REVIEW ARTICLE

MOLECULAR FARMING AS AN APPROACH FOR PRODUCTION OF USEFUL METABOLITES

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Abstract: Recently, through modern biotechnology, it is now recognized that plants are potentially a new source of pharmaceutical proteins including vaccines, antibodies, blood substitutes and other therapeutic entities. Unlike mammalian-derived rDNA drugs, plant-derived antibodies, vaccines and other proteins are particularly advantageous since they are free of mammalian viral vectors and human pathogens. Advantages offered by plants include also low cost of cultivation and high biomass production, relatively fast "gene to protein" time, low capital and operating costs, excellent scalability, eukaryotic posttranslational modifications and a relatively high protein yield. Crop plants can synthesize a wide variety of proteins that are free of mammalian toxins and pathogens. Crop plants produce large amounts of biomass at low cost and require limited facilities. Since plants have long been used as a source of medicinal compounds, molecular farming represents a novel source of molecular medicines, such as plasma proteins, enzymes, growth factors, vaccines and recombinant antibodies, whose medical applications are understood at a molecular level. Bio-pharming promises more plentiful and cheaper supplies of pharmaceutical drugs, including vaccines for infectious diseases and therapeutic proteins for treatment of such things as cancer and heart disease. This paper provides a brief knowledge about molecular farming and their issues.

Keywords: Biotechnology, Pharmaceutical proteins, Vaccines, rDNA, Recombinant antibodies

INTRODUCTION

Molecular farming is the production of proteins or other metabolites valuable to medicine or industry in plants traditionally used in an agricultural setting. It harnesses heterologous protein expression systems, such as plants, for the large-scale production of recombinant proteins that are therapeutically valuable. Molecular farming is the production of pharmaceutically important and commercially valuable proteins in plants (Franken et al., 1997). Its purpose is to provide a safe and inexpensive means for the mass production of recombinant pharmaceutical proteins. Plant molecular farming is the growing of plants in agriculture to produce pharmaceutical or industrial compounds instead of food, feed, or fiber. The possibilities range from the manufacture of medical products, such as pharmaceuticals (drugs) and vaccines, to the production of products like biodegradable plastics and industrial chemicals.

Molecular farming in plants has the potential to provide virtually unlimited quantities of recombinant proteins for use as diagnostic and therapeutic tools in health care and the life sciences. Plants produce a large amount of biomass and protein production can be increased using plant suspension cell culture in fermenters, or by the propagation of stably transformed plant lines in the field (Kamenarova et al., 2005). Complex mammalian proteins can be produced in transformed plants or transformed plant suspension cells. Plants are suitable for the production of pharmaceutical proteins on a field scale because the expressed proteins are functional and almost indistinguishable from their mammalian counterparts. The breadth of therapeutic proteins produced by plants range from interleukins to recombinant antibodies. Transgenic plants can also produce organs rich in a recombinant protein (Ma S, 2012) for its long-term storage. This demonstrates the promise of using transgenic plants as bioreactors for the molecular farming of recombinant therapeutics, including vaccines, diagnostics, such as recombinant antibodies, plasma proteins, cytokines and growth factors.

Modern biotechnology is extending the use of plants in medicine well beyond its original boundaries. Plants are now a source of pharmaceutical proteins, such as mammalian antibodies (Hiatt et al., 1989) blood substitutes (Magnuson et al., 1998) and vaccines (Walmsley & Arntzen, 2000). The feasibility of precise plant genetic manipulation, high-scale expression of recombinant proteins, rapid and easy scaling up, convenient storage of raw material and less concern of contamination with human or animal pathogens during downstream processing have attracted biotechnologists to Plant Molecular Farming, especially plastid and chloroplast engineering for this purpose (Breyer et al., 2012, Sparrow et al., 2013 and Ma S, Wang 2012).

Why plant should used as a metabolite production system

Plants have the natural ability to make human and animal proteins. This means that mass production is greatly simplified by just increasing the acreage of

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the plants under cultivation. Existing farming equipment can be used in the processing of the plants and thus reduce the costs involved in making the product. Often costs can be reduced as much as 1/30 of that necessary when using animal cell culture and at least by 1/3 when compared to microbial culture systems. Products produced in plants can be stored for long periods without refrigeration if they are expressed in seeds or leaves which can be stored dried.

