PLANTIBODIES PRODUCTION FOR PUBLIC HEALTH

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Acknowledgement

I have immense pleasure in presenting this project on plantibodies for public health.

Thank you Dr. Pritam chattopadhyay sir for give this Dissertation topic (plantibodies for public health).

The subject is an interesting one. It give me an opportunity to have detailed study on the subject and showed how thing work in the practical world. I came to understand and analyze the importance and the role of plantibodies production.

I had a great time working on the project and I have provided information to the fullest of knowledge and findings.

Summary

Nowadays demand of antibody production is increased to cure different diseases including diabetes, hepatitis and cancer. For that different types of systems are used for the expression of antibody production. But these were not improved the antibody production.plants cells have several benefits in comparison with other eukaryotic cells. As compared to the human cell or other microorganisms, the plant cell is safe and decrease the contamination of antibody production. Plants are more economic than the all others froms of creating antibodies and the technology for obtaining and maintaining them is already present. Plantibodies are safer in use because plants reduce the chance of coming in contact with pathogens. Plantibodies can be made using plants such as tobacco, Tomato, potato, soyabean, alfalfa, rice and wheat. The methods of production and purification of plantibodies as well as the various types of pharmaceutical antibodies produced in transgenic plants.

19.07.2021

Certificate

*This is here by certified that the dissertation entitled "***PLANTIBODIES PRODUCTION FOR PUBLIC HEALTH***"submitted by the student of B.Sc. semester six (University Roll No. 180611610032) for partial fulfilment of the B.Sc. degree is done under my supervision. I wish her every success in life.*

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INTRODUCTION

Plantibody is an antibody produced by genetically modified crops. It is encoded by animal genes but produced in and by the plants. Plantibody was first reported in(Hiatt et al. 1989)with a mouse antibody made by tobacco plant.

Plant + antibodies = Plantibodies

Antibodies are a group of complex glycoprotein and antibodies are produced by specialized white blood cells called B-lymphocytes or B cells and present in the serum and tissue fluids of vertebrates. When an antigen binds to the B cells surface, it stimulates the B cells to divide and mature into a group of identical cells, called plasma cells. As antibodies circulate, they attack and neutralize antigens that are identical to the one that triggered the immune response. Antibodies attack antigens by binding to them. Individual and specific binding activity allows antibodies to be used for a variety of applications, including the diagnosis, prevention and treatment of disease. The basic unit is composed of two identical heavy (H) chains, which are held together by sulphide bonds to form a flexible "Y" shape. Each chain is composed of a variable (V) region and a constant (C) region.

For the production of pharmaceutical antibodies various strategies have been developed. Vaccines are mainly produced in animals for their therapeutic value. Plants do not naturally make antibodies but plantibodies have been shown to function in the same way as normal antibodies. 1st antibodies were expressed in plants in the mid- 1980s by two German graduate students. In present time molecular farming of antibodies using plants becomes commonly when compared to the transgenic animals. Production of recombinant proteins in plants and their administration provides an added margin of safety and scalability (Stover et al, 2014).Six important plant antibodies that have been developed as human therapeutic, six antibodies are- CaroRx, scFvT84.66, Anti- HSV, 38C13, PIPP, EpCAM and NeoRx.

Plantibodies functions in a similar way to mammalian antibodies. It offers numerous unique advantages over conventional methods. First, plants are less prone to mammalian pathogens. This property reduces screening costs of plantibody for bacterial toxins, prions and viruses.

DIFFERENT TYPES OF PLANTIBODY

From the trees we get different types of plantibodies . Below is a description of all those plantibodies. Here are some types of plantibodies:-

- Expression of full length antibody
- Expression of antibody fragments (Fab)
- Expression of single chain scFv or single domain (VL) genes.(Figure-1)

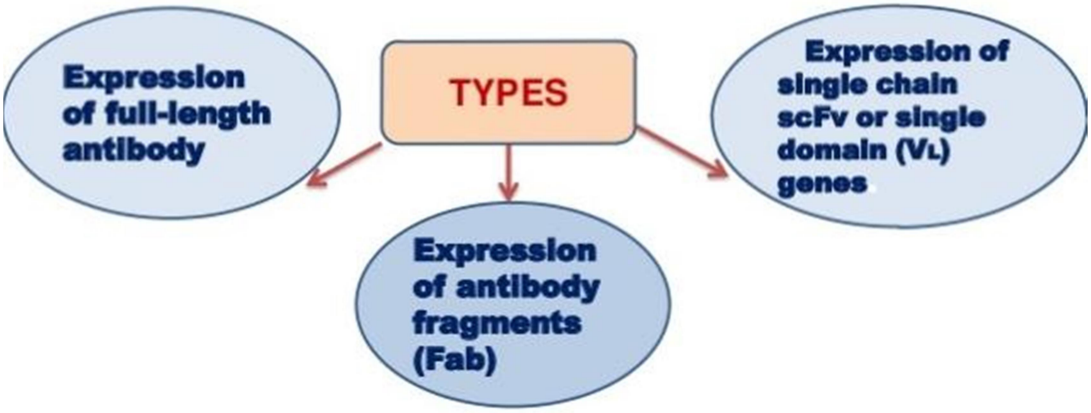


Figure 1:- Different types of plantibodies produced in plants.
(Source:- https://images.app.goo.gl/Yn5pbRw6ZWRDsDea9)

