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Research review paper

Tobacco, a highly efficient green bioreactor for production of therapeutic proteins

Reynald Tremblay [a], David Wang [b], Anthony M. Jevnikar [c], Shengwu Ma [a,c,d,*]

[a] Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7
[b] Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014, USA
[c] Transplantation Immunology Group, Lawson Health Research Institute, London, Ontario, Canada N6A 4G5
[d] Plantigen Inc., 700 Collip Circle, London, Ontario, Canada N6G 4X8

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ABSTRACT

Molecular farming of pharmaceuticals in plants has the potential to provide almost unlimited amounts of recombinant proteins for use in disease diagnosis, prevention or treatment. Tobacco has been and will continue to be a major crop for molecular farming and offers several practical advantages over other crops. It produces significant leaf biomass, has high soluble protein content and is a non-food crop, minimizing the risk of food-chain contamination. This, combined with its flexibility and highly-efficient genetic transformation/regeneration, has made tobacco particularly well suited for plant-based production of biopharmaceutical products. The goal of this review is to provide an update on the use of tobacco for molecular farming of biopharmaceuticals as well the technologies developed to enhance protein production/purification/efficacy. We show that tobacco is a robust biological reactor with a multitude of applications and may hold the key to success in plant molecular farming.

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1. Introduction

The worldwide demand for recombinant proteins is growing faster than traditional systems can keep pace. This includes valuable pharmaceutical proteins such as antibodies and vaccines as well as industrial enzymes and secondary metabolites. Traditional recombinant production systems such as bacterial and mammalian cell culture are limited in their scalability and production cost, due in part to requirement for complicated fermentation equipment and expensive downstream processing. Recombinant protein production in plants, on the other hand, offers a solution to the rising demand and provides opportunities that are not feasible with other systems.

Plants offer several advantages as "Green Bioreactors". First is the ability to perform eukaryotic post-translational modifications such as glycosylation and disulfide bridging that are often essential for biological activity of many mammalian proteins (Ma et al., 2003;

* Corresponding author. Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7. Tel.: +1 519 685 8500x75855; fax: +1 519 432 7367.
E-mail address: sma@uwo.ca (S. Ma).

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Horn et al., 2004). Green bioreactors do not suffer the same risk of pathogen contamination as seen in mammalian cell culture as there are no known cross-kingdom pathogens. Plant growth requirements are simple and inexpensive compared to traditional cell culture systems, allowing for inexpensive and nearly unlimited scalability. In cases where plant cell culture is used, it has much more simple growth requirements than mammalian or insect cell culture and is able to utilize light as its main energy source, further reducing costs. Additionally, plant systems are robust and inert, allowing for simplified handling/purification and the ability in the case of pharmaceutically relevant proteins to be administered orally with minimal processing. For the establishment of a green bioreactor, there are seemingly many plant species to choose from, each with its own advantages and disadvantages depending on the type of application desired.

Tobacco (*Nicotiana tabacum*) has been and will continue to be a major platform for green bioreactors. Despite a traditionally negative view due to its strong ties to smoking, tobacco offers several unique advantages over other plant species. Tobacco is often referred as the "white mouse" of the plant world, as it is amicable to genetic modification and has become the primary vehicle for proof-of-concept work in recombinant protein production for the last 20 years. Tobacco is a leafy plant, having high biomass yield (up to 100 t of leaf biomass per hectare) and high soluble protein levels compared with many other model and crop species, an attractive feature for a protein production platform. Tobacco also offers various ways of expressing proteins of interest, such as transient based expression via agrobacterium or viral induction and stable nuclear or chloroplastic genome based expression. The availability of low-nicotine low-alkaloid tobacco varieties such as cultivar "81V9" has made tobacco plants even suitable for direct oral delivery of recombinant antigens in plant material or crude protein extracts (Menassa et al., 2001). Tobacco is neither a food nor feed crop, thus reducing the likelihood of transgenic material contaminating the food or feed chains. These features have made tobacco particularly well suited for plant-based production of biopharmaceutical products. Indeed, the number of therapeutic proteins produced in transgenic tobacco plants is increasing steadily, with several tobacco-derived products having advanced to human clinical trials. The goal of this review is to discuss the application and potential of tobacco as a green bioreactor for recombinant therapeutic protein production.

2. Production of pharmaceutical proteins in tobacco

Tobacco is proving to be an attractive bioreactor for the production of pharmaceutically relevant proteins. In addition to the economical advantages of tobacco bioreactors, it has the ability to produce a wide range of therapeutic proteins including antibodies, vaccines and immunomodulatory molecules such as cytokines. Moreover, by using transient protein expression, tobacco is able to generate significant quantities of protein in a short period of time that is necessary for rapid response to disease outbreaks, such as the recent influenza A/H1N1 pandemic, and for the patient-specific treatment of cancer.