The potential of using plants as a production system for recombinant pharmaceuticals was established between 1986 and 1990 with the successful expression of a human growth hormone fusion protein, an interferon and human serum albumin (Barta et al., 1986; De Zoeten et al., 1989; Sijmons et al., 1990). A crucial advance came with the successful expression of functional antibodies in plants in 1989 (Hiatt et al., 1989) and 1990 (Düring et al., 1990). This was a significant breakthrough for it showed that plants had the potential to produce complex mammalian proteins of medical importance. By analogy to the production of insulin in bacteria, the production of antibodies in plants had the potential to make large amounts of safe, inexpensive antibodies available.

Advances in recombinant DNA technology, plant transformation technology and antibody engineering are major reasons why plants have emerged as an expression system. Antibody expression in plants showed proof that plants were capable of expressing functional mammalian proteins (Voss et al., 1995) and further progress has made it possible to produce chimeric mouse–human therapeutic antibodies in plants in sufficient quantities for pre-clinical trials (Zeitlin et al., 1998). Plant expression systems are attractive because they offer significant advantages over the classical expression systems based on bacterial, microbial and animal cells. Firstly, they have a higher eukaryote protein synthesis pathway, very similar to animal cells with only minor differences in protein glycosylation. Contrastingly, bacteria cannot produce full size antibodies (Menkhaus et al, 2004) nor perform most of the important mammalian post-translational modifications. Secondly, proteins produced in plants accumulate to high levels (Ziegler et al., 2000) and plant derived antibodies are functionally equivalent to those produced by hybridoma (Voss et al., 1995).

Technique of plant cell cultures

In whole plants, the possibility of contamination with agrochemicals and fertilizers must be considered, as well as the impact of pests and diseases, and the variable cultivation conditions due to local differences in soil quality and microclimate (Jelaska et al., 2005). Plant cell culture as an expression system for recombinant proteins avoids these problems while retaining the advantages (Valkova et al., 2013). Like microbes, plant cells are inexpensive to grow and maintain, but they are higher eukaryotes they can carry out many of the post-translational modifications that occur in human cells. Plant cells can be maintained in simple, synthetic media, but like animal cells they can synthesize complex multimeric proteins and glycoproteins (Twyman et al., 2012), such as immune-globulins and interleukins. Recombinant human glycoproteins synthesized in plants show much greater similarity to their native counterparts in terms of N-glycan structure compared to the same proteins produced in yeast, bacteria or filamentous fungi (Obembe et al., 2011). One of the most important driving factors has been yield improvement, as product yield has a significant impact on economic feasibility. Strategies to improve the recombinant protein yield in plants include the development of novel promoters, the improvement of protein stability and accumulation through the use of signals that target the protein to intracellular compartments (Schillberg et al., 2013), and the improvement of downstream processing technologies (Menkhaus et al, 2004).

During the last two decades a diverse upstream (production) and downstream (purification) technologies, such as table nuclear transformation, stable plastid transformation, plant cell-suspension cultures and transient expression systems (Agro infiltration method, gene gun technology, virus infection method and magnification technology) were developed in Plant Molecular Farming, and thousands of plant-derived biopharmaceutical proteins including antibodies, vaccines, human blood products, hormones and growth regulators were produced at laboratory and pilot levels, and some of them reached the late stages of commercial and are expected to be marketed soon (Ahmad et al, 2012 and Twyman et al., 2012). Also some of them, such as Caro RX previously have been commercialized (Twyman et al., 2012).

Advantages of using higher plants for the purpose of protein production

According to Horn (Horn *et al.*, 2004) the advantages of using higher plants for the purpose of protein production include:

i. Significantly lower production costs than with transgenic animals, fermentation or bioreactors;
ii. Infrastructure and expertise already exists for the planting, harvesting and processing of plant material;
iii. Plants do not contain known human pathogens (such as virions, etc.) That could contaminate the final product;
iv. Higher plants generally synthesize proteins from eukaryotes with correct folding (obembe *et al.*, 2011) glycosylation, and activity; and
v. Plant cells can direct proteins to environments that reduce degradation and therefore increase stability.

