

**PLANT MOLECULAR FARMING: MUCH MORE TO KNOW**

**Anup Mishra<sup>1</sup>, Vikas Mishra<sup>1\*</sup>, Pervej Akhter<sup>2</sup>, Roma Agrahari<sup>3</sup>, Wakar Ahmad<sup>4</sup>,  
Shivangi Khare<sup>5</sup> and Alok Raghav<sup>6</sup>**

<sup>1,2,3,4,5</sup>Department of Biochemical Engineering, HBTU, Kanpur.

<sup>6</sup>Biological Sciences and Bioengineering Indian Institute of Technology, Kanpur-208016,  
India.

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**\*Corresponding Author****Vikas Mishra**

Department of Biochemical  
Engineering, HBTU, Kanpur.

**ABSTRACT**

Plant Molecular Farming is an utilization of recombinant DNA technology for the genetic manipulation of plants for the production of proteins and chemicals that are being used for medicinal and commercial purposes. Recently, through 21st Century biotechnology, it is now understood that plants are possibly another wellspring of pharmaceutical proteins including edible vaccines, antibodies, blood substitutes and other helpful compounds. Plants can produce a wide variety of proteins that does not contain any mammalian toxins and infectious agent. They can produce a lot of biomass at a very low cost

and require limited facilities. Since plants have for some time been utilized as a source of medicinal compounds, Plant Molecular Farming provides us with an opportunity in which production of compounds such as, antibodies, medicines, vaccines, growth factors, enzymes and recombinant plasma proteins, whose medicinal applications are comprehended at a molecular level. Plant-made pharmaceuticals" [PMPs] are created by genetically modified plants to produced particular compound or component of a bigger compound, generally proteins, which are isolated and purified after harvest. As used here, the terms molecular farming and PMP do exclude normally happening plant products or nutritionally enhanced foods.

**KEYWORDS:** Molecular farming, Biotechnology, Plant Made Pharmaceuticals [PMPs].

**INTRODUCTION**

Plants have been providing people with valuable compounds for a very long time, but only in last two decade has it become possible to utilize plants for the production of particular

heterologous proteins.<sup>[1]</sup> Production of various metabolites or proteins important to medicine or industry in plants by using RDT is known as molecular farming. It is the use of complete organism or part of it (organs, tissue, cells) as a bioreactor for the production of proteins and chemicals for pharmaceutical and other commercial purpose by application of biotechnology to genetically modify crops.<sup>[2]</sup> Genetic engineering has recently opened up new opportunities for to use plants as production factories for biopharmaceuticals. Human growth hormone [GH] was one of the first pharmaceutically important protein that were expressed in transgenic tobacco.<sup>[3]</sup> From then on transgenic plants expressing vaccines, antibodies, nutraceuticals, digestive support enzymes, therapeutic proteins and other pharmaceutical proteins have been produced. The thought of utilizing plants for the synthesis of human proteins was at first met with incredible doubt. In 1989 recombinant antibodies were produced for the first time in plant by using molecular farming.<sup>[4]</sup> Before this result was published, there was some support for the idea that plants can be used to produce therapeutic proteins. After 1990s, it has been shown that transgenic plants are very flexible and they are being used for production of a wide range of pharmaceutical proteins.<sup>[5]</sup> Plants have many advantages over microbe and animal in the production of protein and other biopharmaceuticals like vaccine and plantibody in plants which can be achieved at much lower costs and much lower risk of contamination from human or animal pathogens. additionally, plants possess natural protein storage organs and their seeds are easily distributed, allowing local production.<sup>[6]</sup>

The advantage of using plants as bioreactor for the purpose of production of complex organic compound includes.<sup>[7]</sup>

- The cost for the production of protein is low as compare to transgenic animals, Fermentation or bioreactors.
- Planting, harvesting and processing of plant material can be done easily because Infrastructure and expertise required for that are already exists and well known.
- The chances of contamination form human pathogens such as prions, virions, etc are reduced exponentially.
- Proteins synthesize in higher plants have correct folding, glycosylation, and higher activity because of post translation modification.
- Plant cells can direct proteins to environments that reduce degradation and therefore increase stability.