PLANTS USED FOR PLANTIBODIES PRODUCTION

Tobacco can be used in the production of antibodies and other different crops can be used to produce antibodies. These crops are rice, wheat, maize, pea, soyabean, alfalfa, Tomato and potato. But choosing the right crops, must be need many factors consider for the choosing the right ones. Not all threes can be used for plantibody production. The main reason plants are being used to produce antibodies is for treatment of illnesses such as immune disorder, cancer and inflammatory diseases, given that the plantibodies also have no risk of spreading diseases to human. Plant systems for the production of antibodies have clear advantages such as flexibility

to rapidly scale up, reduced input costs and absence of potential risks of contamination with human pathogens. Due to having no side effects fruits, vegetables, plant, seed these we can use easily for plantibodies production.

- CRITERION FOR SELECTING HOST PLANT:- Before choosing the plant we need to look at several important aspect . They are as follows -

  1] The tree has been selected that plant should be easily genetically engineered.

  2] Production level that plant should be high.

  3] You have to look at different aspects of the selected plants like no side effects on the animal body.

- SOURCE OF TRANSGENIC PLANTS :-

  I. Tobacco:- The tobacco plant is notable among the transgenic plants. Because tobacco is the most biomass yields per hectare, so they can be cropped several times a year(Fischer et al, 2003). Tobacco plants grow quickly. As a result relatively enough antibodies are available from here. Tobacco is one of the most important plants used for plantibody production. Because tobacco is most biomass yields per hectare, so they can be cropped several times of a year.

  II.

Figure 2:- Tobaco plant for plantibody productions

Source:-(https://images.app.goo.gl/eQMtYd11tY1jFCDj8)

All these plantibodies are used different types of transgenic plants are used to produce plantibodies. Tobacco is notable among them. Above is a picture (figure:-2) where we can see transgenic Tobacco Plant used for plantibodies production.transgenic tobacco plants are also ideal model organisms for the study of basic biological functions, such as plant pathogen interaction, environmental response, growth regulation.

II Alfalfa and other legumes:- For the production of antibodies from plantibodies alfalfa and soyabean is another a leafy crop. A lot of antibodies are also available from these trees. Alfalfa is a perennial crop from which antibodies are available for a long time. Alfalfa is often the highest yielding forage plant. Other legumes also like that pea a grain legume is a useful plant for production of antibodies. It's content high protein in the seed. The strong advantages of this crop is its tendency to synthesize homogeneous N – glycans, which improves the consistency of recombinant proteins batch to batch (Bardot et al.,2003).

_ Figure 3:- Alfalfa plants used for plantibodies production
Source:-(https://images.app.goo.gl/tiftQeJEij8tMFPy9)

Above is a picture (figure:3) where we can see transgenic alfalfa plants used for plantibodies production.

III. Cereals, seeds and Tubers:- Cereals, seeds and tubers can be used if we need long term plantibodies storage. Rice, wheat, soyabean, pea are widely used as an plantibodies. Rice, wheat, soyabean these are good at room temperature and convenient to transport. Antibodies derived from these grains can be stored at normal temperature for several months and one year(Stoger et al., 2000).

Figure 4-: seeds used for plantibody production

Source:-(https://images.app.goo.gl/5CS2SaiDcEMe28Bt9)

Above is a picture (figure:-4) where we can see the different types of seeds from which the plantibodies are produced.

I. Fruits and vegetables:- We can also get a lot of antibodies from fruits and vegetables. Fruits and vegetables that are eaten raw or partially row from theme passive oral immune therapy available. Here are some examples of fruits and vegetables like – tomatoes, bananas, potato etc from here we get antibodies. Some fruits and vegetables have outstanding properties for pharmaceutical protein production. Tomato is an example of such a fruit. Tomatoes were first used to produce a plant – derived rabies vaccine. Transgenic Tomato based developed edible vaccine expressed cholera toxin B against cholera. Like tomatoes also have high biomass yield, but the production cost is much higher than tobacco. Antibodies released on tobacco and other cereals grains are lasting at normal

temperature for months or even years, but before that tobacco leaves must be dried or frozen. Potato have been used widely for the production of plant- derived vaccines and been administered to humans in most of the clinical trials so far. First demonstrated the potential of potato tubers for antibody production and recently this crop has been investigated as a bulk – production system for antibodies (Wilde et al.,2002).

