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Production of Monoclonal Antibodies in Transgenic Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Author YB managed the literature searches, organized and wrote the primary manuscript. Author PM involved in proof reading the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Antibodies are one of specialized immune protein and innovative therapeutic agents that provides effective alternatives to treating various human diseases. The existing fermenter based process of production of great value antibodies that have biomedical importance is costly, tedious and low yield obtained via purification process. So far different plant types such as vegetables, cereals and legume plants were used to produce important therapeutic proteins. Expression of antibody in transgenic plants might be a solution to successfully scale up therapeutic antibodies, and lower the production costs. This is due to cheap production cost of plants and large amount of yield would be obtained. This review aims to high light on the possibility and comparison to efficiently produce antibody in different plant organs. Also the review extends to over view the role of plants as a flexible expression system for antibody production, which we foresee to progress alongside the production platforms to manufacture specialized antibodies using transgenic plants.

Keywords: Antibody; expression system; innovative therapeutic; transgenic plants.

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1. INTRODUCTION

Antibodies are a specialized immune protein that are produced because of the introduction of an antigen that cause infection or diseases into the body. Specific high quality antibodies against one specific epitope, called monoclonal antibodies (mAbs). Monoclonal antibodies can be produced using hybridoma technology, involving the fusion of spleen cells from an immunized mouse with immortalized myeloma cells [1]. Today, this platform contributes to some of the major demands of monoclonal antibodies in research and diagnostics. However, for therapeutic application, the use of mouse monoclonal antibody is discouraged due to immunogenicity, lack of effector functions and short serum half-life. Hence, for human application, humanized or at least partially humanized antibodies are needed [2].

1.1 Importance of Antibodies in Innovative Therapy

The *in vitro* technology for the development of human monoclonal antibody directly from a single B cell derived from an ampule of peripheral blood mononucleocytes has been well established by sequestering the specific antibody encoding genes using state of the art molecular biology tools [3].

Antibodies are one of innovative therapeutic agents providing effective alternatives to treating various human diseases. So far variety of antibodies are produced for treatment of multiple sclerosis, lymphoma, variety types of cancer (carcinomas of the breast, ovarian, lung, colon, and pancreatic cancer) and squamous cell carcinoma of the head and neck, immunity therapy, autoimmunity and inflammation. The innovative therapy with antibodies has proved be very effective against acute antibody-mediated rejection of organ transplant. Humanized monoclonal antibodies such as Herceptin and Rituxan have been very clinically significant in the treatment of breast cancer and non-Hodgkin's lymphoma treatment respectively [4,5].

Therefore, antibodies should be synthesized and supplied in large amount in repetitive form to help prevention from infection. For many years, mammalian cells such as Chinese hamster ovary cells were the only viable platform for complex human proteins such as glycoproteins, and naturally the regulations for drug manufacturing had developed [6]. However, the production system yields increased about 5–10 g/L can be achieved in commercial antibody manufacturing processes. However, stable transformation of mammalian cells is a lengthy process. As an alternative, transient expression with viral promoters has empowered production of hundreds of milligrams of antibodies [7]. Though the existing fermenter based process of production of great value antibodies that have biomedical importance is costly, tedious and low yield obtained via purification process.

The major focus of antibody engineering in the past three decades has been to reduce immunogenicity and to improve production. With recombinant engineering technology and understanding of disease biology and mechanisms of action of antibodies, an array of novel classes of antibody molecules are emerging as promising new generation therapeutics [8].

The current manufacturing technology for antibodies can be divided in development and optimization of upstream and downstream processing. [9] reported that development of technological platforms were possible in process development and consequently in manufacturing of monoclonal antibody. Downstream processing practices and platform technologies will need to advance to keep pace with increases in cell culture titers, increasing regulatory requirements and the need to control costs. Downstream processes must be adapted to cope with higher titer cell culture processes and advances in expression systems in existing plant infrastructure [10].

Expression in transgenic plants might be a solution to effectively scale up therapeutic antibody, and lower the production costs. This review aims to extrapolate different plant type uses as a flexible expression system for antibody synthesis. In addition, it visualizes the progress alongside the characterization and production platforms to manufacture specialized antibodies in plants.

