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Plantibodies: Advancements In The Frontier of Plant-Based Vaccines

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Abstract

Plant based vaccine production has emerged as a very feasible alternative for the biopharmaceutical industry in recent times since plants prove to be very competent and cost-effective system for generating therapeutic products. Precisely, plant-based production systems are focussed on the field of glycoengineering as it leads to development and production of diverse therapeutic molecules like antibodies or vaccines. These monoclonal antibodies will be really vital for developing disease resistance in humans and animals against existing or continuously evolving disease-causing agents like bacteria, fungi or viruses. This review provides an overview of the recent work done in the area of plant based vaccines, particularly plantibodies, by highlighting selected case studies and provides an overview of the possible applications, advantages and limitations of plantibodies. Interestingly, many plantibodies have been developed, while only some plantibodies have undergone clinical trials and very few have been approved for worldwide usage. There are many research possibilities to be explored and great number of plantibodies need to be developed in order to combat the healthcare problems prevalent worldwide. In this context, further improvisation of technologies are required so that maximum number of the plantibodies with specific advantages can be easily assessed and accepted for use, all across the world.

Keywords:plantibody, bioreactor, transgenic plant, bioreactor

Introduction

Animals and plants are afflicted by numerous diseases across the globe. There areseveral causative agents of dreadful diseases and development of disease resistance among organisms has remained one of the major challenging areas of research since the past five decades.The discovery of antibodies and understanding of their mode of action gave rise to a new avenue of research [1]. The invention of hybridoma technology by Kohler and Milstein in 1975 further led to the possibilities of development of antibodies having specificity and definite properties [1].

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Monoclonal antibodies in conjunction with drugs or toxins emerged as major components for therapeutic use in clinical medicine and were reported to serve as inhibitors of protein function. The advent of gene technology immensely eased the process of antibody engineering, allowing production of antibodies with desired functional properties [1]. Further, the need for antibody productionhavinggood yield and in cost effective manner, triggered researchers to explore various systems which could be used for antibody production. Initially, the production of antibodies was attempted successfully in yeast, myeloma cells lines and later in plant systems as well [1]. In recent times, the concept of development of human and animal vaccines from plant system has brought a major shift in research endeavours worldwide [2].

Historical Perspective

Initially, Hiatt et al., (1989) reported the production of antibodies in plant system. In this study, genes for heavy and light chains of immunoglobulins were cloned in plant expression vector containing 35S constitutive promoter and transformation of recombinant constructs was done in tobacco plants [3]. The transformants expressing either of the chains were crossed in order to obtain plants which had expression of both the immunoglobulin chains. Accumulation of functional immunoglobulins was reported in selected tobacco progenies, however, details about their assembly and in situ localization was not stated. Further, During et al. in 1990, demonstrated the production of antibodies in tobacco plants, [4] and also examined the correct assembly and in situ localization of these antibodies using anti-idiotypic antibodies [1, 4]. In this study, recombinant construct contained both the light and heavy chains of immunoglobulins in addition to β-amylase leader peptide coding sequence under the regulation of pNOS promoter [1]. The site of accumulation of assembled antibodies was found to be in endoplasmic reticulum and also in chloroplasts [1,4]. From the preliminary studies, it was deciphered that critical optimization was required in case of antibody production in plants [1]. It was also inferred that molecular chaperones may play vital role in assembly of immunoglobulins in plants [1]. The recombinant antibodies expressed in suitable expression vector and produced in plants were named as “plantibodies” [5].