Below is a table (table -1) showing that antibodies we get from trees and what diseases are those antibodies used for.scFV, diabody, chimeric, IgGI antibody get from tobacco plants used for diagnostic/ contraceptive application. ScFv – fusion antibody get from Barley, potato, tobacco plants used for diagnostics application. SigA/G(caroRx) antibody from tobacco plant used for therapeutic applications. IgG antibody form Corn plant used for contraceptive application. IgG antibody form Arabidopsis plant used for diagnostic application. IgG antibody form Alfalfa plants used for diagnostic application. IgG antibody form Tobacco Plant used for therapeutic applications. IgG antibody form Soyabean plant used for therapeutic applications. ScFv immunotoxin fusion antibody form Tobacco cell culture used for therapeutic applications. One – chain antibody form Algae chlamydomonas chloroplast used for therapeutic applications. IgG antibody form Tobacco Plant used for therapeutic/ diagnostic application. SIgA antibody form Corn plant used for therapeutic applications. scFv diabody from tobacco virus vector used for personalized vaccines.

Table 1:- pharmaceutical antibodies produced in transgenic plants

Antigen	Plant	Antibodies from	Application
Human chorionic gonadotropin	Tobacco	scFV,diabody, chimeric,iggi	Diagnostic/contraceptive
Glycophorin	Barley, potato, Tobacco	ScFV-FUSION	Diagnostic (hiv)
Streptococcus surface antigen sai/ii	Tobacco	Siga/g(CaroRx)	Therapeutic (topical)
Sperm	Corn	IgG	Contraceptive (topical)
Rhesus d	Arabidopsis	IgG	Diagnostic
Human igd	Alfalfa	IgG	Diagnostic
Rabies	Tobacco	IgG	Therapeutic
Herpes simples virus	Soyabean, rice	IgG	Therapeutic (topical)
Cd40	Tobacco cell culture	ScFv immunotoxin fusion	Therapeutic
Herpes simplex virus	Algae chlamydomonas	One chain antibody	Therapeutic
Colon cancer antigen	Tobacco	IgG	Therapeutic/diagnostic
Carcino embryonic antigen (cea)	Tobacco, rice, wheat,pea, Tomato	scFv, diabody, chimeric, iggi	Therapeutic/ diagnostic
Herpes simplex virus	Corn	sIgA	Therapeutic
Non- hodgkins lymphoma idiotypes	Tobacco virus vector	ScFv	Personalized vaccines
Clostridium difficile	Corn	IgG	Therapeutic (oral)
Hepatitis b virus	Lettuce	IgG	Vaccine
New castle disease virus	Corn	SURFACE GLYCOPROTEIN F	Vaccine
Cholera	Tomato	CHOLERA TOXIN B SUBUNIT (ctb GENE)	Oral vaccine
Enterovirus	Tomato	SERUM IgG VP1	Oral vaccine
Porcine reproductive and respiratory syndrome virus	Banana	IgG AND IgA	Oral immunization

PRODUCTION AND PURIFICATION OF PLANTIBODIES

- METHODS FOR PLANTIBODIES PRODUCTION

  FOR THE PRODUCTION OF PHARMACEUTICAL ANTIBODIES IS Done through different methods where plants as bioreactors.

I. Conventional methods:- This is a stable transition for plantibody production. Desired DNA from the transformed host cell is isolated and purified, it can be injected into the embryo of a maturing plant, which we want to use for plantibodies. After using desired gene followed by the propagation of plants in open field allow large scale production of plantibodies.

   Production of plantibodies through conventional method offers several advantages over other methods of antibody production, including low cost of production, high yield of antibodies. This is a safe and effective approach to bring the product to the market within a short time (Doran,1999).

II. In vitro cell and tissue culture:- This method is another economically important method for plantibodies production. Here the plant is harvested at different stages of tissue culture and harvested in the form of cell suspension or micro-propagated plants. Both invitro cells and micro-propagated plants produce enough recombinant proteins within a very short time.

   Many advantages of plantibodies production can be seen through in vitro cell and tissue cultures. Through this method there is no need for sexual reproduction. But this process cannot be used to make edible vaccine production. In this process transgenic stability is increase because crossing over is not seen in this process. In this method the new plantlets can be grown in a short time. The new plantlets and plants are more likely to be free of viruses and diseases. In vitro methods are usually the methods of choice for large scale production by the pharmaceutical industry because of the ease of culture for production, compared with use of animals and because of economic consideration.

