Current Trends in Biotechnology and Pharmacy
Vol. 15 (3) 315-324, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.32

Molecular Pharming: A New Approach for a Healthy Future by a Vast Development in the Pharmaceutical Industry

Suhashini Marian Silva. S. P[1]*, Kithmini Siridewa[1]

Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo.Sri Lanka
*Corresponding Author: susymarian@gmail.com.

Abstract

Molecular pharming is a process in which different biopharmaceuticals are produced from genetically modified organisms (GMOs) such as microbes, transgenic animals (TAs) and transgenic plants (TPs). Out of these, transgenic plants are more advantageous as they are safer, cheaper and they do not require cold storage systems. The advent of the biotechnological arena have paved the way to transform genetic material to host organisms such as plants, thereby to produce recombinant proteins (RPs) in a large scale. It could be observed that the current techniques used to produce pharmaceuticals are not much safer and they are not affordable by developing countries. Therefore the production of RP by molecular pharming is essential. Current demand for RPs is increasing rapidly for diagnosis and pharmaceutical purposes, while scientists have developed edible vaccines and drugs for certain diseases. Currently new approaches are been carried out to develop vaccines against deadly diseases such as Dengue and Ebola using molecular pharming. Therefore molecular pharming have generated new hopes in the minds of the suffering population with these deadly diseases.

Keywords: Molecular pharming, biopharmaceuticals, GMOs, transgenic animals (TAs), recombinant proteins (RPs).

Introduction

What is "Molecular pharming"?

"Molecular pharming" is a particular process in which pharmaceuticals are produced by the use of genetically modified organisms (GMOs), which are generated by the insertion of genes to numerous host or bioreactor systems such as plants, microbial cells and animals (1).

Man has been using plant products to treat different illnesses since ancient times. The development of biotechnological arena have paved the way to transform genetic material between organisms thereby to generate new recombinant proteins (RPs) (1). Usually, traditional vaccines are produced by the use of attenuated or inactivated pathogens and it is capable of reverting to virulence, as a result it is not entirely safe (2). Therefore use of RPs to produce vaccines is essential. Not only pharmaceuticals and vaccines even antibodies and cytokines could be produced by this technique (3).

The selection of a particular bioreactor system to produce essential proteins is determined by purity, cost and on the expression of those proteins in the host system (4). There are advantages as well as disadvantages in using each of these host systems and out of these plants seem to be more advantageous.

Even different parts of plants could be used to express these recombinant proteins and seeds seem to be more beneficial (5).

Historical background

The first use of living cells for the production of drugs was in 1940 when penicillin was produced by fermenting of mould *Penicillium notatum* (6). The birth of DNA technology and recombinant technology was in 1970, while the first protein produced in tobacco and potato plants was human serum albumin produced in 1990 (7). The first sheep clone was produced in 1997 by nuclear transfer (NT) of a somatic mammary gland cell into an oocyte and this paved the way for the production of RPs by transgenic animals (TAs) (8).

Types of organisms used for molecular pharming

Microbes

Bacteria and yeasts are the commonest type of microbes used to produce RPs (4). While E. coli K12 is the frequent used recombinant E.coli system to produce RPs, as it is relatively simple, inexpensive, easily genetically manipulated and express high levels of heterologous proteins (6). The different types of biopharmaceuticals produced using it are tissue plasminogen activator (tPA), insulin, α,γ-interferons, interleukin-2, Granulocyte colony stimulating factor (G-CSF) and human Growth Hormone (hGH) (6).

The main drawback of prokaryotic expression systems is that certain mammalian post translational modifications such as glycosylation is not carried out in these systems therefore the production of RPs by microbe bioreactor systems has been limited (9).

Transgenic animals (TAs)

Body fluids such as milk, egg white, blood, urine, seminal plasma and silk gland of the TAs such as cows, pigs, sheep, rabbits, goats and silk worm are used to produce RPs out of which RPs produced in the mammary glands of TAs are more prominent (10). Even, certain human proteins secreted in the milk of TAs are similar to human plasma proteins and also, glycosylation occurs in mammalian species as they are phylogenetically similar to humans (11).

Also, according to a particular research it was observed that around 100–500 ng/mL of hGH was found in the resulting urine when hGH gene was expressed in the urothelium of mouse by uroplakin II gene promoter (10).