Production of biopharmaceuticals in transgenic plants

Biopharmaceuticals are drug products (proteins, including antibodies) produced in living systems and used for therapeutic or diagnostic purposes or as dietary supplements. Manufacturing pharmaceutical products in crops has been one of the promised benefits of plant genetic engineering for the past 20 years. The using of biotechnology, sometimes known as "pharming," "bio-pharming," or "molecular farming," (Kamenarova et al., 2005) has migrated from speculation to the testing phase in fields and greenhouses across the country. Through genetic modification, it is now recognized that plants are potentially a new source of pharmaceutical proteins (Twyman et al., 2003) including vaccines, antibodies, blood substitutes and other therapeutic entities (Ahmad et al., 2012). Unlike mammalian-derived rDNA drugs, plant-derived antibodies, vaccines and other proteins are particularly advantageous since they are free of mammalian viral vectors ((Schillberg et al., 2013) and human pathogens. Advantages offered by plants include also low cost of cultivation and high biomass production, relatively fast "gene to protein" time, low capital and operating costs, excellent scalability, eukaryotic posttranslational modifications (Valkova *et al.*, 2013) and a relatively high protein yield. Some examples of plants used for biopharmaceutical production (Table: 1) and companies involve in molecular farming (Table: 2) are given in Table 1 and Table 2 respectively. Simplified representation of molecular Farming is given in Figure 1.

Table 1. Some examples of plants used for biopharmaceutical production

S. No	Category	Plants used
1	Model plants	Arabidopsis thaliana
2	Leafy crops	Tobacco, lettuce, alfalfa, clover
3	Cereals	Maize, rice, wheat, barley
4	Legumes	Soybean, pea, pigeon pea
5	Fruits and vegetables	Potato, carrot, tomato, banana
6	Oil crops Oilseed	Rape, Camelina sativa
7	Simple plants:	Lemna sp. Physcomitrella patens, Marchantia polymorpha, Chlamidomonas reinhardtii

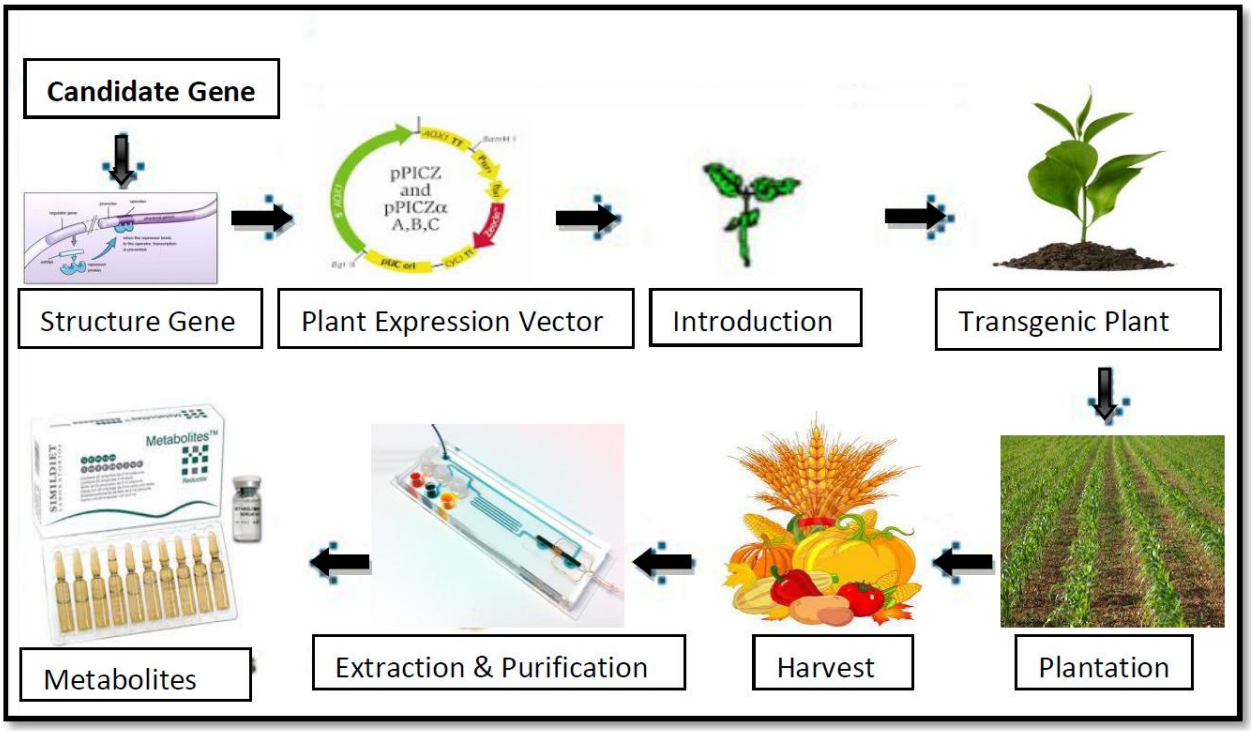

Fig. 1: Simplified Representation of Molecular Farming

Recombinant proteins expressed in plants

Horn ((Horn et al., 2004)) categorizes proteins currently being produced in plants for molecular farming purposes into following broad areas:

