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Application of transgenic plants as factories for producing biopharmaceuticals

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Abstract

Antibodies (Ab) are glycoprotein compounds produced by the vertebrate immune-system. They recognize and bind to their aim antigens with high affinity and specificity, which enable them to be used for numerous applications, such as the diagnosis, prevention, resistance to pathogens of plant, and cure of human and animal illness. The importance of antibodies as an *in vitro* investigation tool has been incorporate *in vivo* applications in basic researches of proteins and other compounds. Recently, the production of antibodies in plants because many reasons, including low-cost production, ease to storage large-scale production, safety and etc. is proposed. This technology is referring to the plantibody. Plantibody approach is one of the new events in molecular technology field. Plants as a bio-factory can be also used for biopharmaceutical and drugs production. Pharmaceuticals that derived from plants are poised to become the next major commercial development in plant biotechnology. But, however, there are some concerns in this context. In the present article, we try to summarize the recent advances made in the use of transgenic plants as biological factories for the production of drugs, vaccine, and biopharmaceuticals also discuss the plantibodies as a low-cost, efficient method for the production of biological materials with demonstrated utility to the pharmaceutical industry or medical community. In addition, a referring to applications of antibodies produced in plants and has been compared with other expression system.

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Introduction

Human being always acquaintance with medicinal plants and its thousands years that human uses them as a raw material source and kind of plant medicine. From establishment of the first civilization, human use the plant extract or essence for disease cure and pain cure (Ghasempour *et al*., 2007). Attention to the plants as a factory of edible pharmaceutical production (Giddings *et al*., 2000; Danielle *et al*., 2001) was important from the first, and they are full of advantages. At the present time, plants are the end source of many combinations with medicine effect. But proteins medicines bring from animal source (plant or animal cells) or microbe, and biopharmaceutical industry with using mammalian cells that they work as a Golden standard for producing of these kinds of combinations (Fischer *et al*., 2006). Applying plants as medicinal intentions comeback to more than thousands years ago, but plant genetic engineering tries to produce suitable biopharmaceutical medicinal that they don't have a long antiquity and it comes back to recent years. Requesting for biopharmaceutical medicine with the reason of available scale is increased these days and of course it is because of the low cost of these drugs. These days limited factor of availability of these medicines are the cost of them. Plants-derived biopharmaceutical have these characteristics: cheap cost of production, easy maintaining and easy produce to high level and bring easier than animals (Danielle *et al*., 2001; Ma *et al*., 2003). Plant-derived drugs category in three parts: Antibody, biopharmaceuticals medicine and vaccine (Walmsley and Arntzen, 2000) that produce antibodies in plants are named to Plantibody. The important point is, how can we put a medicine material in the same level with production antibody from every another system in agriculture industry and of course production and formulation as a drug part is important. Using details in every stage of this process should explain with creditable factories (GMP or Good Manufacturing Practice) (Ghasempour *et al*., 2007). In addition one of the methods of the production of resistance plant to disease (Stoger *et al*., 2002; Fischer *et al*., 2003) is plantibodies production. High cost of production of the proteins with high quality (in the scale of Kg) may be reason of the establishing a new industry for production of recombinant protein in transgenic plants or animals (Larrick *et al*., 1998). Recently molecular farming is introduced as an industry in the word-wide (Danielle *et al*., 2001; Ma *et al*., 2003; Larrick *et al*., 1998; Jaeger *et al*., 2000; Lico *et al*., 2005) and we use transgenic animals as a bioreactor with the aim of protein production with the cure effect and clinical goals (Larrick *et al*., 1998). Selection of these expression systems high depends on the kind of protein and its application (Ma *et al*., 2003). For paying attention to applying production system for this important point should concentrate on some another subjects. In the period of the time, animal system suffer from long development time line and possible contaminations of purified proteins with animal viruses and perions (Larrick *et al*., 1998). But further more Abs may produce in mammalian cells or transgenic animals. Because in these shown case proteins fold correctly and they do correct pattern of glycosylation (Schillbergi *et al*., 2002). Glycosylation is a process that is influenced of enzymes that monomer sugar and oligosaccharides add to residue amino acids (Stoger *et al*., 2002; Larrick *et al*., 1998; Fischer *et al*., 2003) and folding operation is essential for protein with the aim of their functional. In this review paper, attempt to pay attention to produce Abs in plants as a medicine source, also refer to advantage, disadvantage, application, production systems comparison, bottlenecks and some another points for more introducing with this technology.

Plantibodies; importance, history and some applications in this field

Plantibody derived from two words: plant and antibody (anti-core, antidote and in general name immunoglobulin) with this meaning that we call antibody or antibody fragments production in plants, and they are full of applications like *in-planta* and *ex-planta* (Jaeger *et al*., 2000). Antibodies are glycoprotein complex that they produce with immune system and because of their individuals capability;

they are able to detection and binding to antigens (Fischer *et al.*, 2003). Ab and Fab (fragment antibody) are one of the most important recombinant protein because of high specify and affinity for with rank of antigens product in large scale. Antibodies or antibody fragments after extraction and purification from plant tissues can be use of industrial progress, diagnostic tools for immune-chromatography or in clinical diagnoses (Jaeger *et al.*, 2000) (Fig. 1). Although Abs expression was done with two Germany university students but the first reported in this case referred to 1989. From that time different reports from plantibodies were published. Most of the times Abs with the use of A. Tumefaciens transfer to plant cells, and plant regeneration do with the use of tissue culture. Although this tissue is suitable for transferring the mono chain Ab, but for producing plants with multiple chain need to cross planting or can be used in biolistic transformation (Larrick *et al.*, 1998).

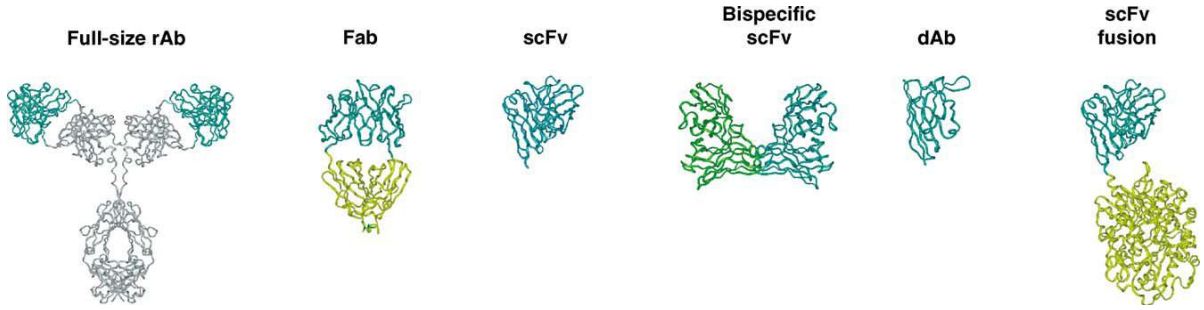

Fig. 1 Recombinant antibody Types expressed in transgenic plants: rAb, recombinant antibody; Fab, fragment antigen binding; scFv, single chain Fv fragment (Fischer *et al.*, 2003).