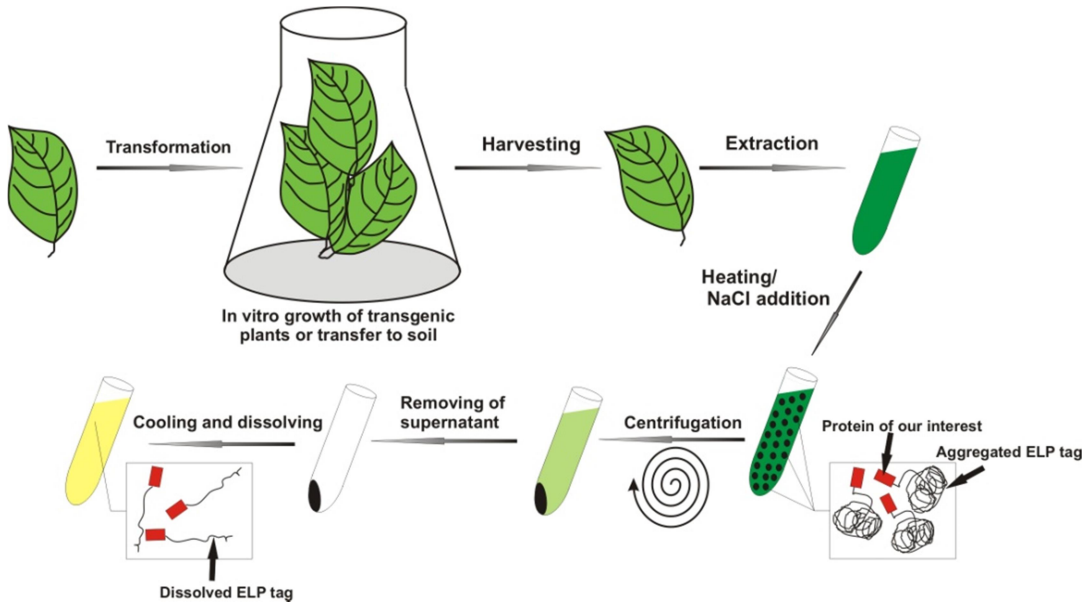
III. Transgenic seeds:- In the case of plantibodies production, if transgenic seed is used instead of green plant tissue, the antibody is stored for a long time. Green plant tissue cannot store antibodies for long periods of time because they contain proteases that degrade the recombinant protein. Moreover transgenic seeds can store antibodies because transgenic seeds contain a low level of proteases(Larrick et al.,1998)

Many advantages and disadvantages of plantibodies production can be seen through transgenic seeds. The main disadvantages include allergic reactions, emergence of super pests and loss of biodiversity. The main advantages of transgenic plants include larger yield, resistance to diseases and pests and capable of growing under stressful condition. Since the advantage is more than the disadvantage, and the disadvantage is rarely seen, use of transgenic seeds for plantibodies production is still a common practice.

- PURIFICATION

The antibodies obtained from the plant can be easily purified and the process of this purification is simple. It is easy to separate plantibody from seed because of the limited range of endogenous proteins. Easy purification of plantibodies makes biopharmaceutical production more economic (Arntzen,1998).As a result of purification, antibodies came out from the cell inside the plant. Also noxious chemicals and contaminants helps antibodies are also found in the trees. The absence of human pathogens in plants eliminates expensive validation of virus removal steps during purification. The main techniques used for the purification of plantibodies are filtration, immuno fluorescence, chromatography, defiltration, polymer fusion and protein asepharose chromatography. Some other techniques used for purification of plantibodies are RIA (Radioimmunoassay), northern blot technique, ELISA (Enzyme linked immune sorbent assay), western blot analysis.

Advantage of plantibody over antibody includes cheap downstream processing. Platibodies are produced without application of antigens or infection microorganisms. Therefore, many seeds of plants allow ample storage capacity without risk of transmitting



diseases to humans.

Figure 5: Purification of plantibodies after production
Source:- (https://images.app.goo.gl/GPzfHUYXswzRvi8M8)

APPLICATION OF PLANTIBODIES

The use of transgenic plants has been observed in that particular special case, they are therapeutic, diagnostic or vaterinary applications.the application of plantibodies are increasing because recombinant DNA is very useful in creating proteins.this technology is a very good for the pharmaceutical industry.

I. Therapeutic Application:- Treatment of infection disease, inflammation, autoimmune disease or cancer.

- Tobacco produced mAB IS MORE VIABLE ALTERNATIVE TO mAB produced in mouse ascites fluid for the large amounts needed for purification of hepatitis b vaccine.

- World's first clinically tested plantibody CaroRx (Fischer et al.,2006)binds specifically to streptococcus mutants, the bacteria that cause tooth decay, and prevents the bacteria from adhering to teeth.
- CaroRx is intended for regular topical prevention administration by both dental hygienists and patients allowing a thorough cleaning and intervention for any existing decay.
- A plantibody based rabies vaccine produced (Ki et al.,2003)in tobacco was experimentally administrated in hamsters to check whether it could effectively target rabies.
- The plantibody proved to be safe and economically feasible alternative method compared to the current antibody production in animal system.
- Tobacco derived plantibodies were experimentally administrated in mice against the Lewis y antigen found on tumour cells in mice and also in lung, breast, ovarian and colorectal cancer.