1.2 The State of Art in Production Antibody

Promisingly, the current molecular techniques in the field of in planta expression have enabled high-level production of a variety of antibodies in different plant organs, like roots/tubers/fruits, leaves and seeds, of a variety of plants providing

a very wide range of possible plant-based therapies.

Plants have successfully been used for the production of different proteins for therapeutic and technological applications [11]. Plants could potentially be developed as promising biofactory systems for the large-scale production of antibodies [12]. This is due to high production capacity, is inexpensive to cultivate on a large scale, easily scalable, low downstream processing requirements, can be grown under containment conditions, and avoidance of ethical problems associated with transgenic animals [13,14]. It has been estimated that for plant-based antibodies expressing up to 1% of total soluble protein, the production cost would be 0.1% of that of the mammalian cell culture system and up to 2-10% of that of microbial systems [15].

Another advantage is that many plant species regarded as safe status, since they do not contain mammalian viruses or pathogens, or produce endotoxins. Despite showing a potential antibody production in plants may bring allergic reactions to plant proteins that is incapability of human N-glycosylation, culture parameter being uncontrollable, and risk of contamination (soil, bacterium and pollen contamination [16,17].

Modification of glycans of antibodies has also been achieved in comparative expression systems like mammalian cell cultures, but it has been seen that glyco-engineered plants have a much higher degree of glycan homogeneity [18, 19]. The higher degree of desired glycosylation demonstrated in case of h-13F6, an anti-Ebola virus monoclonal antibody which can lead to higher product quality and clinical efficacy [19, 20]. The plant-derived version of h-13F6, bearing the complex N-glycosylation and devoid of the core fucose, showed higher potency than the original version derived from mammalian cells.

Thus, plants are considered to be a potential alternative to compete with other systems such as bacteria, yeast, or insect and mammalian cell culture. Plant production systems, particularly therapeutic antibodies, are very attractive to pharmaceutical companies to produce the antibodies in demand.

2. SELECTION OF PLANTS FOR ANTIBODY PRODUCTION

The selection of plant species should be carefully considered for successful production of antibodies, since each plant species has its own physical and physiological characteristics affecting the expression and glycosylation of recombinant glycoproteins [21]. Even though plant tissue have innate capacity to assemble complex antibodies, plants do not have sialic acid residues on their glycan structures, which is essential for glycoprotein stability [22].

2.1 Leafy Plants

Among leafy plant species tobacco and alfalfa have been developed for production of antibodies. The first proof of concept for functional antibody production in plants was provided in 1989, when two transgenic tobacco plants, each expressing light or heavy chains, were produced by Agrobacterium-mediated transformation of tobacco leaf discs [23]. Crossing these two transgenic tobacco lines led to the expression of assembled functional IgG antibodies, accumulating up to 1.3% of total soluble protein.

In tobacco, the main advantages are the high biomass yield and the rapid scale-up by high-volume seed production compared to other plant species [21]. Transient expression process yields as much as 4 g of recombinant protein (green fluorescent protein) per kilogram of fresh leaf biomass in *N. benthamiana* and up to 2.5 g/kg of tobacco (*N. tabacum*), can be applied to other plant species [24,25].

In current report, the level of expression recombinant proteins in tobacco stems was similar to that of leaves, thus suggesting that the whole tobacco plant biomass can be used for production of recombinant therapeutic proteins, eventually increasing the upstream production cost efficiency [26]. In addition, tobacco is a non-food, non-feed and well characterized as an expression system excluding human pathogen contamination, which can attenuate biosafety concerns. One of the most successful examples of a tobacco-made anti-body for passive prophylaxis is the secretory antibody CaroRx™ (www.planetbiotechnology.com). The secretory antibody is used for the prevention of dental caries in European Union [27].