Plants

Plant molecular pharming is the use of agricultural plants to produce plant derived metabolites (PDMs), while the major types of plants in which gene transformation had occurred successfully are industrial plants, cereal crops, legumes, vegetables, fruits, tropic plants and medicinal plants (12). Out of the different parts of plants used to produce RPs, seeds seem to be more beneficial as it inhibits degradation and provide a constant environment which promotes accumulation of excess proteins in a small volume (5).

Even the accumulation of RPs in seeds could be enhanced by using promoters derived from the genes, by using KDEL tags (which corresponds to amino acids: K-Lysine, D-Aspartic acid, E-Glutamic acid, L- Leucine) causing long term storage (5). The first food and drug administration (FDA) approved plant derived enzyme is ELELYSO™ (taliglucerase alfa) which was molecular pharmed in carrot cells and this enzyme is currently used to treat patients with Gaucher disease which is a rare metabolic genetic disorder (13, 14).

Types of plants currently used

Maize

It's a larger grain and has a higher proportion of endosperm which provides high biomass yield per hectare, therefore it has been used to produce the first successful plant derived RPs, avidin and β-glucuronidase (5).

Rice

Rice is a self-pollinating plant therefore it carries a lower risk of gene flow to nonpharmaceutical crops (3). Also, the rice endosperm contains special protein storing bodies which is capable of storing proteins, therefore these compartments could be used to accumulate RPs (5).

Tobacco (Nicotiana tabacum)

Compared to other plants Tobacco (*Nicotiana tabacum*) is a beneficial plant to produce PDMs as it's capable of producing a biomass of 100 ton/hectare and also, the probability of its contamination within food chains has been decreased since it's a non-food crop (12).

Arabidopsis

There are many advantages of Arabidopsis in the process of transformation and regeneration. Also, thousands of seeds could be produced using this plant since this plant consists of a short generation cycle, therefore currently this plant is used to test the efficiency of molecular pharming strategies (5).

Table 1 depicts certain biopharmaceuticals produced in maize, rice, tobacco and Arabidopsis plants.

Virus like particles (VLPs) made in plants

At present viral pathogens are used to make recombinant vaccines and as a result high safety precautions should be taken to avoid pathogenicity due to incomplete inactivation therefore VLPs are a suitable vaccine candidate as they do not contain infectious genomic material even though they look like viruses (16).

Process of producing RPs in plants

Different methods are used to introduce suitable traits into the host genome without altering the individual characteristics of the plant (12).

The process of plant molecular pharming consists of mainly 5 steps as shown in figure 1.

Construction of the transgene of interest

The initial process of molecular pharming using transgenic plants is the identification of the gene which codes for the desired protein and next this gene should be sequenced and isolated (15).

The production of RP in transgenic plants is carried out by transcribing DNA to produce mRNA and then, through combination of activities of mRNA, tRNA and rRNA produced mRNA is translated to yield a RP (12).

Transformation of transgene to host plant

Next this transgene could be transformed to the plant of interest mainly by two transformation methods such as,

- Nuclear transformation
- Chloroplast transformation

Nuclear transformation

According to this technique the transgene of interest is produced and it is integrated into the plant nuclear genome as shown in figure 2, leading to the production of RPs.

The accumulation of target proteins could be increased by adding specific signal sequences, optimizing certain molecular factors and using suitable promoters, which contain sites with core sequences to which RNA polymerase and transcription factors could bind (2).

Also, in monocot and dicot plants promoters such as the cauliflower mosaic virus 35S (CaMV35S) and the maize ubiquitin-1promoter, have been utilized respectively but tissue-specific and inducible promoters produces a better gene expression (12).

Currently different viral vectors have been developed to produce efficient plant derived metabolites (PDMs) such as bean yellow

dwarf virus (BeYDV) based single-vector DNA replicon system as shown in figure 3, composed with CaMV 35S promoter and incorporated with multiple DNA replicon cassettes, has produced around 0.5 mg of antibody per gram in tobacco leaves within four days after infiltration (14).

When the plant is propagated, the transgene is transmitted to the plant's progeny and large amount of plants with the transferred gene could be formed (3).

Chloroplast transformation

According to this technique foreign genes are inserted into the chloroplast genome using site-specific integration of homologous recombination and eliminating the expression caused by gene silencing as shown in figure 4 (2).

In this transformation gene silencing is not observed since the chloroplast is prokaryotic in nature and also as chloroplasts are maternally inherited cross pollination is avoided therefore transfer of genes to other plants does not occur (9).

