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PLANT MOLECULAR PHARMING: THE FUTURE OF PHARMACEUTICAL BIOTECHNOLOGY

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ABSTRACT: Plants are the natural sources of a vast diversity of molecules, exploited for the benefit of human health. They are the factories synthesizing proteins, carbohydrates, secondary metabolites, and biopolymers. Further, converting these molecules into antioxidants, pharmaceuticals, food, and biofuels is a major industrial process. Plants are becoming significant sources for producing pharmaceutically essential proteins, metabolites, vaccines, industry-based drugs, and bioplastics and are explored for large-scale production. Plant molecular pharming will enable the production of the desired therapeutic proteins. Plant-based pharmaceutical products are advantageous as they are free of vectors and pathogens utilized to transfer genes. Biopharming offers augmented breeding conditions, high biomass productivity, and excellent yield at low cost, making the plants an attractive platform for pharmaceutical production. The ability of plants to produce pharmaceutical-related products and the methodology involved in the production were discussed in this article. Technologies implemented for increased productivity of molecular pharming were also discussed, along with biopharma products, applications and limitations.

INTRODUCTION: Molecular pharming uses crop plants to produce pharmaceutically essential, commercially valuable proteins and metabolites beneficial to human health [1]. This modern pharming system exploits heterologous expression systems such as plants to provide a safe and cost-efficient means for the pilot-scale production of recombinant proteins and therapeutics. Molecular pharming cultivates agriculturally essential plants to produce a wide range of pharmaceutically and industrially crucial compounds, including drugs, vaccines, industrial chemicals, and biodegradable plastics.

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The molecular pharming approach has complete recombinant proteins for diagnostics and therapeutics [2]. Plants have the unique ability to make proteins that belong to other organisms. An increase in their cultivation will simplify mass production to a greater extent. Existing equipment for pharming will reduce the cost of making a product to 1/30 while using animal cell culture and 1/3 of using microbial cell culture. Plant pharmaceutical products are stored for long durations without freezing or refrigeration by expressing the desired product in seeds or leaves.

The utilization of plants to produce recombinant proteins and pharmaceuticals was started in 1986, after the successful expression of human growth hormone in the plant system [3]. Successful expression of functional antibodies in the plant system during 1989 is the crucial breakthrough for the demonstration of plants with the potential to produce mammalian proteins of clinical

significance [4]. Further advancements in plant transformation technology, recombinant DNA technology, and antibody engineering have made plants emerge as a significant source of expression systems. Analogous to the insulin production in the bacterial system, the plant system can prepare large amounts of safe and inexpensive antibodies. The expression of mammalian antibodies in plants has proved that plants can express functional mammalian proteins. In contrast, bacteria cannot produce full-size mammalian antibodies and cannot perform mammalian post-translational modifications [5]. Accumulation of proteins produced in the plants is at high levels similar to that of animal cells. Plant-derived antibodies are functionally equivalent to those produced by using hybridoma. The main objective of molecular pharming is to make beneficial products for human society. It is an upcoming technology used to mass-produce therapeutic recombinant substances [6].

Methodologies Implemented for Molecular Pharming in Plants: Two processes are implemented for achieving molecular pharming in plants. 1. Transient expression and 2. Permanent expressions.

Transient Expression: production is considered one of the best and most efficient systems for manufacturing recombinant and therapeutic compounds [7]. Many biotech companies employ this method to produce a significant amount of required proteins [8]. *Agrobacterium tumefaciens* is widely used in the pharming sector to produce many valuable compounds. The transfer of DNA achieves the recombinant Agrobacterium suspension into tobacco leaves into the cells and the expression of transgenes at a higher rate [8]. Some transfers are executed even without a proper and stable gene transfer [9]. This method is helpful during the production of bio-clinical medicines at an extensive rate [10]. *Agro bacterium* and other viral vector transfer methods overtly suffer from the co-expression of many polypeptides required to produce proteins [11]. Development of a novel approach known as Magnicon technology to overcome this involves removing systemic proteins and non-competent proteins in the virus and increasing the proliferation, leading to the co-expression of several polypeptides resulting in the manufacture of a protein by a considerable difference. This method requires compatibility between plant and virus functioning as a vector without affecting its genome in a destructive way [12].

Permanent Expression: Also known as part of stable expression systems, the permanent expression of proteins transforms the chloroplast through plant cell culture and nuclear transformation. Stable plastid transformation is a unique solution for achieving the permanent expression of desired proteins as this process consists of many positive utilities, including the prevention of escaping transgenes [13]. The absence of chloroplasts in the pollen will reduce the undesired transfer of pollen to other plants in the surroundings, thereby minimizing the environmental concerns [14]. Altered stability of the protein during its storage in refrigerators for a long duration is the only limitation of this method, overcome by using plant cell suspension culture. This method removes the plant cell wall and suspends the protoplasts in culture for gene transfer. Suspension cultures help to reduce different types of polypeptides, resulting in a decreased variety of types and sizes of cells [15]. The plant cell suspension is a fast, simple, and cheaper technique where the cultures produce biomedicine [16, 17]. Despite its applications, this method falls with a drawback of feedback inhibition as the product obtained using cell suspension culture will limit recombinant proteins [18]. Nuclear transformation is the technique applied to amalgamate genes into the plant nuclear genome, altering the genetic material and structures [19].