Table 2: Therapeutic antibodies from transgenic plants

Plant	Antibodies	Treatment
Soyabean	IgG AGAINST HSV-2	HSv treatment
Tobacco	HYBRID IgA-G	Anthrax
Rice	ScFv AGAINST CEA	TuFor HIV
Tobacco	IgG AGAINST HIV	For HIV
Cereal	ScFv AGAINST CEA	Clinical test
Tobacco	IgG AGAINST EBOLA VIRUS	For Ebola virus
Tobacco	IgG AGAINST RSV	For RSV

Plantibodies are now used for large scale medical and veterinary sectors for the treatment of immune disorders, cancer, inflammatory diseases, for the production of vaccines and also for the diagnostic purpose.

Many monoclonal antibodies are used to treat cancer. Monoclonal antibodies are a type of antibody made in the laboratory that can be used in diagnostic or treatment. In cancer treatment, monoclonal antibodies may kill cancer cells.(figure-6) Directly they

may block development of tumor blood vessels, or they may help the following shows how can plantibodies used in cancer treatment.

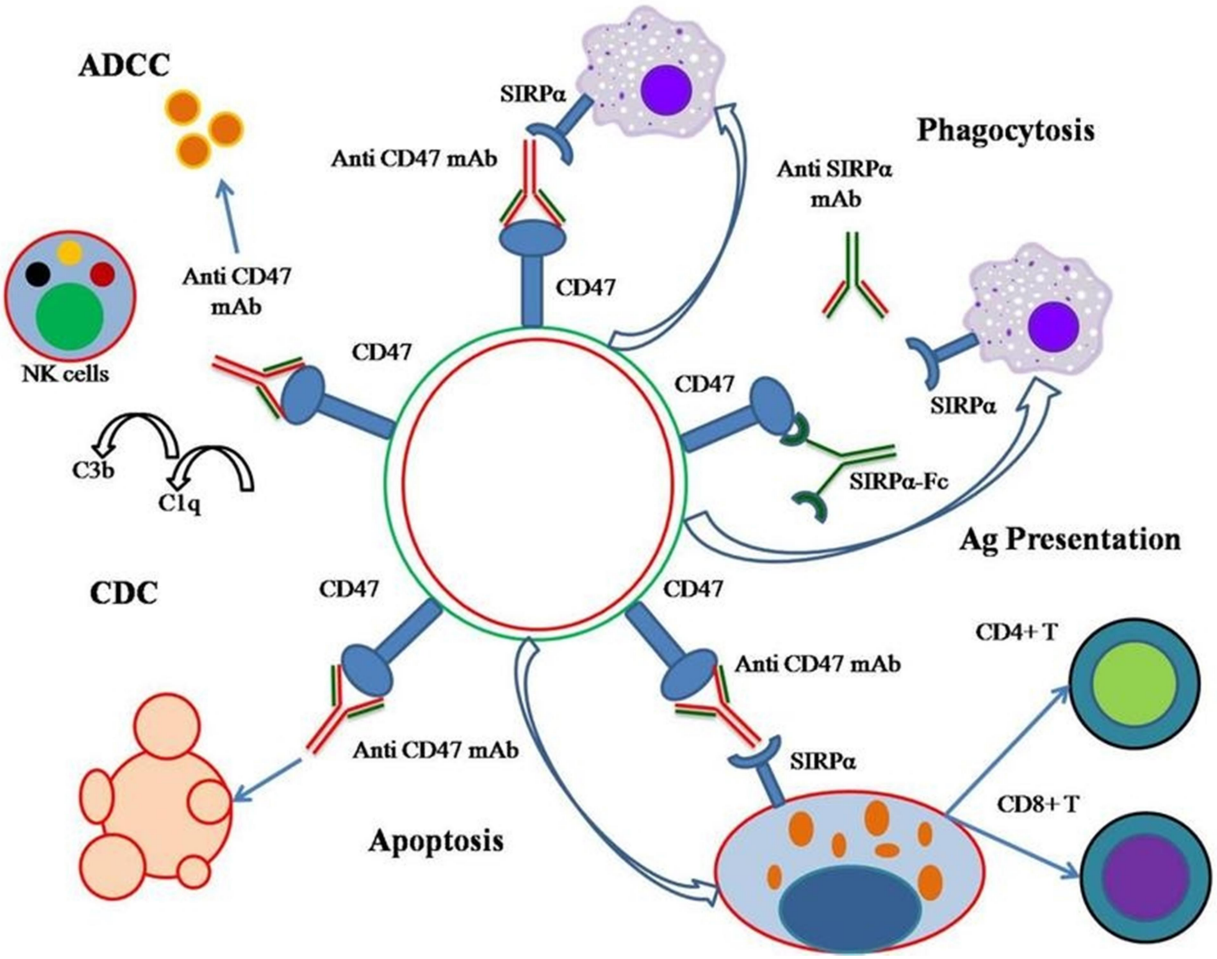


Figure 6:- Use of plantibodies for cancer treatment
Source :-(https://images.app.goo.gl/yP9ryUZmNKTprc7Q8)

