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Review Article

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Review on the Therapeutic Protein Production in Plants

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ABSTRACT

The production of protein therapeutics in plants it is great potential for increasing, improving and developing the number of therapeutic protein production, therapeutic protein help for the prevention of diseases and treatments in animals and human transgenic plants are the most promising system for the production of a human therapeutic protein. The glycoproteins produced from plants are not the same as a native therapeutic proteins produced in mammals. But using the plants has more advantages such as the low cost and the large scale production is safe. Therefore biological active plant protein has become an alternative option to animal cells for the production of the therapeutic protein.

Key-words: Glycoproteins, Genetic engineering, Recombinant, Therapeutic, Transgenic

INTRODUCTION

Therapeutic protein engineered in the laboratory therapeutic protein is a protein-based drug. Protein has a role in cells it has a defence mechanism, when the organism is against invaders therapeutic recombinant protein is the exogenous protein that is expressed in a production organism. These therapeutic proteins are manufactured by using the cell culture, fermentation process, transgenic plants and animals. Monoclonal antibodies are the most promising therapeutic recombinant protein by the hybridoma process [1].

Therapeutic protein production platforms are at high levels of scalability and synthesis of complex protein. Protein cultivation technology has been promoted in the last few decades to scale up the cost-effective therapeutic protein yield and has broad applications in different divisions of the food and chemical industries.

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Naturally, these commercially important protein products are also synthesized from plant species, which seems to be having a big advantage over other platforms [1]. Recombinant technology is also utilized to produce therapeutic proteins; it is natural resources based technology including crop plants.

Most common host bacteria cells like *E. coli* is utilized to produce therapeutic proteins *via* fermentation or it can be possible by using *a* Chinese hamster ovary (CHO) cell lines culture. Nowadays therapeutic protein and industrial enzymes are possible to produce *via* plant tissue culture techniques. [2]

B-glucocerebrosidase production is one of the good example of commercially important protein products that plant cell-based protein product that is; used orally for the treatment of Goucher disease in comparison with other system plants cell and tissue culture have advantages including performing post-translation modifications, low cost of production, Environmental conditions, low possibility of being infected by herbicides, pesticides disease [2].

In this context, another example of therapeutic recombinant protein production from potential and newer tobacco species selection becomes a great achievement in terms of the yield of protein products,

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especially in the pharmaceutical sector. Application of these protein products used as a beneficiary agent against harmful diseases like hepatitis, cancer, diabetes etc [3]. The big advantage of using the cost-effective and optimized growth condition to produce pathogen and toxin ingredients free therapeutic protein production but it has disadvantages associated with regulatory compliance and limited glycosylation capacity [4].

