# PRODUCTION OF BIOPHARMACEUTICALS, ANTIBODIES AND EDIBLE VACCINES IN TRANSGENIC PLANTS

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## ABSTRACT

The use of plants and other botanicals as a source of medicines exists of the earliest stages of civilization. Despite great advances in synthetic organic chemistry, it is estimated that about one-fourth of present day prescription drugs still have a botanical origin. Recently, and through modern biotechnology, there has been a revival of interest in obtaining new pharmaceuticals from botanical sources. Through genetic modification, it is now recognized that plants are potentially a new source of pharmaceutical proteins including vaccines, antibodies, blood substitutes and other therapeutic entities. Unlike mammalian-derived rDNA drugs, plant-derived antibodies, vaccines and other proteins are particularly advantageous since they are free of mammalian viral vectors and human pathogens. Advantages offered by plants include also low cost of cultivation and high biomass production, relatively fast "gene to protein" time, low capital and operating costs, excellent scalability, eukaryotic posttranslational modifications and a relatively high protein yield.

*Key words: Heterologous proteins, plant-made pharmaceuticals, edible vaccine, molecular farming*

## INTRODUCTION

Plants have provided humans with useful molecules for many centuries, but only in the past 20 years has it become possible to use plants for the production of specific heterologous proteins (1). The first pharmaceutically relevant protein made in plants was human growth hormone, which was expressed in transgenic tobacco in 1986 (2). Since then, many other human proteins have been produced in an increasingly diverse range of crops. In 1989, the first antibody was expressed in tobacco (3), which showed that plants could assemble complex functional glycoproteins with several subunits. The structural authenticity of plant-derived recombinant proteins was confirmed in 1992, when plants were used for the first time to produce an experimental vaccine: the hepatitis B virus (HBV) surface antigen (4).

More recently, the range of recombinant proteins made in plants has extended to include industrial enzymes (5), technical proteins that are used in research (6), milk proteins that are suitable nutritional supplements (7), and new protein polymers with both medical and industrial uses (8).

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## MOLECULAR FARMING SYSTEMS

Molecular farming, biopharming, greening of vaccine technology and plant molecular farming are expressions for the large-scale production of recombinant proteins in living cells or organisms; frequently applied to the use of crop plants (or domestic animals) as expression hosts because of the allusion to agriculture.

There are four methods of protein production from plants: a) stable nuclear transformation of a crop species that are grown in the field or a greenhouse, b) stable plastid transformation of a crop species, c) transient transformation of a crop species by agroinfiltration, and d) stable transformation of a plant species that is grown hydroponically or in *in vitro* systems so that the transprotein is secreted into the medium and recovered (9). A detailed comparison of the economics, processing and regulatory constraints associated with the most common plant production systems is reviewed (10). Table 1 shows advantages and disadvantages of the above mentioned systems.

Different plant species and their parts are used in recombinant protein production (tab. 2). Many of the early, plant-derived recombinant proteins were produced in transgenic tobacco plants and were extracted directly from harvested leaves (11). High biomass yields and rapid scalability make tobacco very suitable for commercial molecular farming. It is also a non-food, non-feed crop, and thus carries a reduced risk of contaminating of feed and human food chains by transgenic material or recombinant proteins contaminating feed and human food chains (12). One of the disadvantages of tobacco is its high content of nicotine and other toxic alkaloids, which must be removed completely during downstream processing steps. Although low-alkaloid tobacco cultivars are available (13) attention has been turned to other leafy crops for pharmaceutical production. These plants include lettuce and alfalfa. Leafy crops are advantageous in terms of biomass yield, however, proteins that are expressed in leaves tend to be unstable, which means the harvested material has a limited shelf life and must be processed immediately after harvest. On the contrary, proteins expressed in cereal seeds can remain stable for a long time even at room temperature (14). Several different cereals, including rice, wheat, barley and maize have been used as potential hosts for heterologous protein production (12, 15). Oil crops (oilseed rape, *Camelina sativa*) are useful hosts for protein production because the oil bodies can be exploited to simplify protein isolation. In the more recent past, plant species have been designed, which can easily be contained, propagated and transformed, to produce recombinant proteins. Mayfield et al. (16) developed a protein expression system that is based on the green alga *Chlamydomonas reinhardtii*. In this system, chloroplast-targeted transgenes were used to express an antibody that recognized herpes simplex virus glycoprotein D. Other simple plants that have been accepted as bioreactors include *Physcomitrella patens*, *Lemna* (11) and *Marchantia (*17).