The other leafy plant have been used for production antibody is alfalfa which has comparative advantage over tobacco plant. According to [26], tobacco contains nicotine or other toxic alkaloids desires additional extraction procedure and tobacco produces

heterogeneously N-glycosylated antibodies [28]. Also alfalfa has a high yield of biomass and a homogeneous glycan structure [29]. Although alfalfa contains oxalic acid compounds, which affects downstream processing and produces lower amounts of leaf biomass than tobacco, the high protein level in alfalfa leaf tissues maximizes accumulation of recombinant antibodies per plant biomass. However, alfalfa is used as animal feed though it raises biosafety concern. The major drawbacks of these leafy crops comes from the storage and distribution of active and complex metabolism leaf product, high protease activities toward degrading certain proteins which is instable unless the leaf tissue is frozen or processed [30,31,32]. Seeds contain a low level of proteases that allows proteins to be stored without degradation [33]. This experiment suggested that seeds can be used as bioreactors and as natural storage organs.

2.2 Cereal and Legume Crops

Alternatively, cereal crops and legumes such as maize, soybean and rice have been used for antibody production in their seeds. Maize is favoured amongst the major cereals used for antibody production because of its high biomass yields. However, as maize is a wind-pollinated species, there is a risk of outcrossing to food crops. Antibodies expressed in corn seeds are stable at room temperature for more than three years without loss of activity [14].

The human immunodeficiency virus-1 (HIV-1)-neutralizing activity 2G12 antibody is expressed in maize, which could facilitate inexpensive, large-scale production and in vitro cell assays demonstrated that the HIV-neutralizing properties of the maize-produced antibody were equivalent to or better than those of its Chinese hamster ovary cell-derived counterpart [34]. Thus, seeds are advantageous in terms of the cost of grain storage and distribution. In contrast to leafy crops, after harvesting of corn seeds for antibody extraction and purification might be higher than plant leaf materials therefore a well-established food-processing facility may be able to rapidly start up down-stream processing [35].

Among crops, rice is particularly attractive for production of antibody as shown on Table 1. This is due to high grain yield and stable protein storage, glycosylation makes high flexibility of protein production, the risk of unintended gene flow is minimal compared with other crops [13, 17]. Even though rice is food crops it has peculiar advantages over other plants for production of therapeutic antibody, hence it has been developed for the process of being commercialized. Transgenic rice seed have been produced for production and delivery vehicle of oral tolerogens that can be used to target type I allergies. [36] evaluated oral tolerance of antibodies using mice fed with transgenic rice seed containing allergen specific T-cell epitopes fused to cholera toxin B (CTB) as a mucosal carrier has developed oral tolerance and showed suppressed allergens. HIV-neutralizing antibody 2G12 have been expressed and produced in the endosperm of transgenic rice plants [37].

Table 1. Comparison of different plant species expression system for antibody production

Expression system	Protein expression	Glycosylation	Storage cost	Generation of trans formats	Downstream processing / purification	Protein yield
Leafy vegetables	**In leaves**					
• Tobacco	Medium	Heterologous	High	Easy	Medium	High
• Alfalfa	High	Homologous	High	Medium	Difficult	Medium
• Chinese cabbage	Medium	Heterologous	High	Medium	Difficult	Medium
Cereals and legume	**In seeds**					
• Maize	High	Heterologous	Low	Difficult	Medium	High
• Soybean	Low	Heterologous	Low	Difficult	Medium	High
• Rice	High	Heterologous	Low	Relatively easy	Easy	Very high
• Rape	Low	Heterologous	Low	Difficult	Medium	High

Therefore, effective production of antibodies requires the appropriate plant expression system with optimal combination of transgene expression regulatory elements, control of post translational protein processing, and efficient purification methods for product recovery. The yield of functional antibodies is the first standard to be considered for the choice of plant species.

3. TRANSFORMATION AND REGENERATION PROTOCOL OF PLANTS

There are different transformation protocols are adopted for transformation of plants to produce antibody as indicated on Fig. 1. Among transformation protocol whole plants which can be leafy or cereal plant could be transformed either stably or transiently [38]. Insertion of coding sequence for antibody can be done with the help of biological vector agrobacterium or particle bombardment method [35]. The gene can be inserted in to the chloroplast genome to generate chloroplast transgenic plants expressing and properly folding antibodies with disulfide bonds. Thus, if the proper glycosylation is potentially built in the chloroplast, the chloroplast transformation might emerge as a potential stable expression system for anticancer antibodies [39]. The positive transformants were grown under containment greenhouse conditions. The transgene i.e antibody gene insertion and presence of selection marker gene (hygromycin resistant) plants will be confirmed by PCR and transgene copy number determined by real-time qPCR.

