Molecular pharming for the betterment of crop yielding

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Abstract

Molecular farming is a field linked to plant biotechnology which forms a major part of agricultural biotechnology. It combines the use of plants and techniques of biotechnology for the production of valuable proteins or other metabolites which are pharmaceutically and industrially important. The method helps in the creation, conservation, and utilization of genetic variation for the improvement of crop species. Molecular farming is executed by the methods of stable gene transfer, such as gene transfer to nuclei and plastid; transient transformation of crop species like viral vectors and Agrobacterium mediated transformation; and cell suspension technique. Transgenic plants as bioreactors have been the subject of worthwhile recognition concerning their advantages, such as the safety of recombinant proteins (antibodies, enzymes, vaccines, growth factors, etc.) that are free of mammalian toxins and pathogens, and their potential for the large-scale and low-cost production. For the expanding world population, vigorous agricultural production is necessary to satisfy food requirements, which is somehow fulfilled by molecular farming. This article reviews the principles of molecular farming, various cost-effective technologies, and strategies, their advantages and disadvantages, the technical advances that have helped to optimize the yield and quality of recombinant proteins, thereby making plant-based production systems suitable alternatives to the existing systems. Lastly, we will discuss the issue of biosafety and public acceptance of molecular farming products.

Keywords: Molecular farming, Transgenic plant, Human growth hormone, Protein production, Crop yielding, Cost-Effective techniques.

INTRODUCTION

Crop plants are the best source to produce desired and useful recombinant proteins (Horn et al., 2004). The techniques used prior to this were to extract the required protein from animal or human sources. These methods were quite costly and also contained a risk factor. Keeping the risks in mind and with the aim to increase the yield of crops with the desired characteristics, the method of genetic alteration came to the forefront which is popularized as molecular farming. Molecular farming was done for the first time in early 1989 and the trial gave outstanding results.

Molecular farming (also known as "*biopharming*" or "*gene pharming*") is a biotechnological program that uses whole organism, organs, tissues or cells, or cell culture as bioreactors for the production of important proteins and chemicals for commercial and pharmaceutical purposes (Kumar et al., 2007). It is a cost-effective technique in the field of biomedicine. Generally, production costs can be reduced to just 1/30 the cost of using animal cell culture and 1/3 of the cost of using microorganism culture system. Plants can be used for the production of vaccines and antibodies and also food supplements, biopolymers, industrial enzymes, and proteins in the investigations (avidin, β-glucuronidase, etc.). Plants provide an inexpensive and simple system for the production of valuable recombinant proteins on large scale compared to other sources like animals, bacteria, and fungi (Alireza & Nader, 2015). Human growth hormone from transgenic tobacco is the first protein to be prepared for medical usage in 1986 (Barta, 1986). During the period 1986-1999 many therapeutics produced in plants were reported such as human antibodies (During, 1988); secretory antibodies (Hiatt, K. Bowdish, 1989); egg

proteins with important properties - avidin (Hood et al., 1997)and aprotinin, one of the first molecularly farmed pharmaceutical proteins produced in plants (Zhong et al., 1999; DK Das, 2009).

After all the experiments, it was confirmed that transgenic plants are a much important source in order to produce recombinant proteins (Schillberg et al., 2003). Molecular farming relies on the same method used to produced genetically modified crops- plant genetic engineering. Short peptide chains (less than 30 amino acids) can be synthesized chemically, but for larger proteins production living cells are required. The DNA encoding the required protein is incorporated into the cell and the desired protein is obtained after purification once the cells grow (Kunka Kamenarova, 2005).

Horn classified the proteins produced by plants that could be utilized for molecular farming into the following four categories- i) parental therapeutics and pharmaceutical intermediates, ii) industrial proteins (example- enzymes), (iii) monoclonal antibodies, and (iv) antigens for edible vaccines (Horn et al., 2004). The method of molecular farming can be accomplished in three different ways- (i) stable transformation which includes nucleus and plastid transformation, (ii) transient transformation including Agrobacterium and virus-mediated methods along with magnification system, and, (iii) cell suspension method.

A great diversity of plants is currently being used for PMF, which includes food/feed plants like alfalfa, clover, lettuce, maize, rice, wheat, barley, soybean, oilseed rape, pea, potato, and tomato, non-food plants like tobacco, Arabidopsis as well as duckweed, mosses and microscopic algae (Howard, 2005; Ma et al., 2003; Streatfield & Howard, 2003; Fischer et al., 2004; Goldstein & Thomas, 2004). More than 500 recombinant pharmaceutical products are believed to be in development worldwide, including agents directed against cancer, infectious diseases, and a variety of important medical conditions such as monoclonal antibodies (Daniell et al., 2001).