II IMMUNIZATION:- There are different types of immunization and they are active immunization and passive immunization.Active immunization is a resistance to disease through the creation of antibodies by the immune system.Passive immunization is the resistance transmitted to recipient in readymade form, preformed antibodies are administered.This immunity usually lasting only for days or weeks.Passive immunity provides immediate protection,but active immunity takes time to develop.

Cytokinesis , hormones, enzymes, epidermal growth factors, interferons,human protein and pharmaceutical foodstuff which are considered for oral immunization(Mason and Amtzen ,1995).

In transgenic plants,they express their antigen in edible tissue.This edible tissue is used to make oral vaccine production.so,if take “edible vaccine” to provide passive immunization and disease prevention.Human diseases for life threatening infections such as diphtheria, cholera and acquired immune deficiency syndrome (AIDS) against for this diseases genetically engineered plants and plant viruses are used to produce vaccine.

III IMMUNODULATION:- Immunomodulation is the alteration of immune response which may increase or decrease the immune responsiveness.

Immunomodulations are divided into three types:- type-1, acting on normal immune system, type-2 acting on immunosuppressived immune system and type-3 acting on functionally normal and immunosuppressived immune system.

Immunomodulation is an important tool for studying the function of an antigen in-vivo. When an artificial abscisic acid (ABA) sink was created by the production of an ABA specific scFv in the endoplasmic reticulum of tobacco and potato plants when physiological and morphologicals changes were observed in plants.

IV USES OF PLANTIBODIES IN MEDICINE:- Plants expressing clinical proteins and polypeptide of pharmaceutical importance such as human C protein, interferons, hormones and cytokines are currently used to provide immunization for example, oral immunization. Edible vaccines expressed in edible tissue of plants have provided immunization via. oral mean. Combining these, genetically engineered plants proved to be superior system for vaccination in humans. Plantibodies are now used for large scale medical and veterinary sectors for the treatment of immune disorders, cancer, inflammatory diseases for the production of vaccines and also for diagnostic purpose. Above is a picture (figure-7) from which we can see medicine was made from plant for disease. Thus we use plantibodies for the treatment of various diseases.

Figure7:- Medicine made by plantibody production tecniques.
Source:- (https://images.app.goo.gl/DPC9HU1ZfNsRsLEDA)

RECENT ACCHIVEMENTS

Genetically engineered plants/transgenic plants used as host for plantibodies production represents a huge prospect for the pharmaceutical. Among all the pharmaceutical compounds, maximum contribution is accompanied by recombinant proteins. Presently, clinical trials are going on a number of plantibodies for their therapeutic role. In therapeutics, CaroRx was the first plantibody produced from tobacco(Fischer et al.,2006). It is *Streptococcus* secretory antibody and protects from dental caries(Larrick et al., 2001). Another plantibody was developed in soybean against herpes simplex virus(Zietlin et al.,1998). USDA has approved CaroRx , a plantibody for humans ; expressed in tobacco, against poultry virus. Recently, antibodies against Ebola virus infection have been explored in Tobacco (*Nicotiana benthamiana*).

- TREATMENT OF EBOLA PATIENTS

Recently anti Ebola virus antibodies has been explored in plants. For the production of a mAb (6D8) that protected animals from Ebola virus infection(Chen et al.,2002). Ebola immune complex was produced by fusing Ebola glycoprotein GP1 to the C – terminus of the heavy chain of humanized 6D8 mAb that binds specifically to a linear epitope on GP1 using the