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Different approaches for plantibody production

There are various factors like function of promoter, signal peptide, C terminal peptide and their effect on plant tissues which could affect antibody production in plants [6].The conventional method of plantibody production involves transformation and transient expression of selected genes in plant system. The transformed cell is allowed to multiply and proliferate for mass production of antibodies in fields [7;8]. Moreover, plant tissue culture emerges as a more cost-effective approach for plantibody production. The differentiated plant cells are grown in bioreactors and foreign proteins are harvested from biomass or liquid cultures [7, 8].Another method to obtain desired antibody combination is via breeding practices. For selective accumulation of plantibodies, it is preferable for the antibodies to be targeted to specific cellular compartment via tagging with small peptide sequence to ensure protection of antibodies from proteases. In transgenic approach, plantibody production can be targeted via leaf based or seed based approach [9, 8]. The latter approach is more advantageous since seeds contain lesser amount of proteases than leaves and thus plantibodies stored in seeds have better stability due to lower probability of protein degradation [7,8].

The process of antibody purification is critical since endotoxins and mycotoxins, phenolics or any other source of contamination need to be removed from the antibodies. This process involves different techniques namely filtration (ultra/dia) and/ or chromatography. After purification, the antibodies can be assessed for purity, quality and quantity by radioimmunoassay or enzyme linked immuno sorbent assay or Western blotting techniques [7].

Schematic presentation of plantibody production is shown in Figure 1.

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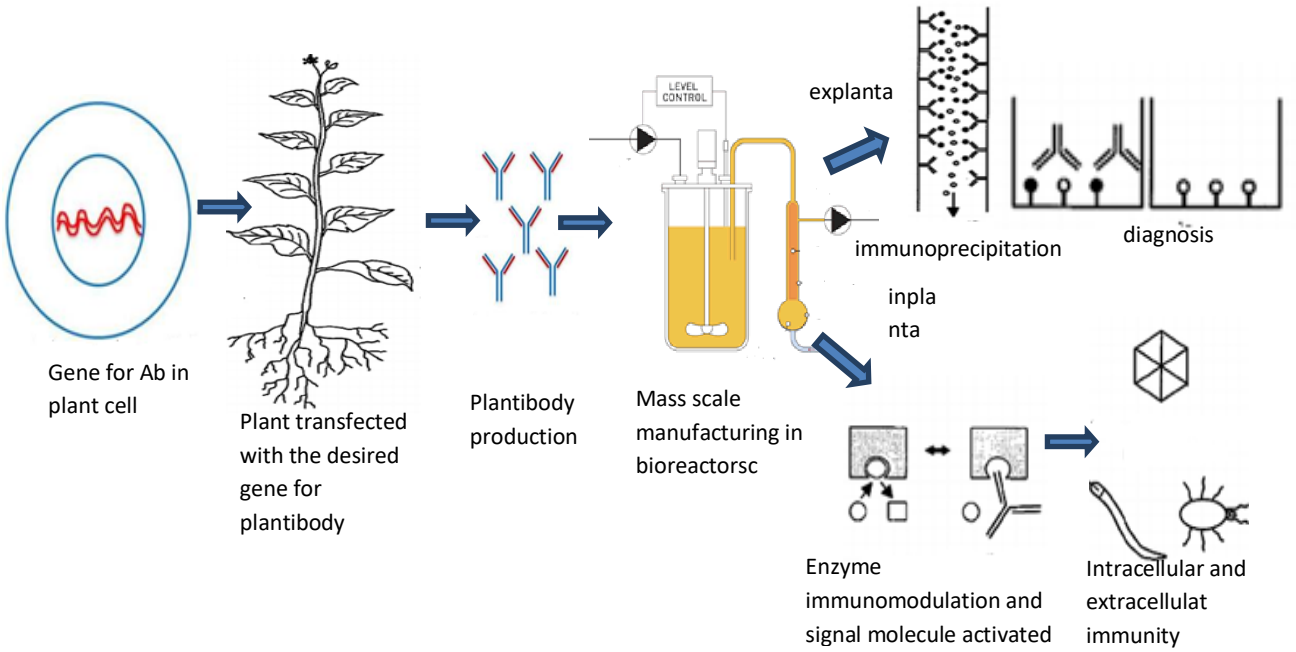