Plant-based platforms are also used for producing subunit vaccines, some of them being in the clinical trial stage (Ma et al., 2005. Not only vaccines related to humans but also plant-based vaccines along with antibodies are produced for practical use in animals (Floss et al., 2007). Plant-made industrial products (PMIs) currently in the pipeline include enzymes that can be used in detergents, bio-plastics, secondary metabolites (phenolics, glucosinolates, tannins, starches, sugars, fragrances, flavors, and alkaloids), fibers, or food manufacturing (Didier Breyer et al., 2009).

Novel secondary metabolites are nowadays being produced mostly by genetic engineering. Functional genomics provides a much better analysis of multiple genes and their expression that becomes important for understanding the regulatory mechanism and biochemical pathways related to secondary metabolites (Khatri et al., 2017) in Table 1.

OBJECTIVES

The objectives of this review paper were as follows- 1) to understand plant molecular farming (PMF) 2) to study the strategies related to PMF 3) to see the advantages and limitations of the process 4) to study the improvement of crops with the help of molecular farming.

MATERIALS & METHODS

Molecular farming is generally done by four methods- i) stable nuclear transformation ii) stable plastid transformation iii) transient transformation of crop species iv) cell suspension (Horn et al., 2004). A variety of crop species have been utilised for molecular farming. However according to a review, tobacco and corn responded best to this technique. Apart from these two species, alfalfa, barley, canola, rice and sunflower are also used for the experimentation purpose (Nikolov & Hammes, 2002). It is further reported that certain edible plants such as beets, peas, beans, tomatoes, carrots, potatoes can also show a positive result (Prakash, 2021) in Figure 1.

Table 1: Examples of recombinant proteins that have been expressed in plants (Source: Twyman et al.)

Species	Recombinant protein
Pharmaceutical proteins: growth factors	
Rice	α- interferon
Pharmaceutical proteins: recombinant antibodies	
Soybean	Humanized IgG, herpes simplex virus
Vaccines	
Tomato	Rabies virus glycoprotein
Industrial/ processing enzymes	
Alfalfa, barley, potato, tobacco	1,4- β-d-endoglucanase
Food additives (nutriceuticals)	
Potato	β- casein
Technical proteins	
Maize	Avidin
Biopolymers	
Tobacco	Synthetic elastin

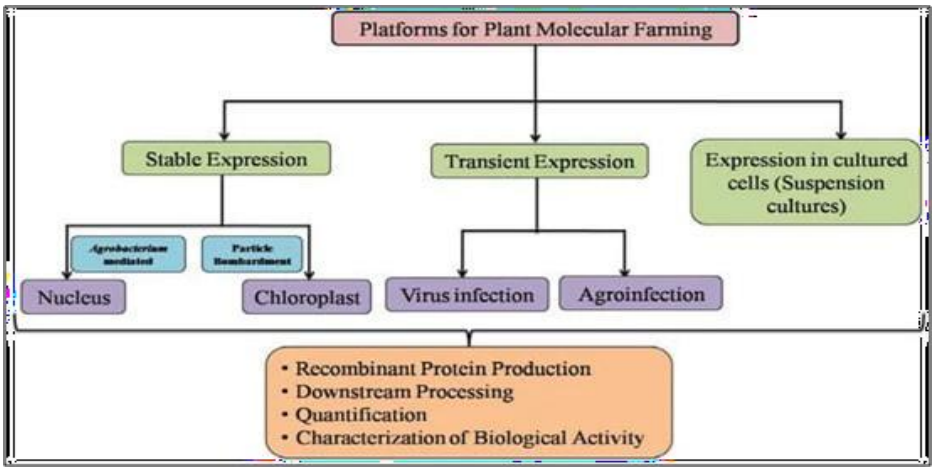

Figure 1. Overview of plant transformation approaches employed for the production of recombinant pharmaceutical and non-pharmaceutical proteins in plants. (Source: Shanmugaraj, B. et al, 2020.Plant molecular farming: a viable platform for recombinant biopharmaceutical production.)

Stable Expression

Stable nuclear transformation- this is the process in which the required genes are integrated into the nuclear genome of the target crop species. This process results in the change in structure and thereby expression of the genes in the target plants. This method has the capability to produce crops which can be grown anywhere.

Advantages of Stable Nuclear Transformation

The protein product of the crop species is generally stored in the seed and harvested in a dry state till processing is completed (Delaney, 2002).

Large acres of land can be utilized at a much effective cost (Horn et al., 2004).