Plant expression system for production and expression antibody coding gene could either transiently expressed or stably expressed. The expression system may involve direct mechanism of gene insertion or organelle transformation mechanism in different organ of plants.

4. CHARACTERIZATION AND PURIFICATION ANTIBODY

In a plant expression system, the plant tissue and cells should be disrupted to release antibodies for purification since antibody is expressed and localized within the cells. In addition, antibodies should be recovered with a removal of cell debris and contaminants. Molecular, immunological and biochemical analyses methods were used for characterization through comparing to its equivalent native protein that is on direct therapeutic use. Currently, for purification of antibody expressed in plants used protein A- or G-based affinity chromatography [40]. Transgene or antibody gene insertion is validated in the regenerated plants by PCR analysis using leaf genomic DNA as the template [41]. Plants transformed with empty transformation vector may be used as control.

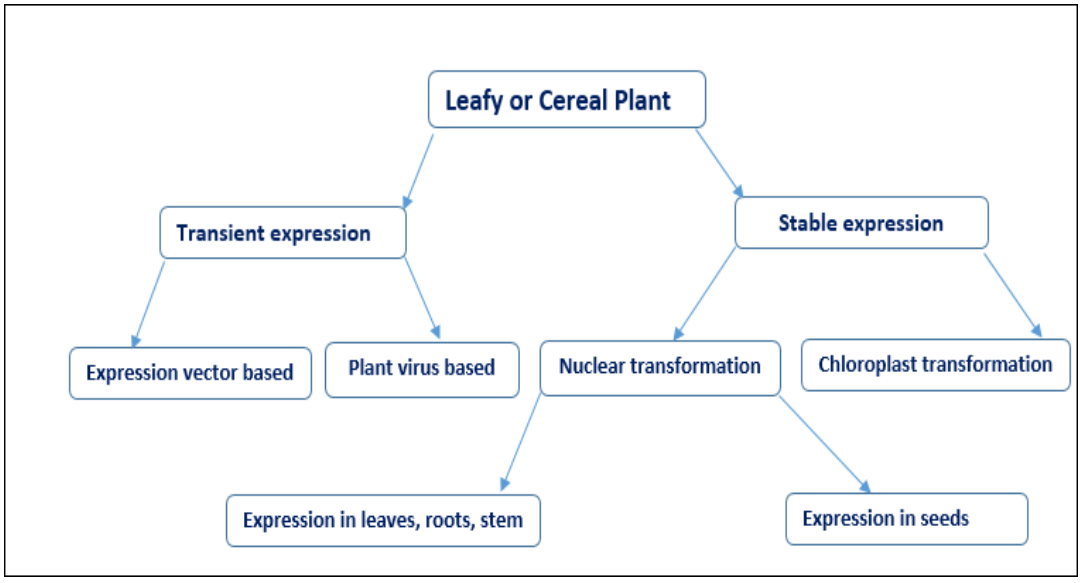

Fig. 1. Different transgenic expression system and protocols

5. CONCLUSION

Plant production systems for therapeutic antibodies are very attractive and have advantage to pharmaceutical companies to produce the antibodies in demand. However, there are only a few antibodies that have made it through the clinical phase. One of the main reasons for this delay in bringing plant-made therapeutic antibodies to the market could be the regulatory issues associated with usage of food crops for antibody production may raise concerns about environment or biosafety issue [14]. As plants for pharmaceutical protein production, non-food and non-feed plants might be the choice to avoid transgene contaminants. There is no ideal choice of plant species since each of the plants has its advantages and disadvantages. Thus, the choice of host plants should be carefully determined. Though, things are now changing mostly due to the development of expression technologies that have enabled transformation of different plant species, some of which can grow fast and produce large amounts of biomass in a short time. Moreover, the advantage of plants not harboring mammalian pathogens, the expression vectors have been improved, which enables high levels of antibody production from a reasonably small plant biomass. There is hope that in the near future the full potential of plants as a cost-effective platform would be realized.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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