Until now use this technology many times and some reported about this case were published. For example refer to these case can be important: production of polypeptides of hepatitis B surface antigen (Ehsani *et al.*, 1997), production of industrial enzymes (Hood *et al.*, 2002), inactivation of the *barely yellow dwarf virus* (BYDV) (Erokhina *et al.*, 2002; Erokhina, 1995), expression of recombinant protein for cure applying (Andersen and Krummen, 2002), production of antibodies (Hiatt *et al.*, 1989), synthesis of monoclonal Ab in tobacco (Düring *et al.*, 1990), production and characterization of recombinant IgA (Chintalacharuvu and Morrison, 1999), production of monoclonal antibody in roots and tobacco seeds (Drake *et al.*, 2003, Fiedler and Conrad, 1995), expression and characterization of an anti-hepatitis B that is glycosilated in tobacco (Ramírez *et al.*, 2003), expression of β-1-4 galactosyl-transfer from human in cultured plant cell (Misaki *et al.*, 2003), production of vaccines and medicine antibodies for veterinary applying in transgenic plants (Floss *et al.*, 2007), applying antibody for plant pathogen resistance (Schillberg *et al.*, 2001), increased of resistance to tobacco viruses with use of antibody (Xiao *et al.*, 2001; Voss *et al.*, 1995; Zimmermann *et al.*, 1998), control of spiroplasma and phytoplasma disease (Chen and Chen, 1998), expression of *acetyl cholinesterase* from recombinant human cells in transgenic tomato (Mor *et al.*, 2001), production of Hepatitis B antigen in plants (Richter *et al.*, 2000), production of antibodies, biomedicines and food vaccines in plants (Danielle *et al.*, 2001), applying potato tubers for production of recombinant antibody, expression of cystatins against Karnal bunt (*tilletia indicia*) (Purwar *et al.*, 2009), and production of Fructose with the method of direct engineering and potato tubers as bioreactor for production of Palatinose (Artsaenko *et al.*, 1998; Beaujean *et al.*, 2000; Bornke *et al.*, 2002). In summary, the most important of events and history of the molecular farming is presented in the following Table 1.

Table 1. The key occurrence in memoir of the molecular pharming (data adapted from Ghasempour *et al.*, 2007; Obembe, 2010; Schillberg and Twyman, 2007)

Year	Event	Reference
1986	Production of the first pharmaceutical recombinant protein in tobacco and sunflower (means that human growth hormone)	Barta *et al.*, 1986
1989	Production of the first pharmaceutical recombinant antibody (IgG Full-length Ab) in tobacco	Hiatt *et al.*, 1989
1990	The first protein with human source –human serum albumin, produced in tobacco and potato	Sijmons *et al.*, 1990
1992	The first vaccine (hepatitis B vaccine) was produce in tobacco	Mason *et al.*, 1992
1992	The first industrial enzyme – α-amylase- was produce in tobacco	Pen *et al.*, 1992
1995	Secretory IgG antibody produced in tobacco	Ma *et al.*, 1995
1996	The first time Elastin artificial polymer produced in tobacco	Zhang *et al.*, 1996
1997	The first clinical test was perform using recombinant bacterial antigen which from transgenic tobacco	Tacket *et al.*, 1998
1997	Commercial production of Avidin in maize	Hood *et al.*, 1997
1999	Performance of the first Glycan analysis of tobacco productive plants	Cabanes-Macheteau *et al.*, 1999
2000	Production of the human growth hormone in plants tobacco chloroplast	Staub *et al.*, 2000
2000	Assembling of the triplet chain and production of human collagen in tobacco	Staub *et al.*, 2000
2001	The first vaccine multiple expressed in potato	Yu *et al.*, 2001
2003	Expression and assembling of one functional antibody in Alga	Mayfield *et al.*, 2001
2003	Commercial production of the cow tripsine in maize	Woodard *et al.*, 2003
2004	Genetic modification of N-glycosilation in Arabidopsis	Strasser *et al.*, 2004
2005	Broadly neutralizing anti-HIV antibody	Cardoso *et al.*, 2005
2005	Production of Plant-derived mouse IgG monoclonal antibody fused to KDEL against hepatitis B in tobacco	Triguero *et al.*, 2005
2006	Cloning and expression of recombinant camelid single-domain antibody in Tobacco	Artsaenko *et al.*, 2006
2006	Optimizating of a human monoclonal antibody glycan in the aquatic plant *Lemnaminor*.	Cox *et al.*, 2006
2006	production of the first commercialized plant-derived antibody or antibody against Hepatitis B (marketed in Cuba)	Pujol *et al.*, 2005
2006	Plastid transformation in the rice (*Oryza sativa*) and transmission of transgenes to their progeny	Lee *et al.*, 2006
2007	Production of an anti-mouse MHC class II monoclonal antibody in tobacco	Shin,Young *et al.*, 2007
2008	Expression of PA gene from Bacillus antracis in Iranian lettuce(*Lactuca sativa*)	Honari, 2008
2008	Production of idiotype vaccines for the treatment of non-Hodgkin's lymphoma :safety and immunogenicity in a phase I clinical study	McCormick *et al.*, 2008
2008	for the first time an antibody vaccine , Caro^{RX} – in order to human use (prevention of tooth decay), to be approved by the EU	Kaiser, 2008
2009	Gamma-Oleosin interferon gene transfer to Canola	Bagheri, 2009
2009	Cloning and transformation of human IFN gene to tobacco plants	Azhdari, 2009
2009	Production of an antibodies recombinant human glucocerebrosidase enzyme expressed in transformed plant cells	Aviezer *et al.*, 2009
2010	Production of heterologous protein in plants:	Desai *et al.*, 2010
2010	production of a foot and mouth disease virus epitope in tobacco	Lentz *et al.*, 2010
2010	Human growth hormone (hGH) production in N. tabacum	Xu *et al.*, 2010
2011	Production of a human papillomavirus 8 E7 protein in plants	Noris *et al.*, 2011

Antibody, structure and mechanisms in plants

In 1989 Hiatt and his co-works for the first time could show that, functional antibodies can be produced in transgenic plants. From that time, researchers try more on stable expression and production of active Ab and Fab in plant cells, and they could produce Ab and different shape of that (Ghasempour *et al.*, 2007). Plantibody approach is one of the new events in molecular technology field for pathogenic infections and plant cell pathways manipulation and analysis (Jaeger *et al.*, 2000).

Antibodies are bioactive molecules that can be used because of their specific effect mean their specific binding to their self's Antigen in the wide-range. This biomolecule know as valuable and extraordinary tool in basic researches because of their transfer ability with metabolic process in organism (Stoger *et al.*, 2002). Antibodies are glycoprotein compounds and they produce in the immune system of body (Fig. 1). On antibody in natural way has Y-shape and it consists of two heavy chain and two light chain that bind to each other with disulfide bonds. Every heavy chain fold to four domains and every light chain fold to two domains. An antibody has two parts: constant and variable (Fischer *et al.*, 2003; Stoger *et al.*, 2002). Every chain from four chains like most of the protein chain have two terminals C and N. domain of N-terminal in every light and chains has variability effect and the reason specific activity for every antibody for ligand is just an antigen (of course about monoclonal antibodies). And it's that part of molecule has the scale of antigen diagnosis. But residue part except variable means constant parts have effectors functional for example diagnose of immune cells and completed effect with this mean that constant parts don't need an antigen for specific binding. In mammalian five class of immunoglobulin can be found (IgG, IgM, IgA, IgD and IgE) (Fischer *et al.*, 2003).