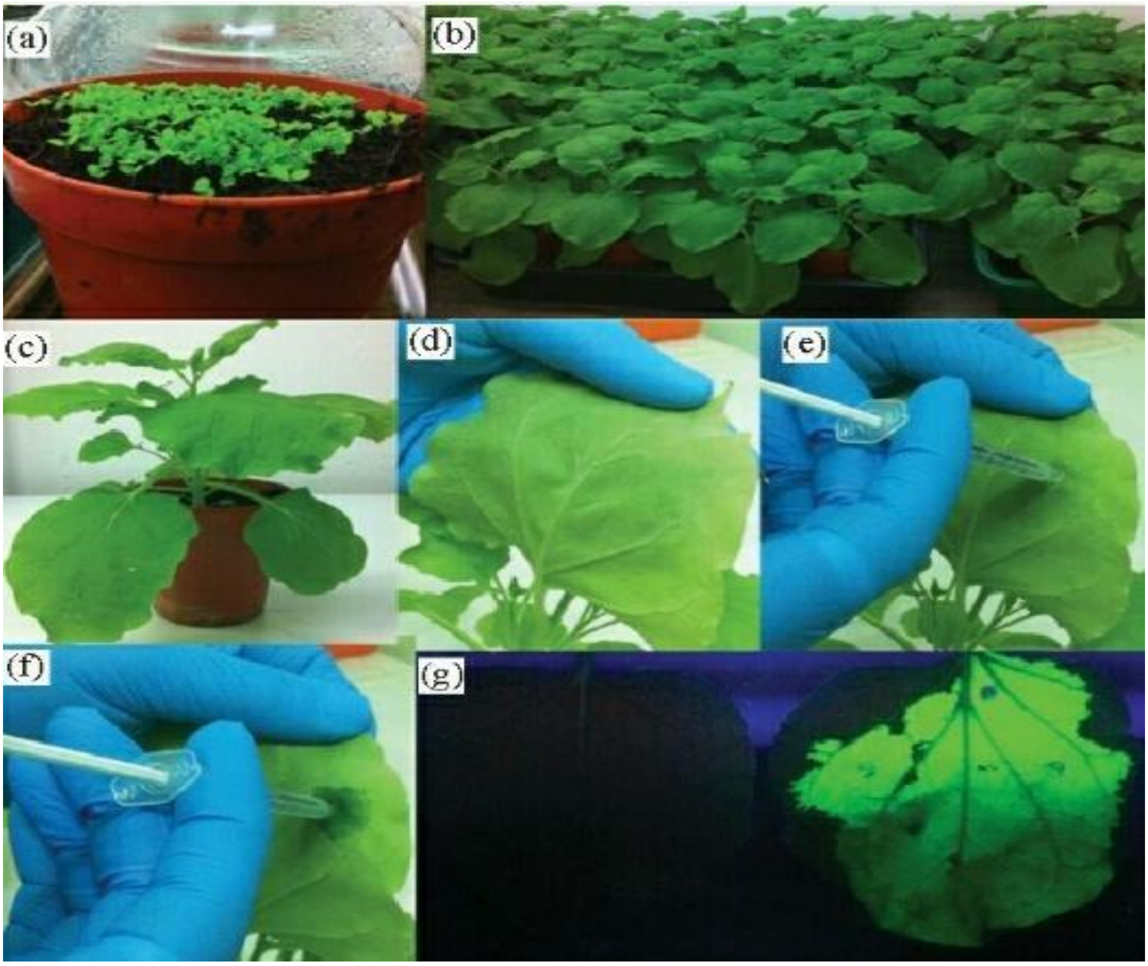

Fig. 1: Produced in plants suspension cell culture [5]

Plants suspension cell culture- Therapeutic proteins include cytokine, antibodies, and vaccines. Of these, the most important recombinant proteins are signalling agents are cytokines. Commercially cytokines produce via using a cell culture-1990 media. Some researchers used cultured human serum named "alumni" for therapeutic protein production formation from tobacco cells [6].

Cytokines, growth hormone and growth factors- Cytokines are secretory proteins, produced by immune cells. Cytokine a signalling molecule, which regulates the innate and acquired immune systems. Interleukin is an important subcategory of cytokines, *eg;* HIL-12 (*Human interleukin-12*) is expressed in rice suspension cell culture media and HTL-10 (*Humaninterleukin-10*) expressed in tobacco BY-2 cell has been reported earlier. Uses of hairy root culture in *Nicotiana benthamiana* as a host system have been used for a long time due to the genetic stability for the production of proteins [1,4].

Therapeutic Enzymes- Transglutaminase is an enzyme that is prescribed for coeliac disease treatment. Acetylcholinesterase is another good example, produced through the hairy root culture of *N. Benthamiana* [6].

Antibody- The usage of monoclonal antibodies as a passive immunization antibody against foreign invaders is one good example of industrial biotechnology. These monoclonal antibodies act against specific foreign antigen-antibody is administered directly as a therapeutic agent in infected individuals. Example monoclonal antibody effectively used against breast cancer, and multiple doses of Herceptin transgenic plant have been used a nowadays for the production of antibody and to prevent dental caries, anti *Streptococcus*

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mutants antibody is a most advanced product to date [7]. Various pathogens have been expressed in the plant from protein antigen to produce an immune response in protection against diseases in humans [8].

Other protein- Milk proteins such as *Lactoferrin* and *beta-casein* are produced in the host plant. Expression of *thioredoxin* in feed materials such as cereal grain would reduce their allergenicity and increase the digestibility of protein. According to recent reports, more than 370 therapeutic proteins are developed and affecting their yielding capacity. It also concludes from these reports that it also experiencing serious stress on mammalian cells utilized to produce recombinant proteins. This can be possible through proteolytic processing, which made an important contribution to pharmaceutical science [9,10] implication of glycosylation specific to plant species. The selection of potential plants for the production of therapeutic proteins has become a well-established strategy [11]. In this respect, transgenic plants were showing a great potent option for the production of therapeutic protein at a wide-scale level [10]. It can lead to enhanced therapeutic efficiency [12]. A combined humanized glycosylation pathway has a role in the discovery of terminal dislocated acid in plants [13].

Functionally expressed antibody was identified through the antibody assay in infected plant leaf extract [14]. Plant-based vaccine reflects the potential to develop enhanced vaccine coverage in children and infants [15] against disease pathophysiology targeting both *in vitro* and *in vivo* antioxidant activity. This assay also contributes to attributing more precise therapeutic value to plants and individuals [16].

The world is facing a growing demand for diagnosing therapeutic protein[s] [17]. The quality and quantity of human recombinant protein produced from transgenic tobacco plants [18] were determined in terms of; the high yield of human therapeutic protein in tobacco [19]. A high yield in tobacco sp. was demonstrated that protein per green leaf biomass is 3-4 times harvested per year [20].