Disadvantages of stable nuclear transformation

a. The time period for production in case of certain plants is quite lengthy (Commandeur et al., 2003).

b. The cost of labour is quite high in comparison to crop yield (Horn et al., 2004).

Stable chloroplast (plastid) transformation- A system for plastid transformation was first described by Svab et al. (1990) using tobacco. Plastid transformation is a remarkable solution since it has numerous advantages includind high levels of transgenic expression, transgenic containment via maternal inheritance and multigene expression in a single transformation event. Highly polyploid nature of the plastid genome will allow the integration of thousands of copies of transgenes per cell. This will result production of very high levels of proteins in trans genic plants produced by plastid transformation. Promoters, UTR sequences, codon optimization, post translational modifications etc. are some factors which participate in regulation of recombinant protein expression. (Meili Gao, 2012).

Advantages of stable plastid transformation

a. It permits introduction of thousand copies of transgene per plant cell. It enhances protein production in the cell (Meili Gao et al., 2012).

b. Absence of chloroplasts in pollen and consequent improbability of their transfer reduces environmental concerns.

Disadvantages of stableplastid transformation

a. Plastid transformation was limited by low transformation frequencies in potato and other crops. (Meili Gao et al., 2012).

b. The technology has not resulted in any product commercialization because the problems in the protein purification still need to be solved. (Meili Gao et al., 2012).

Transient transformation of crop species

This system is widely used in molecular farming. This method is used to check the significant amount of protein in an expression construct (Vezina et al., 2009). It is mainly of three different types:

a. ***Agrobacterium* mediated**- this is one of the most efficient method in gene transfer. This method works by the transfer of T- DNA to the target cells which thereby results in the expression of the transgenes. This process is quite useful in the clinical fields (Regnard et al., 2010)

b. **Virus mediated**- in this method, the carriers of the genes are viral particles. They infect the plant which leads to the entry of the target genes in the respective plant (Porta & Lomonossoff, 2002).

c. **Magnification system**- this method involves the removal of the coat protein of the non- competitive viral particles which are then delivered into the respective plant by *Agrobacterium* (Alireza & Nader, 2015).

Advantages of transient transformation

a. It is most probably the fastest method (Rybicki, 2010).

b. The *Agrobacterium* mediated is widely used in the production of biomedicines (Vezina et al., 2009).

c. The transient transformation method increases the rate of synthesis of protein.

Disadvantages of transient transformation

This method might not be much efficient in conditions that require large protein quantity (Horn et al., 2004).

The viral mediated as well as the *Agrobacterium* method might not always be capable to initiate effective co-expression of polypeptides (Giritch et al., 2006).

Expression in cultured Cells (suspension cultures)

This technique is also used as a alternative to transgenic plants or transient expression systems in molecular farming. This method involves the removal of cell walls and gene transfer to the obtained protoplasts and suspension culture. The cell suspension derived from undifferentiated callus grown in a liquid medium can be scaled up in bioreactors for large scale protein production under an aseptic condition (Shanmugaraj et al., 2020).

Advantages of suspension culture

a. The purification system and its downstream processing are cheaper and easier.furthermore, it is a fast system.

b. The use of suspension culture can decrease heterogeneity in proteins and sugar regarding the uniformity of type and size (Tarinejad Alireza et al., 2015)

Disadvantages of suspension culture

Cell suspension has not yet been suggested as an optimal production choice of production in plant systems due to a belief that the ultimate production and their usability are constrained by reducing the level of recombinant proteins during the stationary phase because of the enhanced proteolytic activity (Tarinejad Alireza et al., 2015).

CROP IMPROVEMENT

Plant Molecular Farming (PMF) over the past two decades, has been applied to improve plant production such as herbicide and pest-resistant crops, as well as nutritional improvement (e.g. Golden Rice), industrial proteins (e.g. cellulase, and trypsin), and pharmaceuticals and vaccines. Other benefits associated with the use of plants are - convenient storage of raw material (in the form of seed), rapid scaling up, and most importantly less concern over human pathogen contamination (Penelope Sparrow et al., 2013).

The improvement of crop species generally deals with the alteration of the genetic composition of the respective crop**.** Crops are improved to satisfy the needs of humans. In order to make a particular crop of one's desired quality, molecular farming has been proved to be quite useful. With the help of this method, the proteins are altered which as a result changes the quality of the crop. This technique has been used to modify the characteristics of the recombinant proteins which has resulted not only in the quality improvisation but also has improved the crop yield.