The basic requirement for expression of full length Ab and Fab are correct folding and assembling of single chains (Gething and Sambrook, 1992). In higher plants this mechanism does with targeting of single chains of endoplasmic reticulum (ER) (Crofls and Denecke, 1998). In mammalian have been at least two chaperon proteins. In plants in endoplasmic reticulum these chaperons can be diagnosed. Attention to this point is essential that these chaperons can be caused of no differentiation folding and assembling in plants and animals. Full length Ab and Fab can assemble in high levels. Although maximum levels of assembly depend on different factors. The interesting point is that an antibody is just 0.004% from all of the soluble proteins of tobacco leaves, and it can be between 1-3% of Arabidopsis leaves (De Neve *et al.*, 1993). So the maximum level of assembling of an antibody depends on plant specifics, tissue and the other parts that on antibody may expression in also when use the other signs like 3UTT we may have the higher level of Fab assembling (to all soluble proteins).

Plantibody applications: production of edible vaccine, bioreactor, resistance to plant disease and bioremediation

In recent years, plant biotechnology can be caused of that agriculture can be antibodies suitable replacement for production of large scale from biomolecule like proteins. Plant engineering for resistance to pathogen and phenotype changing with use of change in plant metabolism (Immunomodulation) can be possible. In addition antibodies developed as antibodies *in vitro* research tool. The applicant of this technology may be: medical diagnosis and therapy, the sensitive detection and removal of environmental contaminants, control of pathogens, and industrial purification processes (Stoger *et al.*, 2002).

Bioreactor

Plantibodies have some application such as, production of vaccine antigen, clinical diagnosis protein, pharmaceutical and industrial proteins, biopolymer, carbohydrates, vitamins, minerals and food (Sharma and Sharma, 1993). These applications not only have been proved in medical science but also they proved in basic agronomy research (Jaeger *et al.*, 2000). In recent years, various plant systems have

been developed in order to using of plants as a bioreactor for the production of recombinant proteins including recombinant antibodies (Stoger *et al*., 2002). Using of plants as a bioreactor, or using them instead of using them as a factory and also using them as antibodies replacement for microorganisms like bacteria to produce human antibodies that they communicate with human health is very important. Firstly, due to large-scale of production efficiency and low cost of production, compared to prokaryotic, plants for production of antibodies are better than prokaryotic. Second, Processes of post-translational such as glycosylation that they are kind of post-translation modifications of proteins, in plants can be done more carefully than bacteria (Ma and Hein, 1995). That this function secretion for antibodies secretion to the apoplast space in transgenic plants is very essential (Stiger *et al*., 1991). Hein and coworkers (1991) reported that processing of recombinant antibodies in transgenic plant cells are similar to mammalian glycoprotein. So the most commonly antibodies that are produced in plant systems have antibodies with high-value products for therapeutic application. On the other hand to producing of complex molecules SIgA, plants as an effective system able to produce high levels from this kind of antibody (Larrick and Thomas, 2001). For example, Caro RX, the most advanced plant-derived antibody, (Fischer *et al*., 2006; Ma and Hiatt, 1996) is a SIg A secretory antibody that produced in tobacco plants and can be caused to prevent Dental caries (Larrick *et al*., 2001). In addition that mentioned before in relation to human health, Plantibody can be used as food additives (Yuan *et al*., 2001). Plant bioreactors can produce up to 10 kg antibody per ha. Compared with other bioreactor production the end price or cost of producing antibodies in plants is about 0.1. Since the advent of this technology, some antibodies with different characteristics was produced such as, produce antibodies to antigens I and II streptococci, human IgG antibody, antibody anti-CFA, anti-sperm antibodies and antibodies of anti-virus. Among the other successful cases include antibodies against CEA (in cancer diagnosis through imaging and treating cancers associated with specific antigen) that expressed in rice and wheat and also anti-tumor antibodies in the treatment of Burkitt's lymphoma (Ghasempour *et al*., 2007).

Resistance to plant diseases

Resistance to plant diseases is another important plantibody application. Generally, two strategies used for engineering of plants resistance to pathogens. The first strategy is based on the using pathogen gene against itself (Pathogen-Derived Resistance) and the second strategy from plant resistance genes were used (Plant-Derived Resistance) for example, with transfer of R-gene (resistance genes) to sensitive plant can be caused of resistance to especial diseases in that plant. The interesting point is that recently, researchers want to use of other animal's genes that this gene can be caused of resistance to plant pathogens (Agrios, 1997). Antibody gene expression strategy of a warm-blooded animal in plant is one of the new methods for protecting plants against pathogens (Plantibody-mediated Resistance). As mentioned before the plants lack of immune system similar to warm-blooded animals, so naturally they aren't able to produce antibodies. Genetic engineers relying on gene transfer technology have made it possible that the antibody is able to produce transgenic plants. Antibody-producing plants are carried out through four main stages, constriction of monoclonal antibodies, cDNA prepared from mRNA antibodies, preparation of appropriate construct from cDNA prepared, and finally stage is plant transformation. Using this technology now a day is developing more and more like that we think and are expected that we use of this technology in production of resistance to other pathogens. Stiger *et al.,* (1991) believed that with use of immunoglobulin can be prevent endogenous enzyme activity, thus it can be antibodies good replacement to antisense technology in the gene silencing.

Production of edible vaccines

One of the most interesting applications of this technology is production of edible vaccines that mechanism of an oral vaccine production has several

stages that are briefly described. First, relating a gene to antigens should be identified and isolated; afterward we attempted to cloning in an expression vector. Then was stable transformations of plant tissue, the transformed plants were selected and should be transgenic plants regenerated, that regenerated plant have expression gene. With extraction of total protein content, antigen of plant tissue detected and isolated, then immunogenicity and safety tests is carried out by feeding the animals. If this test goes successful, in the final stage these substrates for human use are immunization. However many plant-derived Ab or AbF products have successfully improved initially phase of the medical studies trials, various issues and outcome including regulatory procedure and public approval must still be resolved (Stoger *et al.*, 2002, Langridge, 2000). In the following Table 2 plants that were used as the source of vaccine production is shown.

Table 2. Recombinant proteins expressed in plants for pharmaceutical applications.

Vaccine	Host plant(s)	Reference
Rabies virus coat protein	Tomato	McGarvey *et al.*, 1995
Hepatitis B surface antigen	Potato	Richter *et al.*, 2000
Hepatitis B surface antigen	Soybean and tobacco	Smith *et al.*, 2002
Hepatitis B surface antigen	Suspension culture of tobacco cells	Sojikul *et al.*, 2003
Cholera toxin B subunit	Potato	Arakawa *et al.*, 1997
Cholera toxin B subunit	Tobacco	Wang *et al.*, 2001
Human cytomegarovirus	Tobacco	Tackaberry *et al.*, 1999
Glycoprotein B	Tobacco	Tuboly *et al.*, 2000
B subunit of *E.coli* enterotoxin	Tobacco and potato	Haq *et al.*, 1995, Lauterslager *et al.*, 2001
B subunit of *E.coli* enterotoxin	Maize	Streatfield *et al.*, 2001
human papilloma virus vaccines	Tobacco	Biemelt *et al.*, 2003

Immunomodulation

Changing the plants phenotype or the plants metabolism study (Immunomodulation) is a powerful tool for studying or altering the actions of an antigen *in vivo* (Stoger *et al.*, 2002). In addition, there is a molecular procedure that interferes with cellular metabolism or pathogen contamination by abnormal expression (ectopic expression) of genes encoding the antibody or antibody fragments. For this purpose, an antigen, that may be an enzyme or a metabolite, blocked or its efficiency is stable (Jaeger *et al.*, 2000). If the content of an antibody gene was merged into the genome, may be expression of this gene will be with the permanent and non-stop. In recent years, many reports proved the outstanding of this tool in field of plant research about the modulation or simulation of phytohormone interactions or inactivation of plant pathogen infection. Advantageous application of the production of Ab and AbF in plants (or plantibody) approach need distinctive levels of investigation. Physiological and morphological altering was investigated within the plants with creating an artificial source scFv ABA, specific abscisic acid production by the endoplasmic reticulum in tobacco and tomato (Jaeger *et al.*, 2000).