Harvesting of the plants with the transgene

After transforming these transgenic plants the relevant traits are selected as shown in figure 4 and these traits are allowed to propagate in greenhouses or the selected traits are cultured to massively increase the number of cells with the transgenes. Once these plants have propagated, the seeds are collected and the relevant seeds are harvested to increase the number of transgenes (13).

Downstreaming and purification of RPs

Finally by carrying out extraction and purification steps the relevant RP could obtained.

Downstream processing refers to the recovery and purification of RPs produced, the process of recovery could be carried out by fractionating of plant, protein extraction and solid-liquid separation while purification could be carried out by immunoprecipitation, liquid-liquid extraction, membrane filtration and by chromatography techniques (9).

Process of lyophilization

Oral delivery vaccines could be produced by lyophilization by which transgenic plant leaves containing the RPs are freeze-dried in liquid nitrogen, proceeded in low temperature (−50°C) under vacuum to protect proteins which provide benefits such as long-term storage, antigen stability, increase RP content and decrease of microbial contamination (17).

Finally after the process of downstreaming and purification, the relevant metabolite is made.

Production of different metabolites

There are many metabolites which are produced using transgenic plants out of which some common applications are shown in figure 5.

Production of edible vaccines.

The production of plantibodies

Production of tropical applications.

Production of protein metabolites.

Production of edible vaccines

By the use of transgenic plants edible vaccines are produced at a large scale which causes the mucosal immune system to induce protective immune responses (2). Edible vaccines provide many benefits such as complicated purifying and storage methods are not needed and also, it avoid the potential risk of infecting patients with contaminated products as the organisms causing plant diseases do not infect humans (14).

Due to the plant cell wall, these vaccines are protected from acids and enzymes in the abdomen and are digested by the microbes in gut lumen to be delivered to the circulatory or immune system (2). When plant-derived antigens are delivered orally to humans and

mice it induces antigen-specific mucosal IgA and serum IgG synthesis (15).

The production of plantibodies

The process of production of antibodies in plants is known as plantibodies. Currently, monoclonal antibody (BR-96) has been made in genetically engineered soybean targeting against doxorubicin for ovarian, breast, lung and colon tumours (1).

Production of plantibodies could be carried out either by Biolistics or by Agrobacterium mediated transformation methods.

Biolistics:

In biolistic, the genes which encoding for antibodies are impregnated into nucleic acid by coating them with micro-carriers such as high density gold or tungsten microparticles and a gene gun is used to bombard these particles so that they are able to penetrate the cell walls and enter to the cell (1).

Agrobacterium mediated transformation:

The gene of interest is injected into the Ti-plasmid of Agrobacterium Tumefaciens and the bacteria is allowed to infect the plant cells leading to formation of tumours within the plant called as Crown Galls disease of the plants, also it transfers a tumour-inducing (Ti) plasmid into the nucleus of an infected plant cell which contains cancer causing oncognenes is transcribed (1).

Finally proteins are extracted and purified from plant cell cultures in which the plantlet is grown.

Production of tropical applications

Topical application of extracts obtained from the transgenic plants has stimulated the formation of blood vessels and healing of burn wounds in mice. Also according to a particular research it has been found that the topical application of soybean-derived mAbs in human cervical mucus had prevented herpes simplex virus 2 (HSV-2) infection.

Production of protein metabolites

Transgenic plants are used at the moment to produce hydrolases which includes both glycosidases and proteases, also the enzymes which are used in biomass conversion during the process of producing ethanol are candidates for molecular farming (3). Even by the use of transgenic plants certain therapeutic proteins such as anticoagulants, thrombin inhibitors, growth hormones, blood substitutes, collagen replacements, antimicrobial agents and certain drugs to prevent and treat diseases such as neutropenia, anemia, hepatitis, liver cirrhosis, cystic fibrosis, hemorrhage, hypertension, HIV and diabetes are produced (18).

Differences between different host systems used for molecular pharming

Some differences of different host systems are given in table 2. Since plant glycan lacks sialic acid which is found in mammals such as in the mouse monoclonal antibody, would cause potential immunogenicity and allergenicity of plant protein when used as human drugs. Therefore currently trials are carried out to modify plant glycoproteins by introducing mammalian-type transglycosylase gene into plant cells (7).

Advantages and disadvantages of molecular pharming.

Advantages of molecular pharming when compared with traditional production of pharmaceuticals.