Figure 1 schematic representation of plantibody production in plants

Applications of Plantibodies

Several therapeutic applications of plantibodies have been highlighted so far. Plantibodies can be useful for treating bacterial infections in humans. Interestingly, CaroRx and human intrinsic factor, are a few success stories of plant-based biopharmaceuticals being acceptable in healthcare sector of Europe [10]. CaroRx is the world's first clinically tested plantibody. It binds to *Streptococcus mutans*and doesn't allow bacteria to bind to human teeth surface, thereby preventing dental caries. It is recommended for topical application on teeth. In addition, plantibodies produced against different human diseases can be used for immunization programs. Plantibodies are being attempted to be used for immunomodulation, cancer therapies and other therapeutic usage [7,11]. In fact, over the years, tireless efforts are being made to develop potent plantibodies, to assess their efficacy against the dreaded disease, cancer [11]. In addition, plantibodies have been developed to combat several plant diseases caused by different viruses (ACMV, TMV, CMV etc.), fungus (*Fusarium oxysporum*) and bacteria etc [12].

Recent Case Studies: Evidences of Plantibodies developed for disease control

Several breakthroughs have been made in the past few decades and improved efficacy of plantibodies against various diseases have been evidenced till now [13, 14,15].In many cases, pathogen specific antibodies have been produced in transgenic plants in order to mediate disease control [16, 17]. Notably, the expression of at least 32 different therapeutic

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antibodies, viz monoclonal IgG or IgM have been attempted in plant systems with variable success rates [14].

Advancement in plant biotechnological methods ensured enhanced stability of recombinant proteins in plants [18]. In a particular study, transgenic plants expressing light and heavy chains of neutralizing anti-human immunodeficiency virus type 1 monoclonal antibody, 2F5 in conjunction with elastin-like peptides were found to be improve plantibody stability and also led to simpler process of recovery [19]. Earlier, production of antibodies was more preferable in animal systems, but the advent of plantibodies paved way for a more suitable alternative. Further, a recombinant antibody 2G12, expressed in maize endosperm against HIV-1, was found to effectively neutralize it in animal models [20]. Transgenic tobacco plants expressing various versions of anti-HIV-1 antibody, 2G12, associated with elastin-like peptides were reported to have similar kinetic binding parameters as 2G12 antibody produced In Chinese hamster ovary (CHO) cells and it's HIV neutralization were comparable or even better than the antibodies produced in CHO cells [21]. Further, elastin-like peptides attached to anti-human tumour necrosis factor (TNF) single domain antibodies, produced in transgenic tobacco were found to be very beneficial for prevention of lethal septic shock, when tested in humanized TNF mice [22]. Importantly, elastin-like peptide conjugated hemagglutinins, produced in transgenic tobacco, served as potent a plantibody against swine flu virus (H5N1), when tested in mice [23]. In due course of time, it was also demonstrated that haploid technology for plant derived proteins could lead to speedy and effective accumulation of functional antibodies in transgenic tobacco lines [24]. Fusion proteins have been reported to be beneficial for improved plantibody production and in a specific case study, enhanced accumulation and easy purification of hemagglutinin was found when fused with elastin-like polypeptide and hydrophobin under the control of constitutive CaMV 35S promoter in tobacco [25].

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Specific antibodies namely Anti-CD20 against Non-Hodgkin's lymphoma and