Bioremediation

Healthy bioremediation or biological process is one of the benefits of transgenic plants on the environment, in the process of applying disinfection plants or soil or water is clean up (He *et al.*, 2005). For example, mercury (Hg^{2+}) is one of the factors affecting industrial activities, which would later be methylated by bacteria in the form of the higher toxicity of leaves left. MerA gene transfer from bacteria to E. coli can solve this problem. Detoxification of mercury by soil microbes is also shown that the ionic form it into metallic form (Hg0), into a form that can be evaporated. In this case, He and coworks (He *et al.*, 2005) with a codon optimized transmission

(optimizing) found that the tobacco seeds were resistant and 50 micro M of this matter.

Biopharming and its challenges

Ability of gene transfer between species or within species using genetic engineering to say bio-pharming. Biopharming refer to the production of proteins (including antigens, antibodies, enzymes that have very important applications of the treatment, and pharmaceutical industry) or biomolecule that are produced in transgenic plants at the agriculture scale. Most of these proteins have been produced in bacterial systems, fungi or animals. But recently, production of such proteins and biomolecule are preferred in plant expression systems than other expression systems. The use of plants as biological factories is associated with many factors. The production of these proteins in terms of inputs cost such as light, water and minerals is low, in terms of environmental issues, safety and suitable for production of eukaryotic proteins that are require post-translational modifications, oligomerisation and etc., as well as the absence of human pathogens (Dalal *et al*., 2006). Four subjects challenging In this regard are quality and quantity of Product, purification, glycosylation and regulatory-timeline issues. Important limited factor for economical viability of a production system is the level of its production. A recombinant antibody expression level in an expression system by appropriate regulatory elements in an expression construct can be increased; including that by optimizing codon; also using this codon optimizing the expression of an antibody can be increased or stable. Since that Selection of system used for high scale production are depends on the efficiency of expression system, its suitability for increase the scale, storage and downstream processes, So some considerations in this regard should be protected, including value and application of product, anticipate of production scale, production geographic region, required facilities estimate, intellectual property, safety considerations (contamination levels and self-pollination), and economic considerations (Stoger *et al*., 2002). In case of an antibody quality the items that are needed can be pointed to the uniformity, stability, and free-contaminations. First, after the synthesis of a molecule, also after folding and assembling – regardless of the reduced system efficiency, the presence of inactive protein may be due of products degradation. The most effective factor for biopharmaceutical production is easy purification to produce and have a direct impact on the choice of expression system. In this case, chromatography is used for final purification. However, recently an alternative method Oleosin or polymer-fusions used in order to easy purification of recombinant proteins and may also be used for purification of antibody molecules. Antibody glycosylation, the addition of sugar molecules to antibodies, varies according to different production systems. Concerning this point (glycosylation) the important point is that if antibodies produced in plants were used for humans and other organisms do not cause immunogenicity. Differences in glycosylation patterns of proteins produced in plants and humans are more related to the immunogenicity potential of plant-specific N-glycan complexes which located at the heavy chain of plant-derived antibodies. Proven that are the injection of plant-derived antibodies into mice doesn't cause stimulation of immune system response, as well as this action is not done in human. Including solutions for the antibody used in other systems does not cause immunogenicity is deletion of peptide diagnostic sequence for N-glycan. But according to observations, for humans and mice use, don't need to omit this plant peptide sequence.

Advantages and disadvantages of plantibodies and why plants?

Using plants as a bioreactor for the production of recombinant proteins have the advantages and disadvantages compared with other expression systems such as animal systems, bacterial systems, yeast systems and etc. The most important disadvantage of prokaryotic systems is their inability to perform post-translational modification of produced proteins. Antibodies produced by the plants have been affinity absorption and ability of specific

bind to antigen similar to hybridoma cell products and as well as, their properties will be maintained after purification (Ghasempour *et al*., 2007).

High potential of production for large-scale, low cost of biomass production agricultural scale, or economical production in terms of production of recombinant protein in plants than in their traditional system such as the hybridoma cells, controlled conditions, high levels of expression, low risk of contamination produced by mammalian viruses, oncogene, bacterial toxins and pathogens that are carried in the blood, the ability of plant cells to correct folding and correct assembly not only for single-stranded peptides and antibody fragments, but also for multimeric proteins with full size is possible (Ma *et al*., 2003; Sharma and Sharma, 1993; Spoke and Karner, 2008), minimum downstream processes are required for proteins that to have oral, no need for purification of recombinant proteins such as tomatoes that are edible, the ability to introduce new transgenic plants or multiple plants by sexual crosses, produced enzymes by plants can be formulated to the seeds (targeting of their expression in this organ) as a suitable method in terms of transportation and storage costs for an almost unlimited period of time under the environmental conditions, possibility of mass production in plants such as tobacco, alfalfa, etc., ten kilograms per acre, and the antibody production rate is adjusted according to bazaar needs is only part of the benefits of this technology(Ma *et al*., 2003; Spoke and Karner, 2008).

The disadvantages of this method are such as, genes silencing in some cases (Stoger *et al*., 2002, Larrick *et al*., 1998) different patterns of glycosylation (Stoger *et al*., 2002), insufficient expression in some plants, environmental restrictions, allergies or allergic reactions to plants glycoprotein and other plant antigens, mycotoxins produced by impurities, herbicides and plant endogenous metabolites, suspecting of being part of the administration in order to use them as human drugs. Does gene segment or markers segments that used as a marker to be removed? If no, is it harmful? And are they compatible with the environment which is omitted? (Sharma and Sharma, 1993; Rogers *et al*., 1995). Although, the description provided may be realized because of the plant use in order to understand the production of antibodies, anyway following Table 3 provides supplemental and comparison of different systems to produce recombinant proteins are discussed.

Table 3. Comparison of production systems for biopharmaceuticals.

Transgenic plants	Mammalian cell culture	Yeast	Bacterial system	Parameter
Usually correct	Correct	Incorrect	None	Glycosylation
yes	Limited	Limited	Limited	Assemble of protein multimer
Low	Very	Medium	Medium	Production cost
High	High	Medium	Low	Folding protein integrity
Medium	Medium	Very	Very	Protein yield
Low	High	High	High	Large-scale production costs
Medium	Very	low	Low	Timeline of production
Low	Very	Medium	Medium	Requirement workmanship level for growth
High	Very high	Medium	Low	Product quality
Low risk	Viruses, Prions and Oncogenic DNA	Low risk	Endotoxins	Contaminations risks
Very high	Very low	High	High	Scale-up capacity
Very low	Very high	Medium	Low	Overall costs

Source: (Ma *et al*., 2003; Stoger *et al*., 2002; Schillbergi *et al*., 2002; Sharma and Sharma, 1993; Spoke and Karner, 2008; Schillberg *et al*., 2003; Ata Saei *et al*., 2012; Jalali Javaran *et al*., 2009)