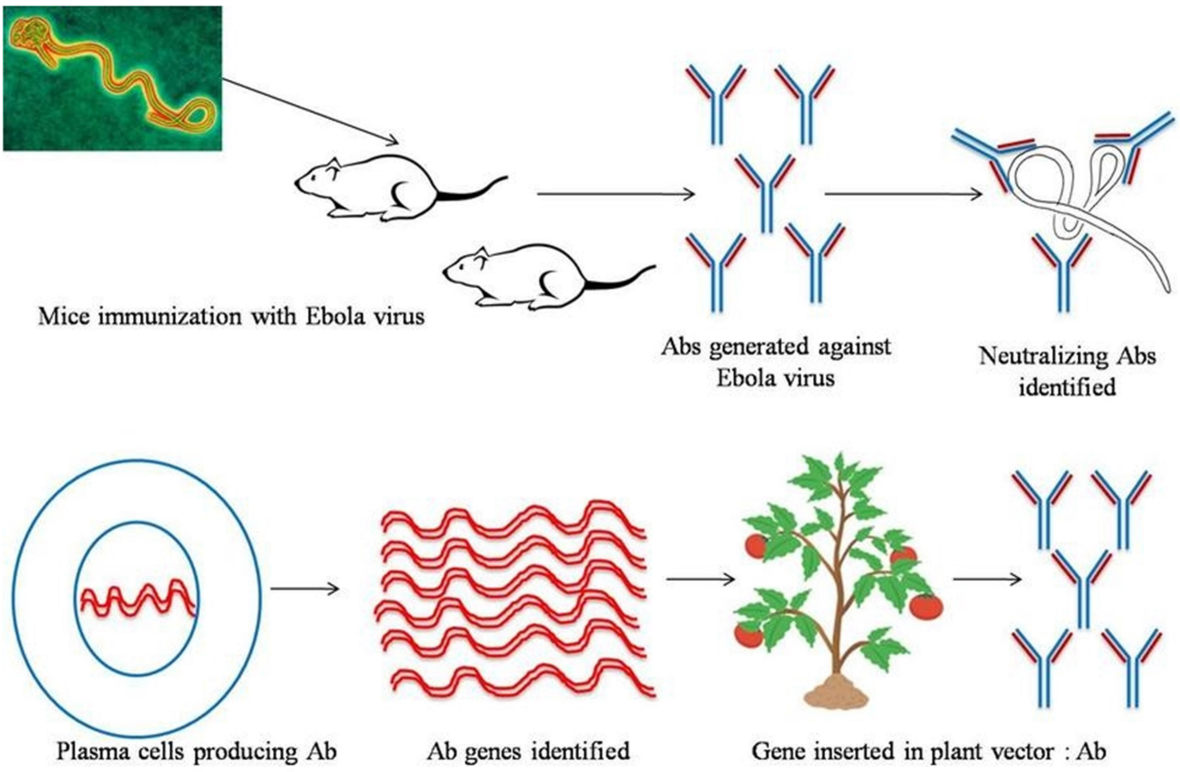


Figure 8:- Use of plantibodies for Ebola virus treatment.
Source:-(https://images.app.goo.gl/CpRj9Py2BZurouHX8)

geminivirus based expression system and *Nicotiana* benthamiana(Bhoo et al.,2011). The recombinant immunoglobulin produced in leaves was then purified by protein affinity chromatography. Subcutaneous immunization of mice with this purified plantibody showed high level of production of anti Ebola virus antibodies that provides protection against Ebola virus. From the picture above (figure- 8) we can see how the Ebola virus is being treated from the plantibodies produced by the plant.

ADVANTAGE OF PLANTIBODY PRODUCTION

Why we used plant for plantibodies production:-

- No ethical issues like animals
- Large scale production without costly instruments
- Low cost and flexible production size
- Easy shipping in form of seeds
- More viable and low risk of contamination.
- Proper post translation modification in ER.
- Ability to express transgenes by crossing

A picture below (Figure:-9) shows why we used plant for plantibodies production.

1. The highest advantage for recombinant protein production has been found from the plant compared with the expression system such as animal systems, Yeast systems and bacterial system.
2. Plants grow quickly and mature after one season of growth for that it is possible to bring the product to the market within a short time.
3. From the tree large scale production of plantibodies for immunotherapy(Stoger, Sack, Fischer ,2002).
4. The cost of producing antibodies from trees is much lower than other system.
5. When producing antibodies from plants toxic compound easily eliminate.