The protein present in transgenic plants is used to produce vaccines from antigen-specific antibodies in serum [21,22]. The majority of therapeutic proteins are produced from bacteria or mammalian cell lines [23]. Many plants are also used to produce a variety of enzymes and proteins, which are utilized against different types of diseases by using transformation in tissues [24] through different genetic modifications of the nucleus, chloroplast and mitochondria [25]. For diagnosis and treatment, different transient genes are involved, which are expressed in the plant and purified these protein[s] [26]. The therapeutic new drugs displayed prominent growth in the market as it approved against diseases [27]. The range of recombinant therapeutic proteins produced in plants includes vaccines, enzymes, and monoclonal antibod[ies] [28]. Foreign protein can be produced in the plant through the regulation of the strong promoter site of *cauliflower mosaic virus* 35 SRANA when the protein-encoding gene is introduced into the plant [29]. Through this regulation protein production time significantly reduced as transient gene expression improved which increased the protein yield [30,31]. The plant cell culture bioproduction system makes it the current biopharmaceutical producer of the whole plant system [28,32]. Therapeutic protein productions are a highly complex process and manufacturing patients most in need of Novak therapies therapeutic protein drug is an important class of medicine serving patients [33]. The USA and other European countries approved more than 300 types of protein-based medicines their functions are determined by the sequence and number of amino acids [34]. Various expression systems, including bioprocess of upstream and downstream recombinant proteins[35]. In 1982 Humulin was first approved recombinant biopharmaceutical through the food and drug administration [36].

Plant breeding includes another method to increase transgene copy number and protein accumulation suited to utilized germplasm [37]. These proteins may be used in the future for therapy or prevention of viral, bacterial or cancer diseases [38]. The first phase 1and 2 clinical trials of a plant-derived therapeutic compound from suspension culture of carrot cell [39]. Chloroplast is a highly efficient vehicle for the potential production of pharmaceutical proteins in plants [40]. Manufacturing high-quality glycoprotein is thus becoming an imperative expression system allowing efficient manufacturing [41]. Pharmaceutical proteins are (e.g. antigen comprising individualized vaccine to treat Non-Hodgkin Lymphoma patient) therapeutic antibodies such as pharmaceutical proteins [42]. In the treatment of chronic diseases, recombinant protein pharmaceuticals are widely used extraction of recombinant protein from *Nicotiana* leaf tissue can be achieved by the collection of secreted protein fractions [43,44].

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Recombinant antibodies, antibody fragments, and antibody fusion protein have become the most common product expressed in plants [41]. The heterologous protein of bioproduction of the technique to genetically modify crop plants has gained more and more interest in plants and has been a possible source of a large amount of cost-effective recombinant protein [45].

Some plants like marigolds have a lot of therapeutic proteins or enzymes to work against many diseases like otitis media infection, cancer, ulcer, skin disorders etc. Apart from this, marigold also produces some therapeutic enzyme which is used to formulate sunscreen (SPF values) [46-48].

This review highlights the advantages and recent progress in plant cell culture technology and the economic feasibility of plant cell-based protein production levels for the engineering processes [49].

Table 1: Advantages and disadvantages of therapeutic protein production in plants [44]

Expression System	Advantages	Disadvantages
Bacteria	Easy to manipulate Low cost High expression Ease to scale up Short turn around time Established regulatory procedures and approval	Improper folding Endotoxin accumulation Lack of post translational modifications, which affects the protein functions.
Yeast	Easy to manipulate post translational modifications of recombinant proteins Rapid growth and scalable Simple and inexpensive media requirements and culture conditions	Difficult in cell disruption due to hard cell wall Hyperglycosylation of proteins
Mammalian cell	Proper folding and authentic post translational modifications Existing regulatory approval	High production cost Expensive media and culture condition requirements
Insect cell	High expression level Proper folding and authentic post translational modifications Ability to produce complex proteins including secreted membrane and intracellular protein	Expensive media and culture condition requirements High cost Time consuming
Plant	Post translational modifications similar Economical Rapid and affordable Optimized growth conditions Free from pathogen and bacterial toxins contaminants	Regulatory compliance Limited glycosylation capacity

Methods for the production of therapeutic proteins- Therapeutic protein production in plants has many methods, the GURT method, Gene transfer method, Partial bombardment, expression, and agroinfiltration method, Gurt method, Partial bombardment, gene transfer method, etc. Those methods are used to produce therapeutic protein in plants [50].

