need for refrigeration [52]. Industrial production of plant proteins can be achieved through increased cultivation of more crops. Plant systems are capable of performing most of the post-translational modifications required for protein stability, bioactivity and pharmacokinetics [54]. Studies have demonstrated that glycosylation, targeting, compartmentalization of simple proteins such as interferons and serum albumins have successfully occurred in plants [47]. In contrast to microbes, plants are capable of expressing and assembling antibodies in their active form [55].

4. LIMITATIONS OF PLANT SYSTEMS

Despite the increasing preference of plants as expression systems, their use for the production of therapeutic proteins still remains at developmental research [52]. Such limitations arise from low level of expression, accumulation and incorrect post-translational modifications of the recombinant protein [56]. Plant proteins lack the terminal galactose and sialic acid residues commonly found in animals, and have α-(1,3) fucose and β-(1,2) xylose which are absent in mammalian systems. Glycoproteins from plants can therefore lead to immunogenicity and affect pharmacokinetic properties [57]. Economically, higher plants are not preferred systems for expressing commercial proteins for many reasons. First, industrial proteins expressed in plants can be synthesized in other systems and the alternatives are more viable technically and economically. Secondly, several studies have shown that higher plants accumulate waste products and heavy metals and these substances can irreversibly compromise the quality of the proteins produced in these plants [58-60]. Thirdly, due to the seasonal growth of most plants, adequate and constant supply of recombinant proteins is always constrained in between the seasons. Lastly, there are possible contamination of plant proteins with pesticides, herbicides and plant metabolites as well varying growth conditions due to differences in soil quality and climate [47].

Other challenges result from the post translational modifications (PTMs) of proteins expressed in plants. The PTMs such as glycosylation and proteolytic processing may influence the stability, function and biological activity of plant therapeutic proteins [20,43]. Studies have confirmed that defects in a number of genes in the glycosylation pathway cause congenital disorders with serious medical consequences [61]. However variation in the glycosylation profiles of specific therapeutic proteins have been recognized in diagnostics as disease markers [62]. Plant protein glycosylation can introduce heterogeneity through the generation of different key oligosaccharides and variable addition of outer arm sugars as the glycoprotein transits the Golgi [63]. The nature and presence of these oligosaccharides may affect the glycoprotein folding, stability, trafficking and immunogenicity including its primary function [64]. The modifications usually range from simple chemical changes, such as the addition of small phosphate or acetate functional groups to complex changes that are enormous in size and mass exceeding the original protein [65,66]. The PTMs are strictly regulated, specific and may be due to developmental stage, location or by certain biotic or abiotic factors, mainly by stress and disease state of a plant [67]. The cleavage of a signal peptide or glycosylation is permanent changes of a protein structure compared to others like phosphorylation that are rapidly and easily reversed [43].

5. CONCLUSION

The use of plants as expression systems has emerged as a promising system for the expression, production and manufacture of proteins of high value to health, food and industrial sectors. There are more advantages of plants compared to other traditional bioreactors. Typically, plants systems are superior in terms of safety, storage, complexity, cost and distribution relative to microbes, animals and insect cell cultures. Glycosylation, targeting, compartmentalization and natural storage stability in certain organs continue to make plants the preferred choice for production of proteins. Production of proteins in transgenic plants for commercial shows a great potential due to the potential economic benefits. Addressing challenges such as expression of proteins that are structurally and functionally similar to their native sources will make the plants ideal system for commercial production of recombinant proteins. It is vital to develop plant protein purifications systems that are safe and cost effective to meet the industrial need. The identification of the right candidate genes, market need and efficient production systems will build a strong link between plant protein screening and commercial utilization.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Giddings G, et al. Transgenic plants as factories for biopharmaceuticals. Nature Biotechnology. 2000;18(11):1151-1155.
2. Ma JK, Drake PM, Christou P. The production of recombinant pharmaceutical proteins in plants. Nature Reviews Genetics. 2003;4(10):794-805.
3. Rech E, et al. Recombinant proteins in plants. In BMC Proceedings. BioMed Central; 2014.
4. Stöger E, et al. Cereal crops as viable production and storage systems for pharmaceutical scFv antibodies. Plant Molecular Biology. 2000;42(4):583-590.
5. Gelvin SB. Agrobacterium-mediated plant transformation: The biology behind the "Gene-Jockeying" tool. Microbiology and Molecular Biology Reviews. 2003;67(1): 16-37.
6. Kusnadi AR, Nikolov ZL, Howard JA. Production of recombinant proteins in transgenic plants: Practical considerations. Biotechnology and Bioengineering. 1997;56(5):473-484.
7. Jervis L, Pierpoint W. Purification technologies for plant proteins. Journal of Biotechnology. 1989;11(2-3):161-198.
8. Porter JE, Ladisch MR, Herrmann KM. Ion exchange and affinity chromatography in the scaleup of the purification of α-galactosidase from soybean seeds. Biotechnology and Bioengineering. 1991;37(4):356-363.
9. Gontia I, et al. Transgenic plants expressing phytase gene of microbial origin and their prospective application as feed. Food Technology and Biotechnology. 2012;50(1):3.
10. Wang Y, et al. Expression of a heat stable phytase from *Aspergillus fumigatus* in tobacco (Nicotiana tabacum L. cv. NC89); 2007.
11. Peng RH, et al. Codon-modifications and an endoplasmic reticulum-targeting sequence additively enhance expression of an *Aspergillus phytase* gene in transgenic canola. Plant Cell Reports. 2006;25(2): 124.
12. Li G, et al. Functional analysis of an *Aspergillus ficuum* phytase gene in *Saccharomyces cerevisiae* and its root-specific, secretory expression in transgenic soybean plants. Biotechnology Letters. 2009;31(8):1297-1303.
13. Greiner R, Konietzny U. Phytase for food application. Food Technology and Biotechnology. 2006;44(2):123-140.
14. Lei XG, et al. Phytase, a new life for an "old" enzyme. Annu. Rev. Anim. Biosci. 2013;1(1):283-309.
15. Yao MZ, et al. Improving the thermostability of *Escherichia coli* phytase, appA, by enhancement of glycosylation. Biotechnology Letters. 2013;35(10):1669-1676.
16. Houschyar KS, et al. Medical leech therapy in plastic reconstructive surgery. Wiener Medizinische Wochenschrift. 2015; 165(19-20):419-425.
17. Wanasundara JP. Proteins of *Brassicaceae* oilseeds and their potential as a plant protein source. Critical Reviews in Food Science and Nutrition. 2011;51(7): 635-677.
18. Rigano MM, et al. Production of pharmaceutical proteins in solanaceae food crops. International Journal of Molecular Sciences. 2013;14(2):2753-2773.
19. Fauteux F, Strömvik MV. Seed storage protein gene promoters contain conserved DNA motifs in Brassicaceae, Fabaceae