## PLANT CELL CULTURES

Although there has been considerable interest for the use of whole plants in production of recombinant proteins, the advantages of agricultural-scale production can be out weighted by the long development times, variations in product yield and quantity, and the difficulty in applying good manufacturing practice (GMP) to the early stages of production (18-20). In whole plants, the possibility of contamination with agrochemicals and fertilizers must be considered, as well as the impact of pests and diseases, and the variable cultivation conditions due to local differences in soil quality and microclimate.

Plant cell culture as an expression system for recombinant proteins avoids these problems while retaining the advantages. Like microbes, plant cells are inexpensive to grow and maintain, but

because they are higher eukaryotes they can carry out many of the post-translational modifications that occur in human cells. Plant cells can be maintained in simple, synthetic media, but like animal cells they can synthesize complex multimeric proteins and glycoproteins, such as immunoglobulins (21, 22) and interleukins (23). Recombinant human glycoproteins synthesized in plants show much greater similarity to their native counterparts in terms of N-glycan structure compared to the same proteins produced in yeast, bacteria or filamentous fungi (24).

*Table 1. Different plant-based production systems*

| *System* | *Advantages* | *Disadvantages* |
|---|---|---|
| Transgenic plants (stable nuclear transformation of a crop species that will be grow in the field or a greenhouse | Yield, economy, scalability, establishment of permanent lines (when accumulated within plants)<br>Containment, purification (when secreted from roots or leaves)<br>Multiple gene expression, low toxicity, containment (transplastomic) | Production timescale, regulatory compliance<br>Scale yield, cost production facilities<br>Absence of glycosylation, some evidence of horizontal gene transfer (transplastomic plants) |
| Virus-infected plants | Yield, timescale, mixed infections | Cost |
| Agroinfiltrated leaves | Timescale | Cost |
| Cell and tissue cultures | Timescale, containment, secretion into medium, purification, regulatory compliance | Cost |

*Table 2. Plants used for biopharmaceutical production*

| | |
|---|---|
| *Model plants* | Arabidopsis thaliana |
| Leafy crops | Tobacco, lettuce, alfalfa, clover |
| Cereals | Maize, rice, wheat, barley |
| Legumes | Soybean, pea, pigeon pea |
| Fruits and vegetables | Potato, carrot, tomato, banana |
| Oil crops | Oilseed rape, *Camelina sativa* |
| Simple plants | *Lemna* sp. *Physcomitrella patens*, *Marchantia polymorpha*, *Chlamidomonas reinhardtii* |

Unlike field-grown plants, the performance of cultured plant cells is independent of the climate, and other environmental changes (soil quality, season, day length, weather). There is no risk of contamination with mycotoxins, herbicides or pesticides (25), and there are fewer by-products (e.g. fibers, oils, waxes, phenolics and adventitious agents). One of the most important advantages of plant cells over whole plants is the much simpler procedure for product isolation and purification (25-27) especially when the product is secreted into the culture medium. Several approaches can be used for the *in vitro* cultivation of plant cells, including the derivation of hairy roots (28), shooty teratomas (29), immobilized cells (30) and suspension cell cultures (31). Suspension cell cultures are the most amenable to good manufacturing practice (GMP) procedures and they can be cultivated relatively easily in bioreactors (32, 33). These cultures have been established from species like *Arabidopsis thaliana* (34), *Catharanthus roseus* (35), *Taxus cuspidata* (36), tobacco, al-

falfa, rice, tomato and soybean (37-41). The cell cultures are grown in liquid culture medium supplemented with plant growth regulators to promote rapid growth and prevent differentiation (42-44). Recently, Hellwig *et al*. (45) reviewed in the detail specific challenges associated with plant cell cultures, and recombinant proteins of medical relevance produced in *in vitro* plant cultures.

In field grown transgenic plants or *in vitro* plant cultures may have the capability of producing any vaccine in large amounts and in a less expensive manner, but purification of the product may require the use of existing or even more cumbersome procedures. Attention has, therefore, been paid to an antigen produced in the edible part of a plant and, applicable as an oral vaccines.