Parental therapeutics and pharmaceutical intermediates

It includes all proteins used directly as pharmaceuticals along with those proteins used in the making of pharmaceuticals. The list of such proteins is long, ever growing, and includes such products as thrombin and collagen (therapeutics), and trypsin and aprotinin (intermediates) (Kamenarova et al., 2005). Many proteins in this category have been expressed in Tobacco. However, there are some notable exceptions for instance rice has been used for the production of human α-interferon (Zhu et al., 1994), Cnola for hirudin (Parmenter et al, 1995) and α-1 antitrypsin (Terashima et al, 1999) and Maize for Bovine aprotinin (Zhong et al, 1999).

Production of antibody in plants

Antibodies are multi-subunit glycol-proteins produced by the vertebrate immune system. They recognize and bind to their target antigens with great affinity and specificity, which allows them to be used for many applications, including the diagnosis, prevention, and treatment of human and animal disease (Fischer et al., 2000). Plants are an alternative expression system to animals for the molecular farming of antibodies (Schillberg et al., 2003). Transgenic plants have been used for the production of antibodies directed against dental caries, rheumatoid arthritis, cholera, E. coli diarrhea, malaria, certain cancers, Norwalk virus, HIV, rhinovirus, influenza, hepatitis B virus, and herpes simplex virus (Thomas et al., 2002).

Production of plant edible vaccines

Edible vaccines have received considerable attention from researchers in both academia and industry. Charles Arntzen (who was the first to use the phrase "edible vaccine"), with Hugh Mason and colleagues have pioneered the field with work on hepatitis B and heat labile toxin, B subunit, in tobacco plants and potato tubers. The main goal of an oral vaccine is the induction of a mucosal immune response (Das, 2009) and a subsequent systemic immune response. Edible vaccines are sub-unit vaccines (Kamenarova et al., 2005) that introduce selected genes into the plants and facilitate the production of the encoded protein. Edible vaccines are mucosal-targeted vaccines that stimulate both the systematic and mucosal immune network takes place. Plant-derived vaccines have been produced against Vibrio cholerae, enterotoxigenic E. coli, hepatitis B virus, Norwalk virus, rabies virus, human cytomegalovirus, rotavirus and respiratory syncytial virus F (Thomas et al., 2002). In some cases, protection has actually been better with the edible vaccine than with the commercially available vaccine (Lamphear et al. 2004). In this way it could be overcome the need for injections and sterile needles and do not require refrigeration. Edible vaccines are being tested in potatoes, tomatoes, bananas, and carrots.

Production of industrial proteins

This group includes hydrolases, encompassing both glycosidases and proteases. Enzymes involved in biomass conversion for producing ethanol are candidates for molecular farming. All of these products are usually characterized by the fact that they are used in very large quantities and must therefore be produced very inexpensively (Hood et al., 1999). Recombinant egg white avidin and bacterial B-glucuronidase (GUS) from transgenic maize have been commercially produced. High levels of expression were obtained in seed by employing the ubiquitin promoter from maize. The recombinant proteins had activities that were indistinguishable from their native counterparts, (Ma JK-C et al., 2003).

Production of other proteins of medical relevance

These include the milk proteins ß-casein, lactoferrin and lysozyme, which could be used to improve child health, and protein polymers that could be used in surgery and tissue replacement (Ma et al., 2003). Expression of thioredoxin in foods such as cereal grains would increase the digestibility of proteins and thereby reduce their allergenicity ((Thomas et al., 2002). It has been shown that human collagen can be produced in transgenic tobacco plants and that the protein is spontaneously processed and assembled into its typical triple-helical conformation.

Genetic aspect of producing of plant made metabolites

To achieve specific protein production in plants, the DNA that encodes the desired protein must be inserted into the plant cells. This can be done as a stable transformation when foreign DNA is incorporated into the genome of the plant. A promoter associated with the inserted DNA then directs the cells to produce the desired protein, often targeting it to accumulate only in specific tissues such as the seed (Fischer et al., 2004). Alternatively, a plant virus can be used to direct expression of a specific protein without genetically modifying the host plant. The transformation and expression systems used to engineer these proteins in plants affect the stability, yield, cost of purification, and quality of the proteins produced (Thomas et al., 2002, Gomord et al., 2004). There are four methods of protein production from plants: a) stable nuclear transformation of a crop species that are grown in the field or a greenhouse, b) stable plastid transformation of a crop species, c) transient transformation of a crop species by agroinfiltration, and d) stable transformation of a plant species that is grown hydroponically or in *in vitro* systems so that the trans-protein is secreted into the medium and recovered (Kamenarova et al., 2005).