Strategies for Increasing the Yield in Molecular Pharming: While using the transgenic plants with gene expression systems to produce desired proteins or metabolites at the industrial level, it is evident that the yield of the recombinant compound is low. Optimization of the protein sequencing for the given conditions will increase the yield [20]. Traditionally, plants' morphology and anatomy are thoroughly studied and segregated into different components. Later, these recombinant proteins are targeted to these components to maximize their yield [21]. Many subcellular and cellular components are available for the formation, accumulation, and mass storage of plant-made pharmaceuticals and recombinant therapeutic components [22]. In

industrial molecular pharming, codon sequencing and optimization have become vital for a maximum outcome from the plant. It is also essential to adjust, edit, correct, and sometimes modify these codons [23].

Technologies Developed for the Improvement of Molecular Pharming:

Geneware System: Developed by Kentucky Bio-Processing LLCs, Geneware is a hybrid derived from the U1, and U5 TMV strains with a sense single-stranded RNA genome of 6400 bases encapsulated in 2100 copies of 17.5 KDa coat protein. This expression system uses independent virus functions, such as cell-to-cell systemic movement activities regulated by a movement protein. Gene ware can produce several mg of coat protein from an infected plant. Gene ware further exploits the strength and duration of viral sub-genomic promoters for re-programming the translational activities of the host plant cell for synthesizing the virus-encoded proteins at high levels like that of coat proteins. A wide range of human enzymes, antimicrobials, cytokines, subunit vaccine components, and immunoglobulin fragments have been produced using a Gene ware expression system.

Magnicon System: This system comprises a distinct minimal virus approach for using a tobamovirus-based vector system for the transient expression of heterologous proteins in permissive hosts by eliminating their systemic functions [24]. Magnicon-based vectors consist of a genetic deletion system to delete the genes corresponding to movement protein (MP) and coat protein (CP). The gene that encodes for pharmaceutical protein is under the control of endogenous coat protein-based sub-genomic promoter for enhancing the capacity to express larger proteins. Magnicon uses an agro-infiltration system to introduce plant viral expression systems as intact vectors or distinct modules containing specific genes or genes of interest. Assembly of the components in the host plant through a distinct module and transcription of the concerned DNA leads to splicing and translation of mRNA resulting in high yields of the expressed protein [11]. Numerous proteins, including cytokines, interferons, bacterial and viral antigens, growth hormones, single-chain antibodies, and mAbs, have been produced using the Magnicon system at 1-10g/kg of plant material. *Nicotiana benthamiana* is an ideally suited plant system for the Magnicon expression.

Products Developed: The main aim of molecular pharming is to produce beneficial products for human society. It is an upcoming and recent technology used to mass-produce therapeutic recombinant substances [6].

Aprotinin: Aprotinin is a 58 amino acids serine protease inhibitor processed from its pre-protein precursor. Clinical implications of aprotinin have been explored for its prophylactic applications in reducing perioperative blood loss and minimizing the need for blood transfusion in patients undergoing cardiopulmonary bypass surgery [25]. However, due to the increased risk of in-hospital deaths, the consumption of aprotinin has been suspended. Despite these adverse events, aprotinin is a prophylactic and therapeutic drug as an alternative active pharmaceutical ingredient to bovine tissue. Several groups have expressed and purified recombinant bovine aprotinin (r-aprotinin) from transgenic corn, duckweed, and tobacco plants with varying yields of 0.17%, 3.7% and 0.5% respectively [6, 27, 28]. Further, purified aprotinin from these transgenic plant sources has a comparable protein size with trypsin inhibitory activity.

Monoclonal Antibodies (mAbs): Monoclonal antibodies represent one of the fastest-growing biopharmaceutical markets, therapeutically used in different areas, including infectious disease, oncology, inflammation, allergy, and cardiovascular disorders [29]. Monoclonal antibodies block the entry of viruses into the cells and prevent infection. CCR5 acts as a co-receptor for HIV-1 entry into human cells. The microbicide that blocks the CCR5 may serve as a strategy for preventing HIV-1 sexual transmission [30]. In 1989, the antibodies were produced and properly folded in plants were demonstrated [31]. However, their production levels were low, as demonstrated in transgenic tobacco plants with 25 mg/ Kg plant tissue [32]. Transient plant expression using Magnicon will considerably reduce the time and cost-efficiency of agriculture production of monoclonal antibodies.