## PLANT EDIBLE VACCINES

Edible vaccines have received considerable attention from researchers in both academia and industry. Charles Arntzen (who was the first to use the phrase "edible vaccine"), with Hugh Mason and colleagues have pioneered the field with work on hepatitis B and heat labile toxin, B subunit, in tobacco plants and potato tubers. Edible vaccines have been shown to induce good mucosal immune responses. Recent reviews of edible vaccines include Carter and Langridge (46) and Streatfield and Howard (47). The main goal of an oral vaccine is the induction of a mucosal immune response and a subsequent systemic immune response. Edible vaccines are sub-unit vaccines that introduce selected genes into the plants and facilitate the production of the encoded protein. Edible vaccines are mucosal-targeted vaccines that stimulate both the systematic and mucosal immune network takes place. Study species include potatoes, tomatoes, bananas, lettuces, rice, wheat, soybean, corn and legumes. Fruits, vegetables and leafy salads can be consumed raw or partially processed, which makes them particularly suitable for the production of recombinant subunit vaccines, food additives and antibodies for tropical passive immunotherapy. Potatoes have been widely used for the production of plant-derived vaccines that have been administered to humans in most of the clinical trials carried out thus far (7, 48-51). Tomatoes were used to produce the first plant-derived rabies vaccine (52), and have proven more palatable than potatoes while offering other advantages such as high biomass yields and the increased containment that is offered by growth in greenhouses. Lettuce is also being investigated as a production host for edible recombinant vaccines, and has already been used in one series of clinical trials targeted at developing a vaccine against HBV (53). Bananas have been considered as hosts for the production of recombinant vaccines, since they are widely grown in those countries where vaccines are most needed. The additional advantage of bananas is that they can be consumed raw or as a purees by both adults and children (54).

Since the HBV was produced and tested, the concept of oral vaccination with raw fruits, vegetables, leaves and seeds has risen in popularity. Edible plants, rather than tobacco, are now in the focus of research targeted at HBV vaccine production in plants (55). Clinical trials have been carried out with the surface antigen that is expressed in potato and lettuce (53).Two further vaccine candidates have reached the clinical trials stage, both of which are expressed in potato: the heat-labile toxin B subunit (LT-B) of enterotoxigenic *Escherichia coli* (ETEC) and the capsid protein of Norwalk virus (NVCP), (56, 57). These antigens, from two important enteric pathogens, might be ideal oral-subunit vaccine candidates, since both are multimeric structures that survive in the extreme conditions of the human gut. Each protein accumulated in high levels in potato tubers and was correctly assembled into oligomers. Clinical trials with the LT-B vaccine showed that the consumption of raw potato tubers that contained 0.3-10 mg of LT-B produced high titres of mucosal and systemic antibodies (56).

The advantages of edible vaccines could be enormous, however, many issues must still be addressed (tab. 3). Researchers have to solve the existing disadvantages such as low yields, immunogenicity, accumulation and stability of the transproteins, and obtaining glycosylation; processes

that are normally observed in humans. The first approved therapeutic products will show the many benefits of transgenic plant technology. These real benefits will help public acceptance and open the way towards a more rapid development of this technology.

## SAFETY CONCERNS

The two main concerns over edible vaccines are the contamination of food crops through cross pollination and of the vaccine itself in plant debris spreading as dust and as pollutants in surface and groundwater. The vaccine antigen may affect browsing animals and humans living in the area drinking vaccine-polluted water or breathing vaccine-polluted dust.

It is imperative that the cultivation and production of pharmaceutical crops should be limited to controlled production facilities such as greenhouses, or in plant tissue culture, that prevent the environmental release of the biopharmaceuticals. The main safety concern is that the oral vaccine preparations will induce "immune tolerance", thereby making the individual susceptible to, for example, the hepatitis B virus.

*Table 3. Advantages and disadvantages of edible vaccines*

| *Advantages* | *Disadvantages* |
|---|---|
| The plants producing the edible vaccines could be grown in the third world countries | Plants are living organisms that changes, so the continuity of the vaccine production might not be guaranteed |
| Plants are regularly used in pharmaceuticals, and there exist established purification protocols | The edible vaccines could be mistaken for regular fruits and consumed in larger amounts than might be safe |
| Growing plants is much cheaper than producing vaccines | The dosage of the vaccines might be variable. For example, different sized bananas will contain different amounts of vaccine |
| Plants can not host most human pathogens, so the vaccines will not pose a danger to humans | If the vaccines were grown in fields or on trees, security would become a big issue |
| Agricultural products can be transported around the world relatively cheaply | Glycosylation patterns in plants differ from those in humans and could affect the functionality of the vaccines |

## CONCLUSIONS

Plants have advantages compared with traditional systems for molecular farming of pharmaceutical proteins. These include: the low cost of production, rapid scalability, the absence of human pathogens, and the ability to fold and assemble complex proteins accurately. Plants might one day surpass other production systems because of the economic and safety benefits, and ultimately, it should be possible to make pharmaceuticals available to everyone who needs them, at a cost that everyone can afford. For the biotech and drug industry, biopharming offers economic and health benefits once the current cycle of product development reaches the commercialization stage. However, for these benefits to be fully realized, the central issue of risk to the food industry and the environment is a critical requirement. A combination of strong and adaptable regulatory oversight with technological solutions are required if the goals of realizing the full potential of plant mole-

cular farming are to be met. For all, plants need to be viewed as a possibility among many for manufacturing therapeutic proteins.

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