For example, the synthesis of glycans in the plant affects biological function, protein stability, and immunogenicity. So, with the help of molecular farming, the genes which encoded for the synthesis of the enzymes- β(1,2)-xylosyltransferase and (1,3)-fucosyltransferase could be knocked out, thereby producing the desired recombinant plant protein which would not participate in the synthesis of glycan (Kaul et al., 2020).

Apart from plant glycan, chloroplasts of certain plants have also been altered in order to acquire desired traits (Daniell, 2004). This approach was successful in overcoming the concerns related to the reduced level of transgene expression, gene silencing, presence of antibiotic-resistant genes. The chloroplast transgenic approach in certain plants has resulted in the increase in the resistance of plants towards disease (Ruiz et al., 2003), drought (Lee et al., 2003). The presence of antibiotic-resistant genes was also overcome in crop species using this technique (Daniell et al., 2002).

RESULT & DISCUSSIONS

Plant molecular farming is in the limelight due to its potential advantages over other systems. These advantages include: i) Uncomplicated and cost-effective culture and processing technology in plants. ii) Ability to perform post-translational modifications required for producing functional proteins (Gomord et al., 2005). iii) Increased safety to human health of products synthesized in plant systems since the risks arising from the contamination with human pathogens or toxins are minimized. iv) Purification processes that can be avoided (when the plant tissue containing the recombinant protein is used directly for product delivery) or greatly facilitated (when recombinant molecules can be targeted or expressed directly into certain intracellular compartments) (Rishi, 2001; Didier Breyer et al., 2009).

In 2006, the first plant molecular farming to gain regulatory approval by the US Agricultural department was a poultry vaccine against Newcastle disease, developed by Dow Agrosciences. In 2012, Elelyso developed by Protalix became the first plant-made pharmaceutical for treating the rare hereditary Gaucher disease (for human use) to gain regulatory approval by the US FDA. Both of these products were developed using plant cell suspensions grown in bioreactors tobacco and carrot cells respectively. In the US, the recent regulatory approval of Enogen corn from Syngenta has paved the way for industrial protein/enzyme commercialization from plants grown in the field. Enogen corn, which contains a heat-tolerant amylase in the starchy endosperm permits reduced cost of saccharification during ethanol production (Penelope Sparrow et al., 2013).

However, the application of genetic engineering also raises some biosafety concerns, in particular regarding aspects such as transgene spread in the environment (as for first-generation GM crops, the vertical transfer of genes from GM plants to non-transgenic populations of the same or related species and the possible negative effects on mammals, birds, insects or microorganisms interacting directly or indirectly with crops) or accidental contamination of the food and feed chains (Didier Breyer et al., 2009). All GM plants, those intended for molecular farming must undergo a thorough risk assessment before they can be used in the field. Commercialization has many hurdles including not only the science and associated intellectual property to protect the inventions but also market realities and government regulations (Penelope Sparrow et al., 2013).

We can limit the potential risks of plant molecular farming by applying complementary strategies. Amongst the most effective strategies are the physical containment of plants or cell cultures, the spatial containment of the GM plants, the development of biological confinement systems, the development of inducible or transient expression systems, and the targeted expression of transgenes. If any GM plant is cultivated outdoor i.e. grown in the field, then monitoring of the production site, encouraged by an appropriate inspection plan, will be required. Additional considerations like handling, transport, equipment, and personnel should be taken care of properly. Therefore, appropriate measures should be taken to handle the waste in order to ensure that the material will not enter the human or animal food chain or impact the environment (Didier Breyer **et al.,** 2009).

CONCLUSION

Trees are the best friends of every human. Plants not only give us food and oxygen but also help us economically and technically. The methods of molecular farming in plants are cost-effective as compared to other techniques. It can also be said that plants are today's best source of synthesizing recombinant proteins. Every technique mentioned above has its advantages and disadvantages but the risk of toxicity, in this case, is quite low. The regulatory concern is also lowered down. For example- we can specify the tissue in which the required protein is to be expressed. If we desire the protein not to interact with the environment, then we can keep the protein in tissues that remain out of the pollen of the flower. Though there remains a possibility of outcrossing, still the problem of regulatory concern can be solved to some extent. It can be said that in the near future, plant molecular farming may arise as one of the best methods in agricultural biotechnology improving the quality of the crop species along with the yield of the crop. Apart from all the above points, it is necessary to bring the importance of transgenic crops to the knowledge of the public. They must be informed about the merits and the traits of the improved crop species. The public must be made aware of the fact that transgenic crops are developed for improving the health conditions of humans. With their realization, the benefits of crops produced from molecular farming will be spread to a larger population. This will thus help the techniques of molecular farming receive more rapid acceptance.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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