**A brief history of molecular farming:** The first recombinant proteins were produced in plants in the 1983 at the same time by the use of *Agrobacterium tumefaciens* as basis for the development of efficient transformation protocols. In this cases, the proteins were the enzymatic products of bacterial selection markers, such as nptII encoding neomycin phosphotransferase.<sup>[8]</sup> Subsequently, plants were transformed with many different genes encoding products that provide us with agronomic advantages such as herbicide tolerance and pest resistance, and only towards the end of the decade did scientists first address the possibility that plants could be used as a production system for recombinant proteins, i.e., with the ultimate objective of purifying the product rather than altering the phenotype of the plant.<sup>[9]</sup> This was the beginning of molecular farming or bio pharming. After 10-year of this breakthrough the first plant-derived recombinant proteins were produced commercially. This time we needed to address technical barriers and make the process economically convenient. There was also considerable enthusiasm for plants to be used for the production of pharmaceutical proteins, a concept sometimes described as “molecular pharming.” This was borne from the appreciation of several key advantages of plants compared to other production hosts, including the low cost of establishing and maintaining pharmaceutical crops compared to industrial-scale fermentation systems, the scalability of plants compared to fermenters, and the safety of plants compared to bacteria and mammalian cells, i.e., the lack of endotoxins and human pathogens that make most crops “generally regarded as safe”.<sup>[10]</sup> In 2012 For the first time, the US Food and Drug Administration (FDA) approved a drug produced in a genetically engineered plant cell for the market.<sup>[11]</sup>

**Why Plants are used as a Production System:** Plants have the natural ability to synthesize human and animal proteins. This means that scale up is simplified by just increasing the hectare of the plants under cultivation. Present farming equipment can be used in the processing of the plants which reduce the costs involved in production of the product as much as 3.3 % when using animal cell culture and at least by 33.33% when compared to microbial culture systems. Also if genes for the required Products are expressed in seeds leaves of plants they can be stored for long periods without refrigeration. The use of plants as a production system for recombinant pharmaceuticals was started with the successful expression of a human growth hormone, Recombinant protein, interferon and human serum albumin between 1986 and 1990, A crucial advance came with the successful expression of functional antibodies in plants in 1989<sup>[12]</sup> and 1990.<sup>[13]</sup> Which established that Plants had the potential to produce complex mammalian proteins of medical importance. As compare to the

production of insulin or antibodies in microbe, the production of antibodies in plants had the potential to make large amounts of safe & cheap antibodies available. This is because as we all know plants are eukaryotes i.e. they contain the machinery required for post-translational modification which is crucial for final packaging of antibodies.

A Plant based expression systems is very promising because they offer significant advantages over other expression systems based on 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<sup>[14]</sup> nor perform most of the important mammalian post-translational modifications. Secondly, proteins produced in plants accumulate to high levels<sup>[15]</sup> and plant derived antibodies are functionally equivalent to those produced by hybridism.

### **Molecular farming Expression systems**

There are currently four methods for expression and production of foreign protein in plants as described below.<sup>[16]</sup>

#### **(a) Stable nuclear transformation**

Stable nuclear transformation of a crop species that will be grown in the field or a greenhouse is the most common of the methods to date, nuclear transformation of a crop species, has produced most of the products available in the marketplace today. This system requires a method for transferring the foreign genes into the plant cells, usually by using *Agrobacterium tumefaciens* or particle bombardment, in which the genes are taken up and incorporated into the host nuclear genome in a stable manner. the main advantages of this system is when performed in a crop species the protein product is normally accumulated in the seed, which can be harvested in a dry state and stored till processing is completed. As an example, dry corn seed has a moisture content of approximately 11–15% and, at that level, the seed is metabolically inert. Thus, long-term non-refrigerated storage of the seed, if properly carried out, can allow the protein to exist without degradation for at least 2 years. These seeds can then be transported to its final destination without refrigeration. Another advantage to this system is that large acreage can be utilized with the lowest possible cost. Since crops such as rice and corn are grown throughout the world, the products have the potential to be produced near the target markets. This system also have some drawbacks which includes, some grains, such as corn, have the potential to cross with native species or food crops. There are technologies that will prevent outcrossing, e.g., genetics-based male sterility. Such

technology generally reduces the cost advantage of the system due to higher manual labor requirements, lower yields, and less effective genetics.