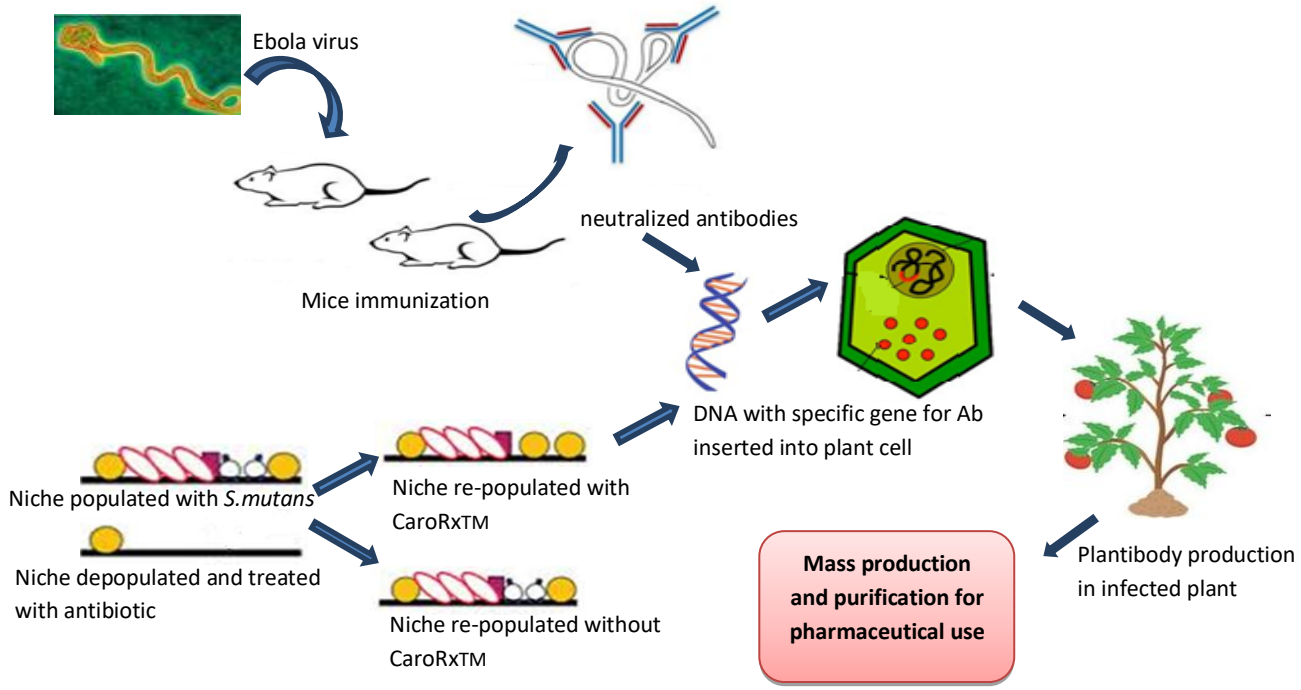

Figure 2 diagrammatic presentation of plantibody production for Ebola virus immunized in mice and CaroRxTM used for tooth infection through S.mutans.

rheumatoid arthritis, Anti-αCCR5 and Anti-HIV gp120 against HIV have been produced via transgenic approach in different plant hosts viz. duckweed, *Nicotiana benthamiana* and maize, respectively and were reported to be in pre-clinical phase [26]. In addition, Anti-Streptococcus surface antigen I/II and Anti-HBsAg scFV antibodies against dental caries and hepatitis B, respectively were produced in transgenic tobacco and have been approved so far [26].

Enterohemorrhagic *Escherichia coli*consists of a virulence factor namely, Shiga toxin 1 (Stx1). Recent research demonstrated that hybrid IgG/IgA, expressed in transgenic Arabidopsis, has therapeutic value and can neutralize the Shiga toxin and thus can be used for immunotherapy against Stx-1 related food poisoning [27].

Ricin is a well-known toxin and agent of bioterrorism in USA. Considerable work has been done to develop somepotent plantibodies which can be used astoxin neutralizing monoclonal antibodies. Among the developed plantibodies, c-PB10 emerged as the most useful therapeutic agent and was successfully tested in mice model [28]. Recently, based on

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in-vitro and *in-vivo* analysis, it was reported that anti-fimbrial protein fimbrillin (anti-FimA) monoclonal antibody, produced in rice cell suspension, can be useful against periodontal diseases caused by *Porphyromonasgingivalis*FimA [29].In recent times, efforts are being made to develop plantibodies against Ebola virus, which has affected huge number of African population [30]. Substantial work is also in progress in order to develop plantibodies against several infectious diseases namely, anthrax, respiratory syncytial virus, West Nile encephalitis, herpes simplex virus and botulism [31] Diagrammatic presentation of ebola vaccine production and *S.mutans* in plants for plantibody production is shown in Figure 2.