Gene transformation methods

In molecular farming desired foreign genes may be inserted, or transformed, into desired plants via a number of methods:

Stable transformation:

Stable transformation into the nuclear genome is done primarily using Agrobacterium mediate transformation or particle bombardment methods (Suslow et al., 2002). In each case, the DNA coding for the protein of interest and an associated promoter to target its expression to a particular tissue or developmental stage is integrated into the genome of the plant. Thus, when the plant is propagated, each plant will transmit this property to its progeny and large numbers of plants containing the transferred gene are readily generated. It is also possible to deliver genes into the separate genome of plastids (chloroplasts and mitochondria) in plant cells. Example: tobacco and potato. Because genes in chloroplast genomes are not transmitted through pollen, recombinant genes are easier to contain, thereby avoiding unwanted escape into the environment.

Recombinant virus vector:

A second method of engineering plant protein expression is transduction, the use of a recombinant plant virus to deliver genes into plant cells. The DNA coding for the desired protein is engineered into the genome of a plant virus that will infect a host plant. A crop of the host plants is grown to the proper stage and is then inoculated with the engineered virus. As the virus replicates and spreads within the plant, many copies of the desired DNA are produced and high levels of protein production are achieved in a short time. A limitation with this system is that the green plant matter must be processed immediately after harvest and cannot be stored (Thomas et al., 2002).

What are the risks, concerns and issues of plant-derived metabolites (PDMs)

The production of plant-derived metabolites introduces several unique challenges for bio-safety regulation and risk-management. Most of these arise from the fact that the plants are generally grown in the open environment. An important environmental concern is therefore the potential gene flow to weeds or related crops through pollination or seed contamination (Horn et al., 2004). In addition, and especially in the case where food crops are used for the production of drugs, there are issues about PDMs accidentally entering the food chain and being consumed by non-target organisms (Breyer et al., 2012).

It is impossible to keep the environmental risks associated with PDMs at absolute zero. A simple approach would be to grow the transgenic plants producing PDMs in physical isolation. However, a more realistic approach would be to minimize the environmental exposure of these proteins (Horn et al., 2004) through a combination of precautionary measures. These could comprise the use of genetic use restriction technologies or GURTs, which prevent the unintended escape of the crop to the environment by engineering plants that produce non-viable seeds (Breyer et al., 2009). Other strategies include the induction of biopharmaceutical production in the plants after harvesting, and the expression of the proteins in a form that must be treated for activation (Obembe et al., 2011). This means that the protein would be in its inactive form in the plant, and only after further modifications or processing the protein would acquire pharmaceutical properties (Jouzani et al., 2013).

A major concern for many developing countries is the lack of bio-safety legislation for genetically modified plants (Salehi, 2012). Without a biosafety framework in place, developing countries cannot perform trials of PDMs. In addition, a major challenge is the high cost of development and of regulatory compliance. Although large scale production of PDM may be more economical, the initial stages of development and bio-safety tests may be prohibitively costly (Valkova et al., 2013 and Obembe et al., 2011). Regulatory requirements for drug development and manufacture, the high failure rate of new drugs, and the protection of intellectual property also contribute to the price of new vaccines (Jouzani et al., 2013). The adoption of PDMs may also raise ethical and religious issues. Among the ethical objections to GM plants, and to genetic engineering in general, are concerns that altering living organisms is like ‘playing God’ (Jouzani et al., 2013).

Economics and commercial opportunities

It is difficult to generalize about the economic viability of molecular farming systems. Each application is different, with variation in farming practices, efficiency of the gene-expression system, ease with which the product can be extracted and purified, and prospects for obtaining useful by-products (e.g., seed oil and meal). One would expect plant systems to be different from animal systems, but there are extreme contrasts even among the plants and animals. For example, tobacco is a very different crop compared to corn, soybeans, or canola. Tobacco has the advantage of producing large quantities of green leaf material per acre, and it is very convenient to work with from a biotechnology standpoint (Valkova et al., 2013). Because of these qualities, tobacco may be the ideal "factory" for products produced in green leaves. In cases where the products need to be produced in seeds, a better choice may be corn, soybeans, or canola because tobacco seeds are extremely small. All crops have different genetic composition, different characteristics, and different production methods, making each a unique "vehicle" for molecular farming (Horn et al., 2004).