#### **(b) Plastid transformation**

The gene of interest can also be incorporated into the plastids genome. The commonly used method to transform chloroplasts involves using a gene gun to incorporate the transgene into the chloroplast genome. The main advantages of this system is level of protein expression exceeds about 40% on a dry weight basis have been reported when tobacco chloroplasts were transformed. Plastid genes are not usually transmitted through pollen so that outcrossing is not a major concern. Main disadvantages of this system include with any fresh tissue molecular farming system, protein stability over time will change even with refrigeration. Extraction and purification must be performed at a particular times following harvest. Tobacco is currently a highly regulated crop and is not edible. Large volume products and edible vaccines would not appear to be feasible using this system. Plastid transformation is also time consuming as it requires the generation of stably transformed plants.

#### **(c) Transient transformation of a crop species**

This system depends on the ability of recombinant plant viruses such as tobacco mosaic virus [TMV] to infect tobacco plants and then transiently express a target protein in the plant tissue. The protein will accumulate in the interstitial spaces. The interstitial fluid can then be collected by centrifugation under vacuum. Advantages of transient transformation of a crop species are rapid genetic manipulation, the infection process is rapid. Small quantities of the target protein can be obtained within several weeks. Probably the ideal system for a large number of custom proteins that are needed in relatively small amounts. The main disadvantages of this system is that its not suitable for any protein needed in large quantities. Product must be processed immediately as storage will cause degradation of the plant tissue. As discussed above, tobacco is a highly regulated crop and is not grown on large acreage.

#### **(d) Stable transformation of a plant species that is grown hydroponically**

In this system, transgenic plants containing a gene coding for the target protein are grown hydroponically in a way that allows release of the desired product as part of the root exudate into the hydroponic medium.<sup>[17]</sup> The main advantages of this system is Plants grown hydroponically are contained in a greenhouse setting and so have reduced fears of unintentional environmental release. Purification of the desired product is considerably easier since no tissue disruption is needed and the quantity of contaminating proteins is low. and the

main disadvantages Include not amenable to producing large [kg] quantities of any protein product. Greenhouse/hydroponic facilities are relatively expensive to operate.

### **Optimizing foreign gene sequences for expressing in plants**

Transgenic DNA can be modified for optimized expression of foreign DNA in plants to increase translation and therefore increasing protein production in plants. This can be achieved by the use of tissue specific promoters i.e. promoters controlling gene expression in a tissue-dependent manner and according to the developmental stage of the plant. These promoter will only initiates transcription of a particular transgenes only where the product of transgene is needed and rest of the tissue in plant will be unaffected by the expressed transgene protein. In case of monocot plants maize ubiquitin-1 promoter is usually used while 35 S promoter is used in case of dicotyledonous plants.<sup>[18]</sup> The use of tissue specific promoters helps in reduce adverse effect on plant growth and development. Protein expression can be increased by the use of introns in the recombinant DNA molecule.<sup>[19]</sup> However, the process by which introns enhance protein expression is not known. It has been found that translation of a transgene can be enhanced by the addition of a leader sequence of alfalfa mosaic virus mRNA 4 that are not translated.<sup>[20]</sup> The stability of mRNA is also influenced by the polyadenylation sites in plant cells.<sup>[21]</sup> These sites protect enzymatic degradation of mRNA. Existence of specific recognition sites also result in RNA degradation. Some of these recognition sites have been discovered and are supposed to be involved in mRNA degradation as a result of their interaction with specific binding factors.<sup>[22]</sup> It may be useful to screen for these sites and remove them by modifying the gene of interest to increase gene expression. The level of amino acid can also be a limiting factor in the expression of particular proteins, it is necessary to alter amino acid synthesis pathways for the expression of some particular proteins.<sup>[23]</sup>