Recombinant proteins targeting to specific tissues

Although most of the proteins targeting remembered as facilitator of recombinant protein purification, but the recombinant protein targeting techniques can also be used to increase the yield of recombinant proteins. In this method, with store of recombinant protein in the cellular components, processes of packaging, assembly and post-translational modifications are affected, that eventually all of these factors was led to the sustainability of proteins and thus increase yield (Bagheri, 2009; Jalali Javaran *et al*., 2009; Aliahmadi *et al*., 2006). It seems reasonable that, targeting of functional antibodies or recombinant proteins to the secretory pathway, the best choice to deliver maximum yield of its (Fischer *et al*., 2003; Zimmermann *et al*., 1998; Conrad and Fiedler, 1998). An example of targeting of gene transfer that in some of the reports referred to, genes is transferred into the chloroplast genome (Ghasempour *et al*., 2007; Wang *et al*., 2009, Danielle *et al*., 2002). This type of gene transfer has advantages such as the possibility of gene escape does not exist in nature, because the pollen is lack of chloroplast. If the gene exists in the chloroplast, the expression level is much higher than nuclear genes (Ghasempour *et al*., 2007, Danielle *et al*., 2001). Although gene expression in plastid organelles such as chloroplasts increases, but there are limitations in this approach such as, chloroplast cannot perform the post-translational modifications (Danielle *et al*., 2002). And also, Safety and environmental concerns like horizontal gene transfer from bacteria to chloroplast that exists in *in vitro* conditions(Kay *et al*., 2002).Therefore, the solution can be imposed is that gene transfer occurs to the nucleus, but used of a signal sequence for protein targeting to chloroplasts (Jobling *et al*., 2003).

Conclusion

Imagination of using of plants as hosts of recombinant antibodies expression (Plantibody) goes back to two decades ago. Antibodies and antibody fragments are one of the most important types of recombinant proteins that because high-specificity and affinity produced in large amounts for a wide range of antigens. The antibodies have therapeutic applications, diagnostic and industrial. In totally, the future production of drugs, vaccine, recombinant protein and biopharmaceuticals in plants looks promising. Because the production of it's in the cell culture cannot be attained economically. Although, production of antibodies in plants can used for alter the biochemical plant metabolism (Immunomodulation) (Jaeger *et al*., 2000) or to obtain pathogen resistance (Jaeger *et al*., 2000, Schillberg *et al*., 2001) but several issues including regulatory guidelines and public acceptance must be resolved. In several practical examples, antibodies in plants have been produced, which represents an important source for the ability of plants to produce herbal medicines with high benefits. Basic need for expression of Full-Length Ab and AbF is the folding correct and the accumulation of individual chains. And this aspect should be considered in various expression systems and science prokaryote system can note to perform this action hence this concern must be pointed for eukaryotic usage. Various factors influenced on the folding and assembly of antibodies in plants that in the genetic engineering of plants it should be pay attention. In higher plants this function is performed by targeting of the individual chains to the endoplasmic reticulum (ER) (De Neve *et al*., 1993). In the plants similar to mammalian, chaperons identified that do this function (Crofls and Denecke, 1998). The researchers think the gene or genes other warm-blooded animals are in the plant immune system, due to lack of immune system in plants, genes that cause resistance to plant pathogens (Agrios, 1997). With regard to aspects of the other systems, the application of these techniques and applications for safe food production appears to be a good option. Use of plants rather than microorganisms such as bacteria to produce human antibodies associated with health for various reasons have been considered. Mass production, low production costs in front amount of product, product safety and the safety of the plants is better than prokaryotes. Perform of Post-translational modification processes such as glycosylation in plants, and its failure to perform in

prokaryotes is other reasons (Ma and Hein, 1995,Stoger *et al.*, 2002). In the last 10-15 years, up to 100 different recombinant proteins are produced in transgenic plants. Plants have many advantages in comparison with other expression systems, especially in economic, safety, operations and production aspects. However, there are some problems for using plants as a bioreactor with the goal of recombinant proteins production that should be considered. Some of these problems are: the quality of final product, extraction and the processing of plant derived pharmaceutical macromolecules and biosafety (Jalali-Javaran *et al.*, 2009). Through genetic engineering vaccine antigens can be produced in the plants edible tissues such as tobacco (Larrick *et al.*, 2001). With manipulation of gene expression, antigen levels can be raised to the extent that an oral dose of vaccines for clinical trials was possible in human. With further advances in the field of science and technology, by increased expression levels of recombinant antigens, can be of greater (even more than one percent of total) as a seed to tissue protein or drug dedicated commercially valuable. Therefore, more attention will be on their way should be shifted to the potential problems of mass production, distribution and manipulation of transgenic plants. Identification of recombinant materials, pollution control, transgenic plants and recombinant proteins may be potential problems are on their way to mass produce vaccines in plants. Quality control should perform be also containing of the antigen. These considerations, when they know the importance of consuming inadequate amounts of vaccine can destroy the property or the use of fixed amounts can lead to immune tolerance to the antigen (Ghasempour *et al.*, 2007).

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References

Agrios J. 1997. Plant pathology. Academic press 365P.

Aliahmadi A, Rahmani N, Abdollahi M. 2006. Plant-derived human vaccines; An overview. Int. J. Pharmacol **2:** 268-279.

Andersen D, Krummen CL. 2002. Recombinant protein expression for therapeutic applications. Curr Opin Biotechnol **13:** 117–123.

Arakawa T, Chong DKX, Merrit JL, Langridge WHR. 1997. Expression of cholera toxin B subunit oligomers in transgenic potato plants. Transgenic Res **6:** 403–413.

Artsaenko O, Kettig B, Fiedler U, Conrad U, During K. 1998. Potato tubers as a biofactory for recombinant antibodies. Mol Breed **4:** 313–319.

Ata Saei A, Ghanbari P, Barzegar A. 2012. Haematococcus as a promising cell factory to produce recombinant pharmaceutical proteins. Mol Biol Rep DOI 10.1007/s11033,012,1861.

Aviezer D, Brill,Almon E, Shaaltiel Y, Hashmueli S, Bartfeld D, Mizrachi S, Liberman Y, Freeman A, Zimran A, Galun E. 2009. A plant-derived recombinant human glucocerebrosidase enzyme-apreclinical and phase I investigation. PLoS One **43:** e4792.

Azhdari H. 2009. Cloning and transformation of human IFN gene to tobacco plants. M.Sc. thesis. Department of Biotechnology, Faculty of Agriculture, Tarbiat Modarres University. Tehran – Iran.

Bagheri KH. 2009. Gamma,Oleosin interferon gene transfer to Canola and study of transgenic plants. PhD Thesis Department of Plant Breeding Faculty of Agriculture Tarbiat Modarres University Tehran – Iran.

Barta A, Sommergruber K, Thompson D, Hartmuth K, Matzke M, Matzke A. 1986. The expression of a nopaline synthase human growth hormone chimaeric gene in transformed tobacco and sunflower callus tissue. Plant Mol Biol **6:** 347–57.

Beaujean A, Ducrocq,Assaf C, Sangwan RS, Lilius G, Bulow L. 2000. Sangwan, Norreel, B,S, Engineering direct fructose production in processed potato tubers by expressing a bifunctional alpha,amylase/glucose isomerase gene complex. Biotech Bioeng **70:** 9–16.

Biemelt S, Sonnewald U, Galmbacher P, Willmitzer L, Müller M. 2003. Production of human papillomavirus type 16 virus,like particles in transgenic plants. J. Virol **7717:** 9211–9220.

Bornke F, Hajirezaei M, Sonnewald U. 2002. Potato tubers as bioreactors for palatinose production. Biotech **96:** 119–124.

