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Plantibodies as biopharmaceuticals: A review

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Abstract
Antibodies are essential part of vertebrate immune system which can now be produced by transforming plants with antibody- coding genes from mammals/humans. Such plant-derived antibodies or plantibodies function as the same way as mammalian antibodies. Production of plantibodies offers several advantages over other methods of antibody production, such as, low cost of production, high yield of antibodies, less time required, etc. Hence, plants are now gaining widespread acceptance as green bioreactors. Several plantibodies are under clinical trials. Agricultural crops such as tobacco, tomato, potato, soya bean, alfalfa, rice and wheat are commonly used for the production of plantibodies.

Keywords: Transgenic, molecular farming, recombinant, applications

Introduction
There was a time not too long ago when most medicinal compounds came from plants: the potent heart stimulant digitalis from foxglove, for example and opium from poppy plant. But beginning about 50 years ago, chemistry took over from botany, with most new drugs being artificially produced in pharmaceutical labs [1]. Nowadays, one of the most promising methods of producing proteins and other medicinal substances such as antibodies and vaccines is the use of transgenic plants. Transgenic plants are attracting interests to provide unlimited amounts of antibodies [2].
A plantibody is an antibody produced by genetically modified crops. The term "Plantibodies" was created to describe the products of plants that have been genetically modified to express antibodies and antibody fragments in plants. Plants are used as factories for large scale production of clinically viable proteins, which can later be purified from plant tissue [3].

Why plants for Molecular Farming of Antibodies?
Plants have several potential advantages over other production systems [4].
1. The potential for large scale, low cost biomass production using agriculture.
2. Low risk of product contamination by mammalian viruses, blood borne pathogens, bacterial toxins and oncogenes. Plants do not serve as hosts for human pathogens. Therefore, there is increased safety.
3. The capacity of plant cells to correctly fold and assemble, not only antibody fragments single chain peptides, but also full length multimeric proteins, since they have similar pathways of protein synthesis, secretion, folding and post translational modifications.
4. Elimination of the purification requirement when the plant containing the recombinant proteins is edible, such as potatoes.
5. Low downstream processing requirements for proteins administered orally.
6. The avoidance of ethical problems associated with transgenic animals.
7. The ability to introduce new or multiple transgenes by sexual crossing of plants.
8. Production size is flexible and easily adjustable to the needs of changing markets.
9. Formulated in seeds, plant made enzymes have been found to be an extremely convenient method for reducing storage and shipping costs, for an indefinite amount of time, under ambient conditions.

Criterion for selecting the host plant
Before selecting a plant for the production of plantibodies, following points should be considered
1. It should be easily genetically engineered.
2. It should be capable of producing high level of specific proteins.
3. It should have a well-established technology for gene transfer and expression.
4. It should have no toxic or side effects on the animal body.

5. Knowledge of agricultural techniques and management, physiology, pest and disease of crop/ plant selected should be known.

Crops and Plants used for Plantibody Production

1. Tobacco- When a large amount of protein is desired from the plant, tobacco leaf is the first choice, as it has a high biomass. Leafy crops such as tobacco has greatest biomass yield per hectare, because they can be cropped several times in a year [5]. It grows quickly and produces comparatively large amounts of antibodies. However, presence of toxic metabolites is hindrance for its use.
2. Cereals, Seeds and Tubers- When long term storage of plantibodies is desired, antibodies are mainly targeted to cereals, seeds and tubers. They can be stored at room temperature and are easily transportable. Most commonly used cereals are rice, wheat and maize along with legumes such as pea, soya bean and alfalfa [6]. Antibodies expressed in potato tubers and other cereal grains are stable at room temperature from months to years together, while tobacco leaves must be dried or frozen for long term storage. However, extraction of proteins from seeds is more expensive than watery tissue, such as tomatoes [5].
3. Fruits and Vegetables- They can be consumed raw or as partially processed material, which makes them suitable for passive oral immune therapy. E.g.:- Tomatoes, bananas, etc. [7]. Tomatoes have outstanding properties for pharmaceutical protein production, such as high biomass yield and advantage of contained growth in green house. Hence, tomatoes were first used to produce plant- derived rabies vaccine [8, 9].