There are numerous advantageous of molecular pharming when compared to the traditional production of pharmaceuticals. Usually, traditional vaccines are produced by the use of attenuated or inactivated pathogens and it is capable of reverting to virulence, as a result it is not entirely safe (2), whereas by molecular pharming since only the relevant transgenes are produced and given in the form of edible vaccines to organisms it is much safer. Also, plant derived products eliminate the risk of

Vol. 15 (3) 315-324, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.32

contamination by pathogens as the organisms which cause infections in plants usually do not cause human infections (14).

Even, edible vaccines reduces the cost of vaccination by eliminating the costs associated with transport, production, purification and other downstream processes which are used in the production of traditional vaccines as the food product itself could be the vaccine (21).

Advantages of using plants for molecular pharming.

The main advantage of using plants is that they only require simple factors such as sunlight, water, carbondioxide and simple nutrients for growth (16). As a result the cost is low. Also,

plants could be cultivated in large acres of land therefore they doesn't require cold storage systems (1).

The cost of plant maintenance for PDMs is very low when compared to E. coli, yeast and mammalian host systems as plants could be grown in a greenhouse or biosafety lab (14). The production cost of PDMs in plants could be reduced by 1/30 when compared to animals and by 1/3 when compared to microbial cultures (3).

Also, plants seems to be more advantageous compared to TAs as there are certain disadvantages in the use of TAs to produce RPs. These include the availability of the limited knowledge regarding the protein maturation and secretion in the animal body and also causation of health impacts to the animal when RPs are expressed in the animal body.

Different parts of the plant such as leaves, fruits and tubers could be used to produce recombinant proteins and out of these seeds seem to be the most advantageous as they are capable to store excess amount of proteins for a long term in a small volume (5).

Disadvantages of using plants for molecular pharming.

Even though plants carry out glycosylation, minor differences could be observed compared to humans. In plant derived glycan chains terminal sialic acid and plant based residues are absent and these differences would cause RPs produced in plants toxic, inactive, or immunogenic (9).

Factors affecting molecular pharming.

Different conditions in the environment would directly affects the amount of RPs produced in plants. Factors such as light, drought, salinity and nutritional deficits have negative effects on the produced plant metabolites. Therefore to protect RPs from these stresses, the light condition should be optimized, also it has been found that drought leads to the accumulation of reactive oxygen species (ROS) in plants causing oxidative stress and thereby decreases the expression of RPs (12).

The 5'-untranslated region of the tobacco alcohol dehydrogenase (ADH) gene has translational enhancer activity and when it was placed upstream of the coding region accumulation of protein has increased by 60–100 times in tobacco and Arabidopsis plants, therefore suitable promoters should be selected in plant hosts as accumulation of proteins depend on the activity of the promoter (7).

Discussion and Conclusion.

Risks and concerns.

There are certain challenges for molecular pharming using transgenic plants such as the amount of pharmaceuticals produced would vary in different plant species or in different plant parts, therefore it has become difficult to determine the appropriate dosage of edible vaccines for children and adult patients, also purifying of PDMs would be difficult due to certain factors such as plant phenolic compounds, plant pathogens, secondary metabolites and fertilizers (14).

Another risk of molecular pharming is that when food crops are used for the production of PDMs, it could be consumed by non-targeted

organisms leading to health issues. Usually it is impossible to keep the environmental risk associated with molecular pharming at absolute zero level. Yet there are certain steps that could be carried out to minimize these risks. One such approach that could be carried out is growing transgenic plants by physically isolating them from normal food crops or growing them in greenhouses, so that the consumption by non-targeted organisms could be avoided.

Even genetic use restriction technologies (GURTs) could be used to prevent the unnecessary exposure of the recombinant traits to the environment by modified plants that produce non-viable seeds and also recombinant protein could be kept in its inactive form in the plant, and only after certain modifications or processing of the protein the required pharmaceutical properties should be provided to the protein (3). By carrying out these strategies the contamination of food crops from transgenic plants could be minimized.

Also, adoption of PDMs raises certain ethical and religious issues, such as strong religious followers think that by alternating the genetic material of living organisms they are changing the will of god. Another common challenge which need to be addressed is, when food crops are cultured with RPs the general public think that it is harmful to human health. Therefore the general population should be educated that there is minimal harmful effects from transgenic plants while there are numerous benefits from molecular pharming such as different treatment options for deadly diseases could be generated.

Future approaches in molecular pharming.