Applications of Molecular Pharming: The human serum albumin was the first recombinant plant-derived protein introduced into transgenic tobacco [3]. At the onset of the new century, the proof to produce many antibodies, blood products, and hormones was patented and was established industrially [33]. By 2003, various plant-derived products were manufactured by multiple plant biotechnology companies that had made their way to the industrial market, used to cure many diseases [33]. Molecular pharming has made its value by bringing many products towards the welfare of society. Bio pharming is also known as the production of plant-made pharmaceuticals, a subsector of the plant biotechnology industry involving genetically modified plants that produce many therapeutic proteins and secondary metabolites [34]. Tobacco has a long history and is an established model system for biopharming. As an alternative to nuclear transgenics, transplastomic tobacco plants have been developed by introducing the desired gene into the chloroplast genome of tobacco. Human growth hormone, serum albumin, tetanus toxin fragment, and cholera B toxin subunit was produced by gene transfer through particle bombardment. One major disadvantage is that chloroplast does not perform glycosylation.

Arabidopsis thaliana is often used as a trial organism for a novel process. At the same time, the actual manufacture of valuable products is carried out on commercial plants such as rice, wheat, potato, *etc.* [35]. Many non-crop plants such as *chlorella* and *Physcomitrella patens* have recently exhibited their potential in producing biopharmaceuticals. The preference of these plants is due to the ease of their utilization in bioreactors. They also reduce a substantial burden of protein purification during the preparation of recombinant proteins [36]. Molecular pharming is gaining more attention with developing novel bioreactors to produce complex compounds using both economically and non-economically hardy crop plants [37].

In modern molecular pharming, plant viruses are incorporated into the plant system and the gene of interest to manufacturing desirable products. Plant viruses such as cowpea mosaic virus (CPMV) were initially used for recombinant protein expression [38]. In an approach to make a specific hybrid virus by combining the genomes of two viruses through homologous recombination and eventually making it genetically and complexly stable [39]. Plant viruses that were first exploited in molecular pharming did not own an envelope and possessed specific subunits that could assemble into filamentous structures by themselves, like the Tobacco mosaic virus [40].

Most commercial crops do not hold any pathogenic agents that may be lethal to human health and well-being, and there is no similarity between the proteins found in plants compared to those of humans. Also, their seeds act as sterile and undisturbed pockets of characters and genetic information that have a long shelf life (Molecular farming, plant bioreactors). In the present situation of the rising population, the demand for pharmaceuticals globally is at a very high level. Molecular pharming was introduced in developing countries by the World Health Organisation (WHO) to make edible vaccines and other valuable products on a pilotscale because these are very cheap in the market and can cure diseases very quickly [33].

Limitations of Molecular Pharming: Molecular Pharming was developed in the modern era to produce pharmacologically related compounds beneficial to humans. However, there are some limitations to this process. Molecular Pharmingin plants are used to develop plant-derived metabolites beneficial to human society. These metabolites pose some special and unique problems and hindrances in bio-safety regulation and affiliation by the government to introduce them into the market [1]. These hindrances arise during the cultivation of plants in an open region, where other crops and plants in the surrounding area are more vulnerable to contamination by various means such as pollination either by insects or by wind [19]. To evade this situation, plants producing the metabolites must be cultivated under specific conditions, isolated from other crop plants but are still within reach of soil and sunlight for maximum growth [19]. Another aspect of these plants being grown in open areas is the flow of genes from the genetically modified crops into other crop plants, leading to weeds and affecting the land [19]. There is also a chance that the pollen of these crops could affect the surrounding commercial crops by

changing their genome and causing various abnormalities [41]. Some safety precautions can avoid these possibilities. Genetic Use and Restriction Technologies (GURT's) are applied to prevent pollen contamination between genetically modified crops and typicalcrop plants [42]. Another major limitation for biopharming is the cost feasibility of developing equipment required for molecular pharming in countries where the foundation of these modern techniques is not laid. It will take a lot of money and labour to begin such new trends in developing countries [43]. In molecular pharming, therapeutic proteins produced are very cheap and affordable. However, the primary and initial tests and experiments are very costly to conduct in special labs in specific conditions and take much time before a particular product obtained from molecular pharming can reach the pharmaceutical market [44].

CONCLUSION: Molecular pharming is an emerging stream in the biotechnology sector and has an enormous scope of innovation, knowledge gain, research, and commercial value in this growing society. It is the best method to produce recombinant proteins and other industrially valuable compounds. This technique is also economically feasible after the initial establishment and standardizations.

Plant gene manipulations and pilot-scale expression of these genes in the desired plants to generate desired recombinant proteins are highly modular and performed at a reasonable time and price. These reasons have attracted many plant biotechnologists and industries facilitating its establishment. There is a concern about contamination of desired genes using external agents such as viruses and other pathogens. There is also a concern about developing genetically abnormal crop plants due to the pollination of genetically modified crop plants. The downstream processing of these recombinant proteins can be achieved effortlessly within a short period.

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