Because of these differences, there is no way to establish the "cost" of generating a product using molecular farming. Some estimates are based on

predictions of crop production costs, necessary capital investments for processing facilities, etc., but these range from rupees/dollars per gram of product produced to thousands of rupees/dollars per gram. However, certain principles are quite clear: for both plants and animals (Horn et al., 2004), profitability requires high expression of the introduced gene, maintenance of product integrity, and the ability to scale up, harvest, recover, purify, and store the target product as cheaply as possible. For products that require high purity, the processing and purification costs are expected to be very high (Jouzani et al., 2013).

The commercial scale-up of molecular farming is now beginning to happen. Many suitable products have been identified, especially in the medical field, and the technology is being perfected for large-scale implementation. High-value pharmaceuticals are expected to be the first products of molecular farming owing to their high profit potential (Obembe et al., 2011) and many such products are under development and in clinical trials from both large and small companies (Valkova et al., 2013). For success in the competitive marketplace, scale-up cost cutting will be critical, particularly in the area of purification and product recovery. Ultimately, if the products and processes of molecular farming are to be commercially successful, they must hold a competitive advantage over existing, alternative products and processes (e.g., pharmaceuticals produced through microbial fermentation). Or, if the products are entirely new (e.g., biodegradable plastics or "biosteel"), there must be a corresponding new market for them (Kamenarova et al., 2005). As this technology continues to develop and molecular farming production becomes more efficient, then larger-scale, lower-value products such as industrial chemicals or biodegradable plastics should lead to larger-scale opportunities for agricultural producers (Paul et al., 2011).

While the commercial progress is very exciting, molecular farming is still very much an emerging industry. The farm-level opportunities will undoubtedly start small, but they will grow over time. Contract production is likely, and profit margins may vary considerably depending on the value of the target and the increased production requirements. But the unique thing about molecular farming is that this opportunity should provide a captive new market for agricultural products (Obembe *et al*., 2011) one that can increase over time and one that should provide the pioneer producers with a clear advantage.

Future prospects

One of the keys to success in the future will undoubtedly be the level of expression of the recombinant protein in plants. This is the one of the most important aspects with regard to economics. The expression level affects the cost of growing, processing, extraction, purification and waste disposal. Clearly there will be a drive towards higher levels of expression and there is much more room for improvement compared to other established systems. Expression is also a major regulatory concern. Whether or not the protein is in specific tissues will enable or nullify exposure to the environment. There has already been work to show that expression can be limited to specific tissues, thus reducing regulatory concerns. As an example, keeping the protein out of pollen can reduce inadvertent exposure to the environment. However, this does not remove the possibility that the pollen will outcross with other plants and intermix with food crops. There are physical isolation requirements imposed by the regulatory agencies to prevent this from occurring. There may be some cases where genetic control of expression is also warranted either for economic or safety concerns, depending on the product. Possibilities including malesterile crops, induced expression, or sequences that prevent germination or the expression of the protein product in non-food products have been discussed. Some combination of these different limitations on expression will most likely find a way into future programs.

CONCLUSION

Plants have advantages compared with traditional systems for molecular farming of pharmaceutical proteins. These include: the low cost of production, rapid scalability, the absence of human pathogens, and the ability to fold and assemble complex proteins accurately. Plants might one day surpass other production systems because of the economic and safety benefits, and ultimately, it should be possible to make pharmaceuticals available to everyone who needs them, at a cost that everyone can afford (Paul et al., 2011). For the biotech and drug industry, bio-pharming offers economic and health benefits once the current cycle of product development reaches the commercialization stage.

However, for these benefits to be fully realized, the central issue of risk to the food industry and the environment is a critical requirement. A combination of strong and adaptable regulatory oversight with technological solutions are required if the goals of realizing the full potential of plant molecular farming are to be met. For all, plants need to be viewed as a possibility among many for manufacturing therapeutic proteins. Attention is now shifting from basic research towards commercial exploitation, and molecular farming is reaching the stage at which it could challenge established production technologies that use bacteria, yeast and cultured mammalian cells. In this review, we highlight not only recent progress in molecular farming and its potential for commercial drug development and production, but also the regulatory control, bio-safety and political impacts of the technology, and its related intellectual property (IP) issues.