Currently only live (Sabin) polio vaccine is delivered orally, therefore PDMs would provide benefits in the vaccination programs in the developing countries by reducing the cost of vaccine production, purification, storage and administration.

Also, in future to increase the amount RPs produced by molecular pharming certain strategies should be carried in selecting the suitable transformation systems, adaptation of codon usage gene silencing, design of recombinant transgenes with appropriate expression, tissue specificity and proper developmental regulation, and subcellular localization of products (14). Also, in future further research need to be carried out to develop new transformation techniques to insert multiple transgenes into plants (12).

There are certain future approaches which could be carried out using molecular pharming to produce new vaccines against certain deadly diseases. Dengue has ranked as one of the top most emerging deadly disease in Asian countries, while a proper treatment option is not available at the moment. Even though different vaccine candidates have been proposed against dengue there are certain draw backs in them such as inability to provide long lasting immunity against all the dengue serotypes, low product yield and also the cost which is not affordable by the developing countries, therefore plant biopharming is a convenient, safe and cost effective production system which is a best treatment option to produce vaccines against this deadly disease (19).

Ebola too is a deadly disease which is progressing rapidly and currently researches have been able to develop monoclonal antibodies (ZMappTM) in tobacco leaves (20) against the Ebola virus outbreak in Africa during the year 2014, yet additional patients suffering with the disease have been unable to receive treatment due to the insufficient supply of ZMapp, therefore further trials need to be carried out to massively produce this drug and also to receive the approval from FDA (14).

Malaria is also another deadly disease and at the moment different repellents have been produced from natural plants to protect against it, also, currently research is been carried out to use transgenic plants to increase the activity of these repellents (21).

Even Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging disease which have affected over 26 countries worldwide while there is no effective drug against it. Currently plant biotechnology of Hayward, USA have been able to produce an immunoadhesin (DPP4-Fc) in transgenic tobacco which exhibits strong binding to MERS-CoV and prevents the virus from infecting lung cells, yet researches are still carrying out further development of this drug to treat patients (14). Also, further research is been carried out to produce mAbs against Non-Hodgkin's lymphoma (1).

Also, it is estimated that by 2020 the world pharmaceutical industry would rise to $1.3 trillion and thereby drugs for life threatening ailments could be produced (6). Therefore by the use of molecular pharming new pharmaceuticals could be produced and even the existing methods could be improved.

Conclusion.

Molecular pharming is a particular process in which different biopharmaceuticals are produced from GMOs. Different bioreactor systems such as microbes, TAs and plants could be used to produce these biopharmaceuticals. Out of these transgenic plants seems to be more advantageous as they are cheaper, safer and do not require cold storage systems (1).

At the moment thousands of people tend to suffer due to the emergence of different deadly diseases which lack an efficient treatment. Even though there are certain treatment options available yet these treatment approaches have become unaffordable by the majority in developing countries. Therefore the quest for newer drugs have been greatly increased.

The development of recombinant technology have paved the way to transform genetic material between organisms thereby to generate new pharmaceuticals at a larger scale and also to modernize the current treatment approaches (1). The approach of plants to act as green biofactories for the generation of RPs has made plants as a suitable candidate for vaccine production.

Current demand for RPs is increasing rapidly for diagnosis and pharmaceutical purposes, while scientists have been able to develop drugs and edible vaccines for certain diseases. This increase in productivity of drugs and vaccines have raised hopes in the minds of underprivileged (1). Yet there are certain concerns which have been raised regarding the impact of molecular pharming to living organisms and the environment. Therefore these concerns should be adequately addressed since molecular pharming provides numerous benefits. Also, it is the responsibility of researches, pharmaceutical production companies and government to proceed cautiously in this area so as to gain recognition from the general public.

Therefore we could hope that in future the suffering population with deadly diseases would be able to live a healthy future in which numerous treatment options would be available at an affordable price.

References.

1. Gangrade, D., Waghmare, N. and Lad, S. (2016) " Pharming " proteins : the future of Panacea. *Journal of Chemical and Pharmaceutical Research*, 8(8) : 133–138.

2. Chan, H. and Daniell, H. (2015) 'Plant-made oral vaccines against human infectious diseases — Are we there yet ?. *Plant Biotechnol J*, 13(8) : 1056–1070.