6. There are no ricks during plantibodies production from plants.

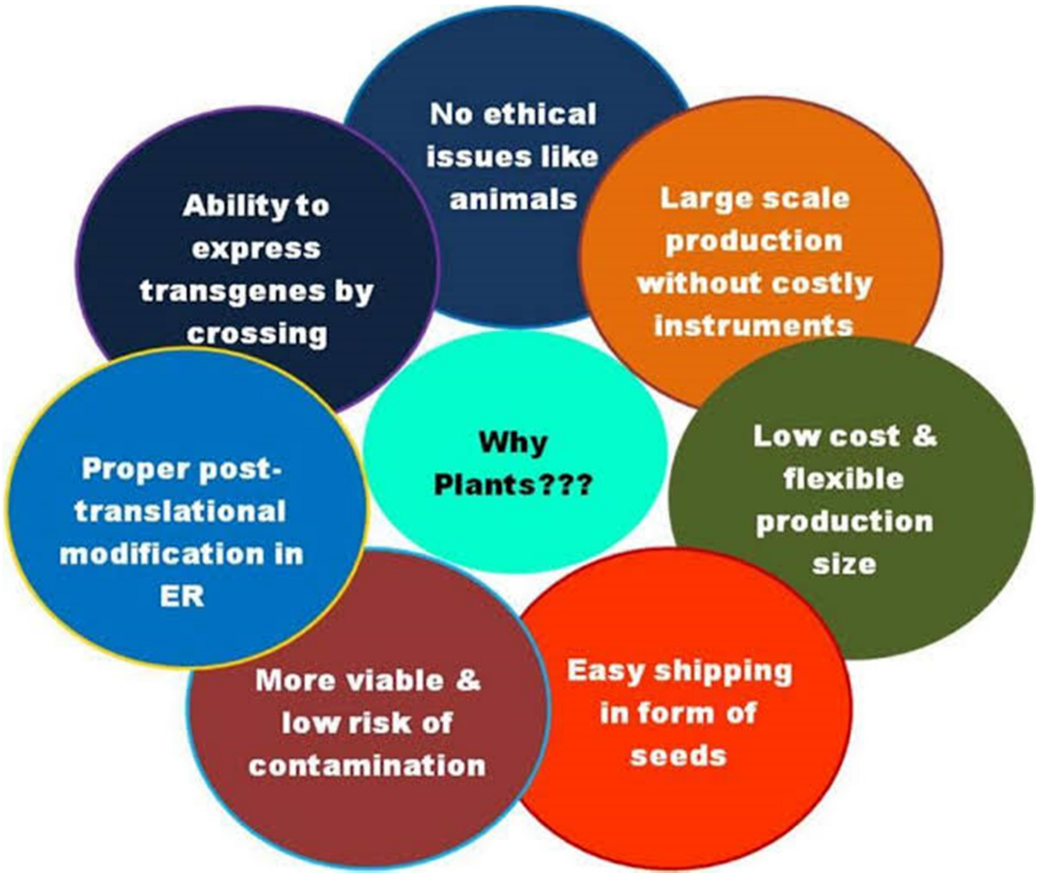


Figure 9:-Benefits of using plants for plantibodies production.
Source:-(https://images.app.goo.gl/XBRShYC5GaBATw3A9)

LIMITATION OF PLANTIBODIES PRODUCTION

There are some advantages to plantibodies production as well as also some limitations. Through several studies people may have negative reaction. This application may have negative reaction to plant derived allergens, fungal contamination and pesticides used during farming. Possibility of plantibodies strains contaminating food crops. We can get around this by using only plants that do not serve for people or livestock. Possibility of transgenic plants to produce allergenic compounds and transfer their antigens into the end products, causing new allergic reactions in the recipient mammal. Other disadvantages of plantibodies production are sporadic transgene

silence, Glycosylation patterns, Inefficient expression, Environmental contamination. Plantibodies are not suitable for infants (Doshi et al.,2013).

NEW APPROACHES FOR PLATIBODY PRODUCTION

I. Targeting and compartmentalization

The different compartment of the cell located in the plant, targeting signals can be used to identify them, such as endoplasmic reticulum, chloroplast, intracellular space etc. Targeting plantibody to the apoplasm via endoplasmic reticulum by the tagging with a small peptide sequence is now achieved to ensure correct folding of the protein(Kusnadi et al.1997).

II. Bioreactors

Plant bioreactors are easy for process scale up and are cost effective also. Human lysosomal enzyme can be produced in plant bioreactors having many advantages. Many plant systems have been developed in order to use plants as bioreactors. Generally the practice has been to used cloned animal cells to make antibodies for use as drugs(Sharma and Sharma,2009). But there is always the remote chance of unwanted allergie reaction due to antibodies of animal origin, and that apart, contamination of the antibody product due to proteins and viruses of animal origin is a distinct possibility. Such problems not arise when plants are used to make antibodies because plants do not generally serve as hosts for human and animal pathogens. For this reason more and more uses of plant bioreactors are coming up these days. These applications were proved in basic agronomy research (Jaeger et al., 2000).

CONCLUSION

Antibodies are the only way we can control viral diseases. Different methods and techniques are used to generate antibodies. Different host cells are used for production of antibody, such as, mammalian cells, insect cells, microorganisms etc. But these methods are expensive, and associated with high health risk such as allergies reaction. On the other hand, transgenic plants are used for recombinant proteins. Transgenic plants have already delivered important therapeutics and edible vaccine. All the antibodies that are produced from the transgenic plants are mostly safe for human and animals. Plantibody technology is now well practiced by the pharmaceutical industries. There are no side effect to used crops and plants for antibodies production. Therefore, according to my openion plantibody provides a better alternatives than any other commercial method of antibody production.

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