REFERENCES

Ahmad, P., Ashraf, M., Younis, M., Hu, X. and Kumar, A. (2012). Role of transgenic plants in agriculture and biopharming. *Biotechnol Adv*, 30: 524-540.

Artsaenko, O., Kettig, B., Fiedler, U., Conrad, U. and During, K. (1998). Potato tubers as a biofactory for recombinant antibodies. *Mol Breeding*, 4: 313-319.

Barta, A., Sommergruber, K., Thompson, D., Hartmuth, K., Matzke, M. and Matzke, A. (1986). The expression of a nopaline synthasehuman growth hormone chimaeric gene in transformed tobacco and sunflower callus tissue. *Plant Mol Biol*, 6: 347–357.

Breyer, D., De Schrijver, A., Goossens, M., Pauwels, K. and Herman, P. (2012). Biosafety of Molecular Farming in Genetically Modified Plants. In: Molecular Farming in Plants: Recent Advances and Future Prospects. *Springer*, 259-274

Breyer, D., Goossens, M., Herman, P. and Sneyers, M. (2009). Biosafety considerations associated with molecular farming in genetically modified plants. *J Med Plant Res*, 3: 825-838.

Carter, J.E. and Langridge, W.H.R. (2002). Plant-based vaccines for protection against infectious and autoimmune diseases. *Crit Rev Plant Sci*, 21: 93-109

Das, D.K. (2009). Molecular farming of Plant Derived Edible Vaccines. *Current Trends in Biotechnology and Pharmacy*, 3(2):113-127.

De Zoeten, G.A., Penswick, J.R., Horisberger, M.A., Ahl, P., Schultze, M. and Hohn, T. (1989). The expression, localization, and effect of a human interferon in plants. *Virology*, 172: 213–222.

Düring, K., Hippe, S., Kreuzaler, F. and Schell, J. (2006). Synthesis and self assembly of a functional monoclonal antibody in transgenic Nicotiana tabacum. *Plant Mol Biol* 15: 281–293.

Fischer, R., Stoger, E., Schillberg, S., Christou, P. and Twyman, R.M. (2004). Plant-based production of biopharmaceuticals. *Curr Opin Plant Biol*, 7: 152-158.

Fischer, R., Hoffmann, K., Schillberg, S. and Emans, N. (2000). Antibody production by molecular farming in plants. *J Biol Regul Homeost Agents*, 14(2):83-92.

Franken, E., Teuschel, U. and Hain, R. (1997). Recombinant proteins from transgenic plants. *Curr Opin Biotech*, 8: 411–416.

Gomord, V. and Faye, L. (2004). Post translational modification of therapeutic proteins in plants. *Curr Opin Plant Biol*, 7: 171-181.

Hiatt, A., Cafferkey, R. and Bowdish, K. (1989). Production of antibodies in transgenic plants. *Nature*, 342: 76–78.

Horn, M.E., Woodard, S.L. and Howard, J.A. (2004). Plant molecular farming: systems and products. *Plant Cell Rep*, 22: 711–720.

Jelaska, S., Mihaljevic, S. and Bauer, N. (2005). Production of biopharmaceuticals, antibodies and edible vaccines in transgenic plants. *Current studies of biotechnology*, Vol IV.

Jouzani, G.S. and Tohidfar, M. (2013). Plant molecular farming: future prospects and biosafety challenges. *Biosafety*, 2:2.

Kamenarova, K., Abumhadi, N., Gecheff, K. and Atanassov, A. (2005). Molecular farming in plants: an approach of agricultural biotechnology. *Journal of cell and molecular biology*, 4:77-86.

Lamphear, B.J., Jilka, J.M., Kesl., Welter, M., Howard, J.A. and Streatfield, S.J. (2003). A corn-based delivery system for animal vaccines: an oral transmissable gastroenteritis virus vaccine boosts lactogenic immunity in swine. Vaccine (in press).

Ma, J.K.C., Drake, P.M.W. and Christou, P. (2003). The production of recombinant pharmaceutical proteins in plants. *Genetics*, 4: 794-805.

Ma, S. and Wang, A. (2012). Molecular Farming in Plants: An Overview, in Molecular Farming in Plants: Recent Advances and Future Prospects, *Springer*, 1-20.