3. Sahu, P. K., Patel, T. S., Sahu, P., Singh, S., Tirkey, P. and Sharma, D. (2014). Molecular Farming : A biotechnological approach in agriculture for production of useful metabolites. *International Journal of Research in Biotechnology and Biochemistry*, 4(2) : 23–30.

4. Yan, N., Fan, C., Chen, Y. and Hu, Z. (2016). The Potential for Microalgae as Bioreactors to Produce Pharmaceuticals. *International Journal of Molecular Sciences*, 17(962) : 1–24.

5. Sabalza, M., Vamvaka, E., Christou, P. and Capell, T. (2013). Seeds as a Production System for Molecular Pharming Applications : Status and Prospects. *Current Pharmaceutical Design*, 19(31): 5543-5552.

6. Abdullah, M. A., Rahmah, A., Sinskey, A. J. and Rha, C. K. (2008). Cell Engineering and Molecular Pharming for Biopharmaceuticals. *The Open Medicinal Chemistry Journal*, 2 : 49–61.

7. Shinmyo, A. and Kato, K. (2010). Molecular farming : production of drugs and vaccines in higher plants. *The Journal of Antibiotics*, 63(8) : 431–433.

8. Maksimenko, O. G., Deykin, A. V, Khodarovich, Y. M. and Georgiev, P. G. (2013). Use of Transgenic Animals in Biotechnology : Prospects and Problems. *Acta naturae,* 5(16) : 33–46.

9. Ahmad, K. (2014). Molecular Farming : Strategies , Expression Systems and Bio-Safety Considerations. *Czech J. Genet. Plant Breed*, 50(1): 1–10.

10. Wang, Y., Zhao, S., Bai, L., Fan, J. and Liu, E. (2013).Transgenic Animal Bioreactors. *BioMed Research International,* 2013(580463) : 1-9.

11. Moura, R. R., Melo, L. M., José, V. and Freitas, D. F. (2011). Production of Recombinant Proteins in Milk of Transgenic and Non-transgenic Goats. *Brazilian Archives of Biology and Technology*, 54(5) : 927–938.

12. Abiri, R., Valdiani, A., Maziah, M., Shaharuddin, N. A., Sahebi, M., Yusof, Z. N.B., Atabaki, N. and Talei, D. (2016). A Critical Review of the Concept of Transgenic Plants : Insights into Pharmaceutical Biotechnology and Molecular Farming. *Curr. Issues Mol. Biol*, 18 : 21–42.

13. Kwon, K. and Daniell, H. (2015). Low-cost oral delivery of protein drugs bioencapsulated in plant cells. *Plant Biotechnol J :* 1017–1022.

14. Yao, J., Weng, Y., Dickey, A. and Wang, K. Y. (2015) 'Plants as Factories for Human Pharmaceuticals : Applications and Challenges. *International Journal of Molecular Sciences*, 16 : 28549–28565.

15. Sack, M., Rademacher, T., Spiegel, H., Boes, A., Hellwig, S., Drossard, J., Stoger, E. and Fischer, R. (2015). From gene to harvest : insights into upstream process development for the GMP production of a monoclonal antibody in transgenic tobacco plants. *Plant Biotechnology Journal,* 13 : 1094–1105.

16. Marsian, J. and Lomonossoff, G. P. (2016). ScienceDirect Molecular pharming — VLPs made in plants. *Current Opinion in Biotechnology*. 37 : 201–206.

17. Kwon, K., Verma, D., Singh, N.D., Herzog, R. and Daniell, H. (2014). Oral delivery of human biopharmaceuticals, autoantigens and vaccine antigens bioencapsulated in n782–799.

18. Thomas, D. R., Penney, C. A., Majumder, A. and Walmsley, A. M. (2011). Evolution of Plant-Made Pharmaceuticals. *International Journal of Molecular Sciences*, 12 : 3220–3236.

19. Pang, E. L. and Loh, H. (2017). Towards development of a universal dengue vaccine – How close are we?. *Asian Pacific Journal of Tropical Medicine*, 10(3) : 220–228.

20. Rosales-mendoza, S., Nieto-Gómez, R. and Angulo, C. (2017). A Perspective on the Development of Plant-Made vaccines in the Fight against ebola virus. *Frontiers in Immunology,* 8(252) : 1–13.

21. Pulice, G., Pelaz, S. and Matías-hernández, L. (2016). Molecular Farming in Artemisia annua , a Promising Approach to Improve Anti-malarial Drug Production. *Frontiers in Plant Science*, 7(329) : 1–17.