The two methods used for the gene transfer in plants are-

1. Victory mediated gene transfer
2. Vectors DNA transfer

Each type of expression system has been used for the production of therapeutic compound expression types are-

- Nuclear expression
- Chloroplast expression
- Transient expression
- Suspension cell

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Nuclear Expression- The expression of a foreign antigen from the nuclear genome introduces into the plant using biolistic gene gun mediated transformation for secretion or organellar storage pipes are used to target [45].

Chloroplast Expression- Using the particle gun method, an introduction of a transgene into the chloroplast genome. The therapeutic protein production is completely free of the glycosylation process which does not occur in the chloroplast [46].

Transient Expression- The protein expression in the host plant involves transgenic technology that offers a unique combination of rapid expression [45]. The agroinfiltration method involves infiltration of recombinant suspension of *A. tumefaciens* leaf tissues under a vacuum. Using this method, the amount of protein (mg) has been increased within a week [49].

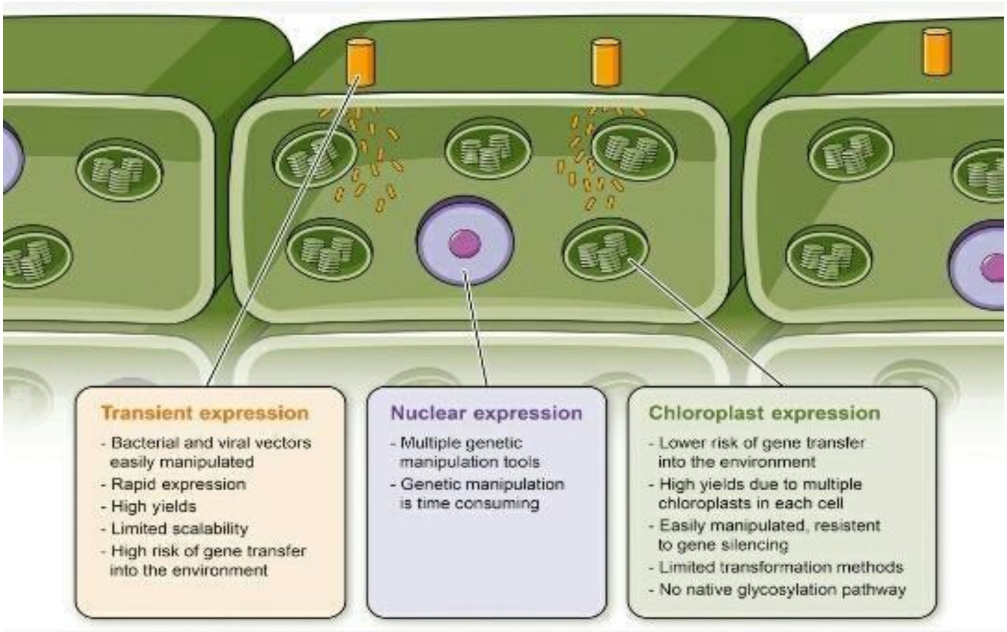

Fig. 2: Transient Protein expression [48]

The plant produces larger therapeutic protein than bacteria plant proves to be the ideal in-between system it has more scalable and cost-effective than the system of the mammalian cell as compared to both systems it helps to reduce the risk of toxic contaminants and pathogens.

Therapeutic Protein production platforms are a high level of scalability and synthesized complex protein, cheap growth conditions existing industry infrastructure the potential for rapid production time scale and human pathogen contamination has low risk the potential market for therapeutic protein is huge with products from enzyme to vaccine and antibody to hormone each type of therapeutic protein has its production challenges. In the last decade therapeutic-based protein has attracted more and more attention for its application in therapy and diagnosis .in the market 94 proteins of human therapeutic Value entered. Different plants are used to produce this type of protein such as Tobacco and marigold.

In the case of ornamental plants marigolds also encompass the higher levels of therapeutic proteins [51,52]. Plant cells as a eukaryotic system pose all the features for the biologically active protein. Plants are the safe host for therapeutic protein and peptide production the specific strength of the plant expression system facilitates the production of therapeutic protein quickly.

CONCLUSIONS

Due to increasing the demand for commercial vital therapeutic proteins in the world market, choosing a plant good expression system is considered to be promising option for the safe, and quality production of a therapeutic protein. In this respect, transgenic plants as a host expression system act as a promising choice and could be a good option to produce cost-effectiveness and reduce the chance of risk of contamination, and easy storage as compared to transgenic animals. Adopting these transient platforms could be a preferable and selective strategy for the rapid

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production of large amounts of therapeutic recombinant protein especially in critical situations. This futuristic technology avoids unwanted results and gathered so many optimistic exceptions.

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Writing article- Shiksha Dwivedi
Critical review- Yashodhara Verma
Article editing- Shiksha Dwivedi
Final approval- Yashodhara Verma

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