Cabanes,Macheteau M, Fitchette,Lainé AC, Loutelier,Bourhis C, Lange C, Vine ND, Ma JK, Lerouge P, Faye L. 1999. N,Glycosylation of a mouse IgG expressed in transgenic tobacco plants. Glycobiology **9:** 65-72.

Cardoso RM, Zwick MB, Stanfield RL, Kunert R, Binley JM, Katinger H, Burton DR, Wilson IA. 2005. Broadly neutralizing anti-HIV antibody 4E10 recognizes a helical conformation of a highly conserved fusion,associated motif in gp41. Immunity **22:** 163–173.

Chen YD, Chen TA. 1998. Expression of engineered antibodies in plants, a possible tool for spiroplasma and phytoplasma disease control. Phytopathology **88:** 1367–1371.

Chintalacharuvu KR, Morrison SL. 1999. Production and characterization of recombinant IgA. Immunotechnology **4:** 65-74.

Cox KM, Sterling JD, Regan JT, GasdaskaJR, Frantz KK, Peele CG, Black A, Passmore D, Moldovan,Loomis C, Srinivasan M, Cuison S, Cardarelli PM, Dickey LF. 2006. Glycan optimization of a human monoclonal antibody in the aquatic plant Lemna minor. Nature Biotechnology **24:** 1591-1597.

Conrad U, Fiedler U 1998. Compartment specific accumulation of recombinant immunoglobulins in plant cells, an essential tool for antibody production and immunomodulation of physiological functions and pathogen activity. Plant Mol Biol **38:** 101-109.

Crofls AJ, Denecke J. 1998. Calreticulin and canexin in plants. Tends plant Sci **3:** 396-399.

D'Arcy CJ, Torrance L, Matrin RR. 1989. Discrimination among luteoviruses and their strains by monoclonal antibodies and identification of common epitopes. Phytopathology **79:** 869–873.

Dalal M, Dani RG, Kumar PA. 2006. Current trends in the genetic engineering of vegetable crops. Sci. Hortic **107:** 215-225.

Danielle H, Khan MS, Allison L. 2002. Milestones in chloroplast genetic engineering, an environmental friendly era in biotechnology. Trends Plant Sci **7:** 84–91.

Danielle H, Stephen JS, Wycoff K. 2001. Medical molecular farming, production of antibodies, biopharmaceuticals and edible vaccines in plants. trends in Plant Sci **6:** 1360-1385.

De Neve M, de Loose M, Jacobs A, van Houdt H, Kaluza B Weidle U, Depicker A. 1993. Assembly of an antibody and its derived antibody fragment in Nicotiana and Arabidopsis. Transgenic Res **2:** 27–37.

Desai P, Shrivastava N, Padh H. 2010. Production of heterologous protein in plants, strategies for optimal expression. BiotechnolAdv **28:** 427–435.

Drake PM, Chargelegue DM, Vine ND, van Dolleweerd CJ, Obregon P, Ma JK. 2003. Rhizosecretion of a monoclonal antibody protein complex from transgenic tobacco roots. Plant Mol Biol **52:** 33-41.

Düring K, Hippe S, Kreuzaler F, Schell J. 1990. Synthesis and selfassembly of a functional monoclonal antibody in transgenic Nicotiana tabacum. Plant Mol Biol **15:** 281-93.

Ehsani P, Khabiri A, Domansky NN. 1997. Polypeptides of hepatitis B surface antigen produced in transgenic potato. Gene **190:** 107–1.1

Erokhina TN, Kastalieva TB, Mozhaeva KA. 2002. Preparing of monoclonal antibodies against BYDV using viruses purified from naturally infected plants, Proceedings of International Symposium. "Barley Yellow Dwarf Disease, Recent Advances and Future Strategies" 1–5 Sept El Batan Texcoco Mexico. 101–103.

Erokhina TN. 1995. Monoclonal antibodies to barley yellowdwarf virus, An immune enzyme test system for virus diagnostics. Bioorg Khim in Russian. **21:** 256–260.

Fiedler U, Conrad U. 1995. High-level production and long-term storage of engineered antibodies in transgenic tobacco seeds. Biotech **1310:** 1090-1093.

Fischer R, Richard M, Schillberg S. 2003. Production of antibodies in plants and their use for global health. Vaccine **21:** 820–825.

Fischer R, Twyman MR, Hellwig S, Drossard J, Schillberg S. 2006. Facing the Future with Pharmaceuticals from Plants. Biotechnology and Sustainable Agriculture and Beyond, Proceedings of the 11th IAPTC&B Congress.

Floss DM, Falkenburg D, Conrad U. 2007. Production of vaccines and therapeutic antibodies for veterinary applications in transgenic plants. an overview Transgenic Res **163:** 315,332.

Gething MJ, Sambrook J. 1992. Protein folding in the cell, Nature **355:** 33-45.

Ghasempour HR, Kahrizi D, Mehdiah N. 2007. New Discussions in Biotechnology. Razi University Press 274p.

Giddings G, Allison G, Brooks D, Carter A. 2000. transgenic plants as factories for biopharmaceuticals. Nature America Inc **1811**: 1151-5.

Haq TA, Mason HS, Clements JD, Arntzen CJ. 1995. Oral immunization with a recombinant bacterial antigen produced in transgenic plants. Science **268:** 714-716.

He Y, Xu J, Tang C, Wu Y. 2005. Facilitation of pentachlorophenol degradation in the rhizosphere of ryegrass *Loliumperenne L.* Soil Biol Biochem **37:** 2017–2024.

Hiatt A, Cafferkey R, Bowdish K. 1989. Production of antibodies in transgenic plants. Nature **3426245:** 76-78.

Honari H. 2008. Expression of PA gene from *Bacillus antracis* in Iranian lettuce *Lactuca sativa*. Ph.D. thesis, Department of Plant Breeding, College of Agriculture, Tehran University. Tehran, Iran.

Hood EE, Witcher DR, Maddock S, Meyer T, Baszczynski C, Bailey M, Flynn P, Register J, Marshall L, Bond D, Kulisek E, Kusnadi AR, Evangelista R, Nikolov ZL, Wooge C, Mehigh RJ, Hernan R, Kappel WK, Ritland D, Li CP, Howard JA. 1997. Commercial production of avidin from transgenic maize, characterization of transformant, production, processing, extraction and purification. Mol Breeding **3:** 291-306.

Hood EE, Woodard SL, Horn ME. 2002. Monoclonal antibody manufacturing in transgenic plants, myths and realities. Curr Opin Biotechnol **13:** 630, 635.

Xiao XW, Chu PWG, Frenkel MJ, Tabe LM, Shukla DD, Hanna PJ, Higgins TJV, Muller WJ, Ward CW. 2000. Antibody,mediated improved resistance to ClYVV and PVY infections in transgenic tobacco plants expressing a single,chain variable region antibody. Mol Breed **6,** 421–431.

Xu J, Okada Sh, Tan L, Goodrum KJ, Kopchick JJ, Kieliszewski MJ. 2010. Human growth hormone expressed in tobacco cells as an arabinogalactan, protein fusion glycoprotein has a prolonged serum life, Transgenic Res **19:** 849–867.

Ismaili A, Jalali Javaran M, Rasaee MJ, Rahbarizadeh F, RajabiMemari H. 2006. Cloning and expression of recombinant camelid single,domain antibody in Tobacco. Iranian Journal of Biotechnology **43:** 162-168.