and Poaceae. BMC Plant Biology. 2009;9(1):126.
20. Walsh G. Proteins: Biochemistry and biotechnology. Wiley; 2002.
21. Moreno FJ, Clemente A. 2S albumin storage proteins: what makes them food allergens? The Open Biochemistry Journal. 2008;2(1).
22. Guerche P, et al. Differential expression of the Arabidopsis 2S albumin genes and the effect of increasing gene family size. The Plant Cell. 1990;2(5):469-478.
23. Bartoszewski G, et al. Modification of tomato taste in transgenic plants carrying a thaumatin gene from *Thaumatococcus daniellii Benth.* Plant Breeding. 2003; 122(4):347-351.
24. Xu Z, et al. Biotechnology and sustainable agriculture 2006 and beyond: Proceedings of the 11th IAPTC&B Congress, August 13-18, 2006 Beijing, China. Springer Netherlands; 2007.
25. Zawirska-Wojtasiak R, et al. Aroma evaluation of transgenic, thaumatin II-producing cucumber fruits. Journal of Food Science. 2009;74(3):C204-C210.
26. Liu JJ, Sturrock R, Ekramoddoullah AK. The superfamily of thaumatin-like proteins: Its origin, evolution, and expression towards biological function. Plant Cell Reports. 2010;29(5):419-436.
27. Liu Q, et al. Modification of the sweetness and stability of sweet-tasting protein monellin by gene mutation and protein engineering. BioMed Research International. 2016;7.
28. Kant R. Sweet proteins–potential replacement for artificial low calorie sweeteners. Nutrition Journal. 2005;4(1):5.
29. Morris JA, et al. Characterization of monellin, a protein that tastes sweet. Journal of Biological Chemistry. 1973;248(2):534-539.
30. Tancredi T, et al. Structural determination of the active site of a sweet protein A 1H NMR investigation of pMNEI. FEBS Letters. 1992;310(1):27-30.
31. Kohmura M, Nio N, Ariyoshi Y. Complete amino acid sequence of the sweet protein monellin. Agricultural and Biological Chemistry. 1990;54(9):2219-2224.
32. Leone S, Picone D. Molecular dynamics driven design of pH-stabilized mutants of MNEI, a sweet protein. PLoS One. 2016;11(6):e0158372.
33. Kim SH, et al. Redesigning a sweet protein: Increased stability and renaturability. Protein Engineering. 1989;2(8):571-575.
34. Templeton CM, et al. Reduced sweetness of a monellin (MNEI) mutant results from increased protein flexibility and disruption of a distant poly-(L-proline) II helix. Chemical Senses. 2011;36(5):425-434.
35. Cai C, et al. Expression of a high sweetness and heat-resistant mutant of sweet-tasting protein, monellin, in *Pichia pastoris* with a constitutive GAPDH promoter and modified N-terminus. Biotechnology Letters. 2016;38(11):1941-1946.
36. Leone S, et al. *S*weeter and stronger: Enhancing sweetness and stability of the single chain monellin MNEI through molecular design. Scientific Reports. 2016;6.
37. Hughes J. Biopharmaceuticals: Biochemistry and Biotechnology. Pharmaceutical Science & Technology Today. 1998;1(3): 94.
38. Mahmood A, Sidik K, Salmah I. Wound healing activity of *Carica papaya L.* aqueous leaf extract in rats; 2005.
39. Murthy MB, Murthy BK, Bhave S. Comparison of safety and efficacy of papaya dressing with hydrogen peroxide solution on wound bed preparation in patients with wound gape. Indian Journal of Pharmacology. 2012;44(6):784.
40. Choudhury D, et al. Production and recovery of recombinant propapain with high yield. Phytochemistry. 2009;70(4): 465-472.
41. Turner P, Mamo G, Karlsson EN. Potential and utilization of thermophiles and thermostable enzymes in biorefining. Microbial Cell Factories. 2007;6(1):9.
42. Bhat M. Cellulases and related enzymes in biotechnology. Biotechnology Advances. 2000;18(5):355-383.
43. Vukušić K, Šikić S, Balen B. Recombinant therapeutic proteins produced in plants: Towards engineering of human-type O-and N-glycosylation. Periodicum Biologorum. 2016;118(2).
44. Zeitlin L, et al. A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes. Nature Biotechnology. 1998;16(13):1361-1364.
45. Shaaltiel Y, et al. Production of glucocerebrosidase with terminal mannose glycans for enzyme replacement therapy of Gaucher's disease using a plant cell