### **Purification and downstream processing of recombinant proteins**

Downstream processing includes processes that involve the recovery Isolation and purification of the recombinant protein from plants. Recovery usually involves processing of the plant tissue, solid-liquid separation and protein extraction, whereas purification consists of liquid-liquid extraction, centrifugation, immunoprecipitation, chromatography, and membrane filtration etc. Processing of leaves requires special attention to prevent degradation of proteins by proteases. Leaves must be processed immediately after harvest or must be frozen, on the other hand chances for the degradation of recombinant proteins expressed in

the seed are less as compare to leave so seeds can be stored for longer periods. The Production recombinant proteins in cell secretion can also be very beneficial because then there is no need to disrupt plant cells during recovery, however, in the culture medium the recombinant protein may not be stable.<sup>[11]</sup> Other ways for protein recovery is to use of affinity tags. Protein tags should be removed after purification to restore the structure of the purified protein to its native state. Oleo sin fusion technology, developed by Symbiosis Genetics Inc.is another system in which the recombinant protein gene sequence is fused to the sequence of an oil body specific endogenous protein oleosin in rapeseed and safflower, after purification the protein is separated by an end protease digestion.<sup>[25]</sup> Problems encountered during protein extraction primarily include proteolytic degradation and structural modification due to the reaction with phenolic compounds. When devising a strategy for heterologous protein production in plants, proper consideration should be given to downstream processing feasibility of the recombinant protein to get optimum protein yield.

**Classes of proteins within molecular farming:** Proteins currently being produced in plants by molecular farming purposes can be categorized into four broad areas.

- Parental therapeutics and pharmaceutical intermediates.
- Industrial proteins [e.g., enzymes].
- Monoclonal antibodies [MAbs], and
- Antigens for edible vaccines.

**Parental therapeutics and pharmaceutical intermediates:** Proteins that can be directly used as pharmaceuticals or can be used in the making of pharmaceuticals are fall under this category. Therapeutics like thrombin and collagen and intermediates like trypsin and aprotinin are examples of these kinds of proteins. Practically speaking, only those proteins with high value will be considered as candidates for molecular farming.

**Industrial proteins enzymes:** This group includes hydrolases, encompassing both glycosidases and proteases. Oxido-reductase enzymes such as laccase, a fungal enzyme used in fiber bleaching and bioglue of wood products, represent a separate class of industrial enzymes. Enzymes involved in biomass conversion for the purposes of producing ethanol are candidates for molecular farming. All of these products will usually be characterized by the fact that they are used in very large quantities and must therefore be produced very inexpensively.<sup>[7]</sup>



**Monoclonal antibodies**

This group includes all antibody forms [IgA, IgG, IgM, secretory IgA, etc.] and antibody fragments [Fv]. They can be produced in plants in both glycosylated and nonglycosylated forms. These plant-derived MABs [plantibodies] have the potential of alleviating the serious production bottleneck that currently exists as dozens of new MAB products attempt to reach the marketplace. Examples of plant-derived MABs in product development include a-caries for prevention of dental decay and aherpes for prevention of herpes transmission.

**Antigens for edible vaccines:** Specific DNA molecule that code for specific amino acid sequence or protein can be introduced in plant and expressed in plants that will induce a cellular immune response when ingested by an animal or human. Protection studies have shown good efficacy when these edible [or oral] vaccines have been used. In some cases, protection has actually been better with the edible vaccine than with the commercially available vaccine.<sup>[22]</sup> Companies involved in molecular farming are depicted in table 1.