Jaeger GD, Wilde CD, Eeckhout D, Fiers D, Depicker A. 2000. The plantibody approach, expression of antibody genes in plants to modulate plant metabolism or to obtain pathogen resistance. Plant Mol Biol **43:** 419–428.

Jalali Javaran M, Mohebodini M, Masoumi Asl A, Saifi Nabi Abad H, Alizadeh H, Mahbodi F, Ismail A, Rajabi Memari H, Moini A, Honari H, Bagheri Kh, Yaghobi MM, Zebarjadi AR, Rasai MJ, Shakib AM, Rahbarizadeh F, Masoumi H, Forozandeh Moghadam M, Sharifi, Sirchi GR, Dymyad S, Sadat Noori SA, Vishlaghi N, Hosseini Pour A, Taheri Javan N, Razmi Sh, Rahimi Far P, Latif B, Abdolinasab M, Azhdari H, Poorkhaleghi M, Razmi A, Khosravi H, Kazemi H. 2009. The success of molecular farming in Iran. J. Agri Biotech **11:** 19-48.

Jobling SA, Cees AM, Van Der Honde P, Punt J. 2003. Immunomodulation of enzyme function in plants by single domain antibody fragments. Nat Biotechnol **21:** 77–80.

Kaiser J. 2008. Is the drought over for pharming? Science **320:** 473,475.

Kay E, Drake PMW, Christou P. 2002. *In situ* transfer of antibiotic resistance genes from transgenic transplastomic. tobacco plants to bacteria. Appl Environ Microb **68:** 3345–3351.

Langridge WHR. 2000. Edible vaccines. Scientific American **2833:** 66-71.

Larrick JW, Thomas DW. 2001. Producing proteins in transgenic plants and animals. Curr Opin Biotechnol **12:** 411–418.

Larrick JW, Yu L, Chen J, Jaiswal S, Wycoff K. 1998. Production of antibodies in transgenic plants. Res Immunol **149:** 603–608.

Larrick JW, Yu L, Naftzger C, Jaiswal S, Wycoff K. 2001. Production of secretory IgA antibodies in plants. Biomol Eng **18:** 87–94.

Lauterslager TGM, Florack DEA, van derWalTJ, Molthoff JW, Langeveld JPM, Bosch D. 2001. Oral immunization of naive and primed animals with transgenic potato tubers expressing LT, B. Vaccine **19:** 49–55.

Lee SM, Kang K, Chung H, Yoo SH, Xu XM, Lee SB, Cheong JJ, Danielle H, Kim M. 2006. Plastid transformation in the monocotyledonous cereal crop, rice (*Oryza sativa*) and transmission of transgenes to their progeny. Mol. Cells **21:** 401–410.

Lentz EM, Segretin ME, Morgenfeld MM, Wirth SA, Dus Santos MJ, Mozgovoj MV, Wigdorovitz A, Bravo-Almonacid FF. 2010. High expression level of a foot and mouth disease virus epitope in tobacco transplastomic plants. Planta **231:** 387–395.

Lico C, Desideio A, Banchieri S, Benvenetu E. 2005. Plants as bio factories, Production of pharmaceutical recombinant proteins. Proceedings of the International Congress “In the Wake of the Double Helix, From the Green Revolution to the Gene Revolution”, Bologna, Italy 577,593.

Ma JK, Drake PM, Christou P. 2003. The production of recombinant pharmaceutical proteins in plants. Nat Rev Genet **4:** 794–805.

Ma JK, Hiatt A, Hein M, Vine ND, Wang F, Stabila P, van Dolleweerd C, Mostov K, Lehner T. 1995. Generation and assembly of secretory antibodies in plants. Science **268:** 716 – 719.

Ma JKC, Hein M. 1995. Immunotherapeutic potential of antibodies produced in plants. Trends Biotechnol **13:** 522–527.

Ma JKC, Hiatt A. 1996. Expressing antibodies in plants for immunotherapy. In, Owen MRL, Pen J eds. transgenic plants, a production of pharmaceutical proteins. John Wiley & Sons, Chichester, U.K. 229-243pp.

Mason HS, Lam DMK, Arntzen CJ. 1992. Expression of hepatitis B surface antigen in transgenic plants. Proc. Natl. Acad. Sci. USA. **89:** 11745-11749.

Mayfield SP, Franklin SE, Lerner RA. 2003. Expression and assembly of a fully active antibody in algae. ProcNatlAcadSci USA **100:** 438–442.

McCormick AA, Reddy S, Reinl SJ, Cameron TI, Czerwinkski DK, Vojdani F, Hanley KM, Garger SJ, White EL, Novak J, Barrett J, Holtz RB, Tuse´ D, Levy. 2008. Plant-produced idiotype vaccines for the treatment of non-Hodgkin's lymphoma, safety and immunogenicity in a phase I clinical study. Proc Natl Acad Sci USA **105:** 10131-10136.

McGarvey PB, Hammond J, Dienelt MM, Hooper DC, Fu ZF, Dietzschold B, Koprowski H, Michaels FH. 1995. Expression of the rabies virus glycoprotein in transgenic tomatoes. Bio Technol **13:** 1484-1487.

Misaki R, Kimura Y, Palacpac NQ, Yoshida S, Fujiyama K, Seki T. 2003. Plant cultured cells expressing human beta1, 4, galactosyltransferase secrete glycoproteins with galactose,extended N,linked glycans. Glycobiology **13:** 199-205.

Mor TS, Sternfeld M, Soreq H, Arntzen CJ, Mason HS 2001. Expression of recombinant human acetylcholinesterase in transgenic tomato plants. Biotechnol Bioeng **75:** 259-266.

Noris E, Poli A, Cojoca R, Ritta M, Cavallo F, Vaglio S, Matic S, Landolfo S. 2011. A human papillomavirus 8 E7 protein produced in plants is able to trigger the mouse immune system and delay the development of skin lesions. Arch Virol **156:** 587–595.

Obembe OO. 2010. Plant Biotechnology on a flight, Is Africa on board?. Life Science Journal **74:** 14-25.

Pen J, van Ooyen AJJ, van den Elzen PJM, Rietveld K, Hoekema A. 1992. Direct screening for high,level expression of an introduced α,amylase gene in plants. Plant Mol Biol **18:** 1133,1139.

Pujol M, Ramírez NI, Ayala M, Gavilondo JV, Valdés R, Rodríguez M, Brito J, Padilla S, Gómez L, Reyes B, Peral R, Pérez M, Marcelo JL, Milá L, Sánchez RF, Páez R, Cremata JA, Enríquez G, Mendoza, Ortega MO, Borroto C. 2005. An integral approach towards a practical application for a plant, made monoclonal antibody in vaccine purification. Vaccine **23:** 1833-1837.

Purwar Sh, Soma S. Marla, US, Kumar SA. 2009. Basal expression studies of cystatins during specific growth stages of wheat spikes for defining their possible role in differential and stage dependent immunity against Karnal bunt *Tilletiaindica*. Mol Biol Rep **37:** 1377–1389.

Ramírez N, Rodríguez M, Ayala M, Cremata J, Pérez M, Martínez A, Linares M, Hevia Y, Páez R, Valdés R, Gavilondo JV, Selman,Housein G. 2003. Expression and characterization of an anti,hepatitis B surface antigen. glycosylated mouse antibody in transgenic tobacco Nicotiana tabacum. plants and its use in the immunopurification of its target antigen. Biotechnol Appl Bioc **383:** 223-230.