system. Plant Biotechnology Journal. 2007;5(5):579-590.
46. Lerouge P, et al. N-glycoprotein biosynthesis in plants: Recent developments and future trends. Plant Molecular Biology. 1998;38(1-2):31-48.
47. Demain AL, Vaishnav P. Production of recombinant proteins by microbes and higher organisms. Biotechnology Advances. 2009;27(3):297-306.
48. Ahmad P, et al. Role of transgenic plants in agriculture and biopharming. Biotechnology Advances. 2012;30(3):524-540.
49. Merlin M, et al. Comparative evaluation of recombinant protein production in different biofactories: The green perspective. BioMed Research International. 2014;1-14.
50. Fischer R, Emans N. Molecular farming of pharmaceutical proteins. Transgenic Research. 2000;9(4-5):279-299.
51. Giddings G. Transgenic plants as protein factories. Current Opinion in Biotechnology. 2001;12(5):450-454.
52. Desai PN, Shrivastava N, Padh H. Production of heterologous proteins in plants: Strategies for optimal expression. Biotechnology Advances. 2010;28(4):427-435.
53. Twyman RM, Schillberg S, Fischer R. Transgenic plants in the biopharmaceutical market. Expert Opinion on Emerging Drugs. 2005;10(1):185-218.
54. Gomord V, Faye L. Posttranslational modification of therapeutic proteins in plants. Current Opinion in Plant Biology. 2004;7(2):171-181.
55. Ko K. Expression of recombinant vaccines and antibodies in plants. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy. 2014;33(3):192-198.
56. Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. Recombinant Protein Expression in Microbial Systems. 2014;7.
57. Malabadi RB, et al. Recent advances in plant derived vaccine antigens against human infectious diseases. Research in Pharmacy. 2015;2(2).
58. Akinola M, Ekiyoyo T. Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Ribila at Odo-nla area of Ikorodu, Lagos state, Nigeria. Journal of Environmental Biology. 2006;27(3):597-599.
59. Auda MA, Zinada IA, Ali EES. Accumulation of heavy metals in crop plants from Gaza Strip, Palestine and study of the physiological parameters of spinach plants. Journal of the Association of Arab Universities for Basic and Applied Sciences. 2011;10(1):21-27.
60. Andráš P, et al. Comparison of heavy-metal bioaccumulation properties in *Pinus sp. and Quercus sp.* in selected European Cu deposits. Web Ecology. 2016;16(1):81-87.
61. Freeze HH. Genetic defects in the human glycome. Nature Reviews Genetics. 2006;7(7):537-551.
62. Holland M, et al. Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA-associated systemic vasculitis. Biochimica et Biophysica Acta (BBA)-General Subjects. 2006;1760(4):669-677.
63. Schneiderhan W, et al. CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in *in vivo* and *in vitro* models. Gut. 2009;58(10):1391-1398.
64. Kobata A. Structures and functions of the sugar chains of glycoproteins. The FEBS Journal. 1992;209(2):483-501.
65. Bosch D, Schots A. Plant glycans: Friend or foe in vaccine development? Expert Review of Vaccines. 2010;9(8):835-842.
66. Stulemeijer IJ, Joosten MH. Post-translational modification of host proteins in pathogen-triggered defence signalling in plants. Molecular Plant Pathology. 2008;9(4):545-560.
67. Webster DE, Thomas MC Post-translational modification of plant-made foreign proteins; glycosylation and beyond. Biotechnology Advances. 2012;30(2):410-418.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/20409