**Biosafety issues in plant molecular farming:** on account of plants molecular farming, the concentration of the evaluation for human, animal and environmental safety is on the risks resulting from accidental exposure to the GM plants by humans and animals since these plants are not intended for food or feed use nor for intentional environmental purposes. In terms of environmental impacts, potential risks concern mainly, 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. A vital concern is the contamination of the food chain with plant made pharmaceuticals (PMPs). This can occur because of different reasons like introduction of DNA from transgenic plants to food crops, use of same equipment for harvesting and processing of food crops that are used for transgenic plants without any decontamination, or growing food crops in the same field where a transgenic crop was grown without any decontamination. To steer clear of the food chain contamination, strict regulation needs to be put in place such as geographical isolation of the transgenic crop, growing in greenhouses instead of open fields, and harvesting and processing transgenic plants using different set of equipment or properly decontaminating the used equipment if the same equipment is also applied to food crops. It is also important to label genetically modified products so that the consumer has the choice to select according to his /her own wish. Regulatory authorities are facing a lot of challenges regarding the control



of genetically modified crops. Every product derived from molecular farming and every host system is unique so each case needs to be handled separately. The 0.5% presence of transgenic material in non-transgenic food or feed has been allowed by the European Parliament and the Council of the European Union in cases where the presence of transgenic material is unavoidable and its negative effects are dominated by its benefits. Efforts to confine transgenic and reduce environmental exposure have been made recently<sup>[23]</sup>, however, these regulations are still in infancy and much more effort is needed to overcome problems regarding regulations of molecular farming products.

### **Future Prospect**

**In Plant molecular farming** One of the keys to success in the future will definitely be the level of expression of the recombinant protein in plants. This is 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 well established systems. Whether or not the protein is in specific tissues will enable or nullify exposure to the environment. There has just been work to demonstrate that expression of proteins can limited particular tissues therefore decreasing regulatory concerns. e.g. inadvertent exposure to the environment can be reduce by keeping the protein out of pollen. 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 might be a few situations where genetic control of expression is also warranted either for financial or safety concerns, depending on the product. Conceivable outcomes including male sterile crops, sequences that prevent germination, or induced expression 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. The other regulatory concern is that the pathway to commercialization for human therapeutics has not been proven. The industry will anxiously await the establishment of a clear road map detailing how this process is similar to, and/or different from, the existing protocols today. With the first approved therapeutic products will also come the realization of the many benefits of transgenic plant technology. These real benefits should also help public acceptance and open the way for a much more rapid acceptance of this technology.<sup>[7]</sup>

**Table. 1: Companies involved in molecular farming over the last several years.**

S. No.	Company Name	Established	Species for Production	Location
1	Large Scale Biology	1987	California. Tobacco	Vacaville
2	Meristem Therapeutics Clermont	1901	France Maize	Clermont- Ferrand
3	Monsanto Protein Tech	1901	Maize	St. Louis Mo
4	Crop Tech	1991	Tobacco	Charleston
5	Ventria	1993	California. Rice	Sacramento
6	SemBioSys	1994	Canada Safflower	Calgary
7	ProdiGene	1995	Tex. Maize	College Station,
8	Epicyte Pharmaceutical	1996	Calif. Maize	San Diego
9	Medicago	1997	Canada Alfalfa	Quebec City
10	PlantGenix	1999	Various Crops	Philadelphia

## CONCLUSIONS

Production of pharmaceutical proteins by using plant molecular farming give us an advantage over conventional system which include cheap production, quick adaptability, the ability to fold and assemble complex proteins accurately(i.e post translational modification) and the absence of human pathogens. Plants molecular farming can even surpass other conventional system because it is very economical and safety benefits, and ultimately, it could be possible to make pharmaceuticals available to everyone who needs them, at a cost that everyone can afford. For the biotechnology and pharma industry, biopharming offers economic and health benefits after the development of product reaches the commercialization of product. However, for these benefits to be fully realized, A combination of strong and adaptable regulatory oversight with technological solutions are required. For manufacturing of therapeutic proteins, recombinant proteins all, plants should be seen as a probability among numerous. Plant molecular farming In the last few years has developed in to its own viable industry. As the industry develops, academic laboratories will need to put more emphasis into downstream process development research. This will complement their fundamental work on protein expression and will provide the basic knowledge to fuel the industry. However, the marketing and delivery of commercial products will necessarily fall to industry. As with any new industry, there have been hurdles to overcome, both technical and regulatory. However, the experience to date has taught us much and the industry is now poised for rapid growth and profitability.<sup>[7,24]</sup>

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