Production Technology of Plantibodies

1. Conventional Method- It uses stable transformation and transient expression to introduce new genes in to a host cell. Once DNA from the host cell is isolated and purified, it can be injected in to the embryo of a maturing plant. The plant can then propagate in an open field allowing for large scale production of antibodies. However, purification of these proteins is long and tedious, since upon isolation of the antibody, several proteins, organic molecules; glycans and herbicides must also be isolated, leading to a complex purification process [10].
2. *In vitro* Cell Tissue Cultures – Plant cells in differentiated and dedifferentiated states (hairy roots) ate grown in a nutrient medium under controlled conditions, with foreign proteins harvested from either the biomass or culture liquid or a combination of both [11].
3. Breeding and Sexual Crossing- This method involves transformation to introduce kappa chains of either light or heavy chains in to the host plant. The same is done with gamma chains of either light or heavy regions. Upon crossing one plant with kappa chains and another plant with gamma chains, an antibody is produced which expresses both chains [3].
4. Transgenic Seeds- Some researchers suggest use of transgenic seeds in place of green plant tissue for production of plantibodies as green plants cannot store antibodies for a long period of time. This is because they contain proteases which degrade the recombinant protein. Thus, transgenic seeds are used which contain low level of proteases that allows protein to be stored without degradation [12].
5. Compartmentalization – Targeting signals can be used to retain recombinant proteins within different compartments of the cells such as endoplasmic reticulum, chloroplasts, intra cellular space, etc. This is done to preserve the integrity, protect them from proteolytic degradation and to increase accumulation levels of the recombinant protein. This is achieved by tagging the antibodies with a small peptide sequence which can be targeted to a specific compartment of the cell [3].
6. Purification and Evaluation of Plantibodies- Purification of plantibodies is done by filtration, immunofluorescence, chromatography, polymer fusion or diafiltration. Once the protein is purified, it can be evaluated by ELISA, Western Blot or radio immunoassay [13].

Application of Plantibodies

Plantibodies produced in plants are cheaper, safe to use and easier to manage than those produced in animals. Hence, plantibodies are now used for large scale medical, veterinary and other commercial purposes. They are used for treatment of immune disorders, cancers and other inflammatory diseases. Several plant produced antibodies are undergoing clinical trials.

1. The first plantibody created from tobacco was called CaroRx. It is a clinically advanced anti- *Streptococcus mutans* secretory IgA plantibody that specifically binds to the bacterium and protects humans from dental caries [14].
2. A humanized antibody against herpes simplex virus glycoprotein B which was expressed in soya bean [15].
3. Antibodies against *Bacillus anthracis* developed in transgenic strains of tobacco and tested in mice which can prove useful in anthrax epidemic in future [16].
4. Antibodies against ovarian, testicular and colon cancer as well as melanoma, B- cell lymphoma and human papilloma virus expressed in transgenic tobacco [5].
5. Tobacco plantibody against Newcastle disease virus in poultry [17].
6. The production of anti- Ebola virus antibodies has been explored in plants. They used a high yielding geminivirus based expression system in tobacco plant, *Nicotiana benthamiana*, for the production of a mAb (6D8) that protected animals from Ebola virus infection [18].
7. Hepatitis B vaccine (CB-Hep.1) in tobacco plants [19].
8. Vaccine against HIV virus [2].
9. T84.66 is a monoclonal antibody that can recognize carcino embryonic antigen which is a tumor associated glycoprotein was produced from transgenic tobacco by agro infiltration [24].

Pharmaceutical companies involved

Medicago, a Canadian biopharmaceutical Company is undergoing Phase 3 efficacy study of Quadrivalent Influenza vaccine- the first plant- based seasonal flu vaccine candidate to reach final clinical stage [20]. Planet Biotechnology, a Californian company has developed the most successful tobacco made antibody for passive prophylaxis for the prevention of dental caries, named as, CaroRx™. It has also developed two other products- DoxoRx™ for drug induced alopecia, a common side effect of cancer therapy and RhinoRx™, for the treatment of cold [21].A number of other companies such as Prodigene, Diversa, Dow, Maxygen, Fraunhofer USA, Kentucky Bioprocessing, Icon Genetics etc. are involved in production of plant based proteins and antibodies.

Limitations of Plantibodies

1. People may have negative reactions to plant derived allergens, fungal contaminations and pesticides used during farming.
2. Inability to perform post translational modification of produced proteins.
3. 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 etc. [22].
4. Gene silencing in some cases and different patterns of glycosylation [23].
5. Regulatory issues, particularly for therapeutic proteins requiring approval for human use [25].

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