Magnuson, N.S., Linzmaier, P.M., Reeves, R., An, G., HayGlass, K. and Lee, J.M. (1998). Secretion of biologically active human interleukin-2 and interleukin-4 from genetically modified tobacco cells in suspension culture. *Protein Expr Purif* , 13: 45–52.

Menkhaus, T.J., Bai, Y., Nikolov, Z.L., Zhang, C.M. and Glatz, C.E. (2004). Considerations for the recovery of recombinant proteins from plants. *Biotechnol prog*., 20:1001-1004.

Obembe, O.O., Popoola, J.O., Leelavathi, S. and Reddy, S.V. (2011). Advances in plant molecular farming. *Biotechnol Adv*, 29: 210-222.

Parmenter, D.L., Boothe, J.G., van Rooijen, G.J., Yeung, E.C. and Moloney, M.M. (1995). Production of biologically active hirudin in plant seeds using oleosin partitioning. *Plant Mol Biol*, 29: 1167–1180.

Paul, M., van Dolleweerd, C., Drake, P.M., Reljic, R. and Thangaraj, H. (2011). Molecular Pharming: future targets and aspirations. *Hum Vaccin*, 7: 375-382.

Salehi Jouzani, G. (2012). Risk Assessment of GM Crops; Challenges in Regulations and Science. *Biosafety* 1:e113.

Schillberg, S., Fischer, R. and Emans, N. (2003). Molecular farming of antibodies in plants. *Naturwissenschften*, 90: 145-155.

Schillberg, S., Raven, N., Fischer, R., Twyman, R.M. and Schiermeyer, A. (2013). Molecular farming of pharmaceutical proteins using plant suspension cell and tissue cultures. *Curr Pharm Des*, 19 (31):5531-42.

Sijmons, P.C., Dekker, B.M.M., Schrammeijer, B., Verwoerd, T.C. van den Elzen, P.J.M. and Hoekema, A. (1990). Production of correctly

processed human serum albumin in transgenic plants. *Bio/Technol* 8: 217–221.

Sparrow, P., Broer, I., Hood, E.E., Eversole, K. and Hartung, F. (2013). Risk assessment and regulation of molecular farming-a comparison between Europe and US. *Curr Pharm Des*.

Suslow, T.V., Thomas, B.R. and Bradford, K.J. (2002). Biotechnology provides new tools for planting. Oakland: University of California Division of Agriculture and Natural Resources, Publication, 8043.

Terashima, M., Murai, Y., Kawamura, M., Nakanishi, S., Stoltz, T., Chen, L., Drohan, W., Rodriguez, R.L. and Katoh, S. (1999). Production of functional human alpha 1-antitrypsin by plant cell culture. *Appl Microbiol Biotechnol* 52: 516–523.

Thomas, B.R., Van Deynze, A. and Bradford, K.J. (2002). Production of Therapeutic proteins in plants. Agricultural Biotechnology in California Series, Publication, 8078.

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

Twyman, R.M., Schillberg, S. and Fischer, R. (2012). The Production of Vaccines and Therapeutic Antibodies in Plants, Molecular Farming in Plants: Recent Advances and Future Prospects, 145-159

Valkova, R., Apostolova, E. and Naimov, S. (2013). Plant molecular farming: opportunities and challenges, *Journal of the Serbian Chemical Society*, 78: 407415.

Voss, A., Niersbach, M., Hain, R., Hirsch, H., Liao, Y. and Kreuzaler, F. (1995). Reduced virus infectivity in N. *tabacum* secreting a TMVspecific full size antibody. *Mol Breeding*, 1: 39–50.

Walmsley, A. and Arntzen, C. (2000). Plants for delivery of edible vaccines. *Curr Opin Biotech* 11: 126–129.

Zeitlin, L., Olmsted, S.S., Moench, T.R.; Co, M.S., Martinell, B.J., and Paradkar, V.M. (1998). A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes. *Nat Biotechnol*, 16: 1361–1364.

Zhong, G.Y., Peterson, D., Delaney, D., Bailey, M., Witcher, D. and Register, J. (1999). Commercial production of Aprotinin in transgenic maize seeds. *Mol Breeding* 5: 345–356.

Zhu, Z., Hughes, K., Huang, L., Sun, B., Liu, C. and Li, Y. (1994). Expression of human alpha-interferon in plants. *Virology* 172: 213–222.

Ziegler, M., Thomas, S. and Danna, K. (2000). Accumulation of a thermostable endo-1,4-b-D-glucanase in the apoplast of *Arabidopsis thaliana* leaves. *Mol Breeding*, 6: 37–46.