Richter LJ, Thanavala Y, Arntzen CJ, Mason HS. 2000. Production of hepatitis B surface antigen in transgenic plants for oral immunization. Nat Biotechnol **18:** 1167-1171.

Richter LJ, Thanavala Y, Arntzen CJ, Mason HS. 2000. Production of hepatitis B surface antigen in transgenic plants for oral immunization. Nat Biotechnol **18:** 1167-1171.

Rogers HG, Parkers HC. 1995. transgenic plants and the environment. J. of Exp. Bot **286:** 467-488.

Ruggiero F, Exposito JY, Bournat P, Gruber V, Perret S, Comte J, Olagnier B, Garrone R, Theisen M. 2000. Triple helix assembly and processing of human collagen produced in transgenic tobacco plants. FEBS Lett **469:** 132-136.

Schillberg S, Emans N, Fischer R. 2002. Antibody molecular farming in plants and plant cells. Phytochem Rev **1:** 45–54.

Schillberg S, Fischer R, Emans N. 2003. Molecular farming of recombinant antibodies in plants. Cell Mol Life Sci **60:** 33–45.

Schillberg S, Twyman RM. 2007. Pharma-Planta, Recombinant pharmaceuticals from plants for human health. In, Engelhard M, Hagen K, Thiele F eds. Pharming, A New Branch of Biotechnology. European Academy **43:** 13-30.

Schillberg S, Zimmermann S, Zhang MY, Fischer R. 2001. Antibody based resistance to plant pathogens. Transgenic Res **10:** 1–12.

Sharma A, Sharma KMK. 2009. Plants as bioreactors, Recent developments and emerging opportunities. BIOTECHNOL ADV **27:** 811-832.

Shin-Young H, Tae-Geum K, Tae-Ho K, Yong-Suk J, Moon-Sik Y. 2007. Production of an Anti-mouse Mhc Class I Monoclonal Antibody with Biological Activity in Transgenic Tobacco. Protein Expr. Purif **54:** 134-8.

Sijmons P.C, Dekker BMM, Schrammeijer B, Verwoerd, TC, Van Den Elzen PJM, Hoekema A. 1990. Production of correctly processed human serum albumin in transgenic plants. Nat. Biotechnol **8:** 217–221.

Smith ML, Mason HS, Shuler ML. 2002. Hepatitis B surface antigen HbsAg. expression in plant cell culture, kinetics of antigen accumulation in batch culture and its intracellular form. BiotechnolBioeng **80:** 12–22.

Sojikul P, Buehner N, Mason HS. 2003. A plant signal peptide hepatitis B surface antigen fusion protein with enhanced stability and immunogenicity expressed in plant cells. Proc Natl Acad Sci USA **100:** 2209–14.

Spoke A, Karner S. 2008. Plant molecular farming opportunities and challenges. European comission press 148p.

Staub JM, Garcia B, Graves J, Hajdukiewicz PT, Hunter P, Nehra N, Paradkar V, Schlittler M, Carroll JA, Spatola L, Ward D, Ye G and Russell DA. 2000. High-yield production of a human therapeutic protein in tobacco chloroplasts. Nat Biotechnol **18:** 333-338.

Stiger M, Neuhaus G, Manma, T. 1991. Self assembly of immunoglobulins in the cytoplasm of the alga Acetabulavia meditennnce. Plant Sci **73:** 181-190.

Stoger E, Sack M, Fischer R, Christou P. 2002. Plantibodies, applications, advantages and bottlenecks. Curr Opin Biotechnol **13:** 161–166.

Stoger E, Sack M, Perrin Y, Vaquero C, Torres E, Twyman RM, Christou P, Fischer R 2002. Practical considerations for pharmaceutical antibody production in different crop systems. Mol Breeding **9:** 149–158.

Strasser R, AltmannF, MachL, Glössl J, Steinkellner H. 2004. Generation of Arabidopsis thaliana plants with complex N-glycans lacking beta1-2-linked xylose and core alpha1-3-linked fucose. FEBS Lett **561:** 132-136.

Streatfield SJ, Jilka JM, Hood EE, Turner DD, Bailey MR, Mayor JM, Woodard SL, Beifuss KK, Horn ME, Delaney DE, Tizard IR, Howard JA. 2001. Plant-based vaccines, unique advantages. Vaccine **19:** 2742-2748.

Tackaberry ES, Dudani AK, Prior F, Tocchi M, Sardana R, Altosaar I, Ganz PR 1999. Development of biopharmaceuticals in plant expression systems, cloning, expression and immunological rectivity of human cytomegalovirus glycoprotein B UL55 in seeds of transgenic tobacco. Vaccine **17:** 3020-3029.

Tacket CO, Mason HS, Losonsky, G, Clements JD, Levine MM, Arntzen CJ. 1998. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. J Nat Med **4:** 607-609.

Triguero A, Cabrera G, Cremata JA, Yuen CT, Wheeler J, Ramírez NI. 2005. Plant-derived mouse IgG monoclonal antibody fused to KDEL endoplasmic reticulum, retention signal is N,glycosylated homogeneously throughout the plant with mostly high-mannose, type N-glycans. Plant Biotechnol J **3:** 449–457.

Tuboly T, Yu W, Bailey A, Degrandis S, Du S, Erickson L, Nagy E. 2000. Immunogenicity of porcine transmissible gastroenteritis virus spike protein expressed in plants. Vaccine **18:** 2023-2028.

Voss A, Niersback M, Hain R, Hirsch HJ, Liao YC, Kreuzaler F, Fischer R. 1995. Reduced virus infectivity in *N. tabacum* secreting a TMVspecific full size antibody. Mol Breed **1:** 39–50.

Walmsley AM, Arntzen CJ. 2000. Plants for delivery of edible vaccines. Curr Opin Biotechnol **11:** 126–129.

Wang HH, Yin WB, Hu ZM. 2009. Advances in chloroplast engineering. J. Genet. Genomics **36:** 387–398.

Wang XG, Zhang GH, Liu ChX, Zhang YH, Xiao ChZ, Fang RX. 2001. Purified Cholera Toxin B Subunit from Transgenic Tobacco Plants Possesses Authentic Antigenicity. Biotech Bioeng **724:** 490-494.

Woodard SL, Mayor JM, Bailey MR, Barker DK, Love RT, Lane JR, Delaney DE, McComas,Wagner JM, Mallubhotla HD, Hood EE, Dangott LJ, Tichy SE, JA Howard JA. 2003. Maize Zea mays.,derived bovine trypsin, characterization of the first large,scale, commercial protein product from transgenic plants. Biotechnol Appl Biochem **38:** 123–130.

Yu J, Langridge WHR. 2001. A plant-based multicomponent vaccine protects mice from enteric diseases. Nat Biotechnol **19:** 548 – 552.

Yuan Q, Hu W, Pestka JJ, He SY, Hart LP. 2000. Expression of a functional antizearalenone single-chain Fv antibody in transgenic Arabidopsis plants. Appl. Environ. Microbiol **66:** 3499–3505.

Zhang X, Urry DW, Danielle H. 1996. Expression of an environmentally friendly synthetic protein,based polymer gene in transgenic tobacco plants. Plant Cell Rep **16:** 174-179.

Zimmermann S, Schillberg S, Liao YC, Fischer R. 1998. Intracellular expression of TMV,specific single,chain Fv fragments leads to improved virus resistance in Nicotiana tabacum. Mol Breed **4:** 369–379.