Advantages and limitations of plantibodies

The possibility of recombinant antibody production exists in yeast, protozoa, fungi, insect cells, animals as well as plants [32]. Among all these organisms, plants serve as one of the best systems as they act as efficient bioreactors for production of antibodies. They are easy to grow and depend on renewable sources of energy like light, water and soil. The complete process of plantibody generation is cost effective and quite fast. There is a great diversity of plants which can be explored in order to select a suitable plant host for plantibody production based on our needs. The improvised technological expertise gives an advantage to us in order to develop further efficient methods for plantibody production. Most importantly, plants are inherently and environmentally safe as animal or human pathogens do not infect plants, so it is a very feasible system for plantibody production [33]. Currently, the thought of developing new generation green bionanomaterials from antibody-based supramolecular structures or scaffolds is also being propagated, keeping in view the rapid technological advancements [33].

However, there are some major limitationswhich act as hindrances inproduction of antibodies [34]. One factor is maintaining the quality and quantity of antibody being produced in plants. The efficiency of large scale antibody production depends on choice of plant system, which will further govern the possibility of improvement in scale-up, effective ways of downstream processing and storage [34]. In addition, there are chances of degradation of recombinant antibodies which are produced in plants. This situation can partially be tackled by using protein stabilizers along with proteinase inhibitors, but the challenge of in planta proteolysis may not be ruled out completely [34]. It has been revealed that physiological state of plants could also affect integrity of antibodies being produced.

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The process of antibody purification is costly and involved huge investments in biopharmaceutical industry. The appropriate choice of plants and the sites of antibody production (for example, seeds of tobacco and corn) can determine how efficiently the plantibody can be purified and used in large scale [34].

The contrasting glycosylation patterns of antibodies produced in animals and plants can invoke different immune response in either system. Thereby, variable glycosylation patterns remain as major challenge towards establishment of disease resistance in health care sector, specifically concerning humans [34, 35]. In an attempt to humanize the plant glycans, Bakker et al. (2001) did stable expression of human β-1, 4 galactosyltransferase in tobacco plants. Crossing of these transgenic plants with another set of plants, harbouring a murine antibody resulted in plants expressing plantibody having partially galactosylated N-glycans [36]. It has been understood that plant-specific glycosylation patters are very critical in context of plantibody production. Since, several therapeutic glycoproteins have been targeted for production in plant systems as alternative to mammalian cell lines, researchers have focused to develop better understanding about N and O-linked glycosylation in plants and how plant-specific glycosylation can be optimized for improvement in production of therapeutic glycoproteins in biopharmaceutical industry [37]. The most fascinating advancement in plant-based systems in context of glycosylation has been the development of plant systems which lack core xylose and fucose transferase activity[10].

Overall, advancements in regulatory issues related to production of plantibodies in pharmaceutical industry needs to be made [34].

Conclusions and Future Perspectives

Transgenic plants have proven to be the most prominent platform for production of therapeutics and edible vaccines. Not only tobacco but corn, moss and soybeans have been used for therapy for multiple diseases. Low production cost along with high safety in plant systems has paved the way for the mass scale production of pharmaceutical productions. Moreover, mass production in bioreactors has proven to be beneficial by reducing the high expenses of plantibody production. Not, only in diagnostics, plantibody use should be explored in the field of veterinary medicine, immunotherapeutic, improved livestock and other areas. The glycosylation pathways of plantibodies and mammalian proteins are well defined and their processing is underway [38].For intravenous applications of plantibodies, extensive research is carried for the benefit of medicinal world.The biological activity of

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plantibodies, homogeneity and authenticity is being improvised. The challenge still remains for the implementation of mass production under GMP (good manufacturing practices) where uncertain regulatory affairs constraints the implementation of plantibodies. However, this can be overcome by small scale production of developed antibody-based therapeutics [39].

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Disclosure statement

There is no conflict of interest by the authors.

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