2.1. Antibody production

Antibodies represent the single largest class of new drug entities under development at this time. The generation of antibodies for pharmaceutical use requires the coordinated creation of both a heavy and light chain and the correct assembly into a single functional unit. Monoclonal antibodies are traditionally generated using hybridoma cell lines, which are obtained by fusion of an immortalized cancer cell with an antibody-producing splenic cell isolated from antigen-challenged host. This process is technically challenging and the growth requirements for maintaining hybridoma cells are expensive. Bacterial production is unfeasible due to its inability to glycosylate and assemble functional antibodies. Plants offer an inexpensive alternative that is able to produce authentic antibodies. Tobacco was the first host chosen to express a functional recombinant full-length monoclonal antibody (mAb), the anti-mouse catalytic IgG_1(6D4), in plants (Hiatt et al., 1989). It was shown that when individual tobacco plants expressing single heavy (γ) or light (κ) chains of 6D4 were generated, simple sexually crossing of two parental transgenic tobacco lines resulted in progeny lines that express and accumulate a fully assembled and functional mouse antibody at high levels (1.3% of total soluble protein (TSP)). Since then, the expression of recombinant antibodies using tobacco as a platform has been extended to include monoclonal antibodies with more complex structures such as secretory IgA, single-chain antibodies and single-chain antibody fragments of different specificity.

Secretory IgA (sIgA) is a complex, multimeric protein composed of two IgA units (2 heavy and 2 light chains), a joining J chain, and a secretory component (Johansen et al., 1999), and is the antibody naturally produced by the body to protect oral and other mucosal surfaces against infectious organisms and toxins. It is generated by a unique cooperation between two distinct cell types: J chain-expressing plasma cells that produce polymeric (p)IgA (mainly dimers), and secretory epithelial cells that express the secretory component (SC) (Johansen et al., 1999). The recombinant production of SIgA in mammalian cells is difficult and expensive, requiring two independent and mixed cell lines and constant monitoring and cell rebalancing. However, tobacco greatly reduces the complexity for its recombinant production. The best example is the tobacco-based production of a humanized secretory murine IgA1 (Guy's 13 SIgA-G). The murine monoclonal antibody IgG1 (Guy's 13) which specifically recognizes the SAI/II protein of *Streptococcus mutans*, the main causative agent for dental caries, has been used successfully to prevent *S. mutans* colonization and the development of dental caries in non-human primates as well as in human clinical trials (Lehner et al., 1985; Ma et al., 1989). Therefore, Guy's 13 IgG antibody has the potential to treat and/or prevent dental caries. To produce recombinant Guy's 13 SIgA-G, transgenic tobacco plants were generated to express independently either the Guy's 13 kappa (light) chain, the hybrid IgA-G antibody heavy chain, murine J chain, or rabbit secretory component (SC). Through a series of sexual crosses between these plants, researchers produced transgenic tobacco plants expressing a functional, high molecular weight secretory murine immunoglobulin (Guy's 13 SIgA-G), with accumulation levels up to 500 μg per gram leaf material (Ma et al., 1995). Human clinical trials showed that plant-derived SIgA/G antibody prevented oral colonisation by *S. mutans* (Ma et al., 1998). Renamed CaroRx™, the tobacco-derived SIgA/G protein became the first plant-made antibody approved for human use in 2005 in the European Union.

Tobacco has also proven to be an efficient system for generating fragments of the antibody, including single-chain variable fragment (scFv), single-chain antibody variable domain (Fv) fragment and antibody-binding (Fab) fragment (see Table 1). These small recombinant synthetic antibodies retain full antigen-binding activity but lack specific assembly requirements. They are being used in diagnosis and treatment (Souriau and Hudson, 2003). Expression levels of scFv or its derivatives in tobacco leaves vary from 0.1% (Fecker et al., 1996) to as high as 6.8% (Fiedler et al., 1997) of TSP. Moreover, by multiple sexual crossing of tobacco lines that is expressing individual non-overlapping scFvs, transgenic tobacco plants expressing more than one non-overlapping scFvs is being developed to provide optimal protective efficacy against multiple infections, such as against the Botulinum neurotoxin and anthrax simultaneously (Almquist et al., 2006). With the risk of such biological attacks, a source of inexpensive and rapidly produced yet effective antibodies is of the utmost importance.