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PLANT-BASED VACCINES TO COMBAT COVID-19: STRATEGIES, STATUS AND PROSPECTS

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ABSTRACT

World over, the COVID-19 pandemic has led to an enormous loss of human lives along with huge economic losses. Several COVID-19 vaccines and therapeutic drugs are being developed using different production systems to combat the spread of the disease. Since time immemorial, plants have been used for isolating and extracting phytochemical substances which are directly used for medicinal purposes or are precursors for the synthesis of useful drugs. In recent years, scientists have developed new plant engineering techniques for efficient transient expression of specific genes of interest in plants. The breakthrough technology allows the plants to be used as bioreactors or factories to manufacture therapeutically useful biological products such as vaccines, monoclonal antibodies, immunomodulatory proteins, and drugs on a large scale within a few weeks. The technology not only offers the low-cost production of the desired biological products such as vaccines but also the safety of products due to the absence of pathogens common to both plants and animals. Currently, the world's first plant-based Covid-19 vaccine, *Covifenz*, was cleared for use in Canada. Few others are in the clinical trials or the preclinical stages. This article reviews the status, strategies, and technologies implemented in the development of plant-based COVID-19 vaccines, and the prospects of these vaccines.

Keywords: Covid-19, vaccines, transient expression system, bioreactors, therapeutic drugs.

INTRODUCTION

The coronavirus (COVID-19) epidemic has wreaked havoc around the world. More than 5 million people have died because of the pandemic, which has pushed health systems, economy, and civilizations across the world to breaking point[1].

Vaccination is thought to be a successful technique for preventing and treating Covid-19, as well as ending the pandemic[2]. Shortly after the pandemic began, the scientific community stepped up to the challenge and developed vaccines to defend against SARS-CoV-2. As per the WHO Covid-19 vaccine tracker and landscape, 9 Covid-19 coronavirus vaccines have been authorised for full use, 196 vaccines are in clinical development, and 153 vaccines are in pre-clinical development stage[3]. The main problem today is to get these vaccines to people all around the world, because the pandemic can only be stopped if people in all countries, not just wealthy ones, are immunised and protected. It is the vaccination, not the vaccine, that will put an end to the epidemic. As a result, large-scale manufacture of Covid-19 vaccines is required to meet the needs of countries all over the world.

Vaccine development is the process of taking a new antigen or immunising agent discovered during the research process and turning it into a complete vaccine to combat the disease-causing organism. Antigens are substances that prompt the body to produce an immunological response, such as a complete virus, protein, viral vector, or nucleic acid.

There are numerous research techniques available, such as the use of pathogens (live/attenuated), but recombinant DNA technology or genetic engineering offers the promise of generating effective vaccines against Covid 19 at a rapid pace[4,8]. Recombinant antigenic proteins can now be harvested using specialised plant systems using modern plant-based molecular pharming methods[5]. Studies indicate that plants, in conjunction with plant molecular- pharming technology, appear to be a promising platform for meeting the demand for large-scale vaccine production[6]. This article analyses the technology, status, and methods used in the development of plant-based COVID-19 vaccines, as well as the future prospects of these vaccines.

STRATEGIES FOR PLANT-BASED COVID-19 VACCINE DEVELOPMENT

Plant-based vaccine development involves several steps, including the selection of a suitable antigen producing gene, the insertion and amplification of the gene into the plant, harvesting of the vaccine/antigen, and the evaluation process.

SELECTION OF THE GENE

Proteins are considered ideal antigens for vaccine production[7,8]. The SARS-CoV-2 coronavirus is associated with four structural proteins: the S (Spike), E (Envelope), M (Membrane), and N (Nucleocapsid). Out of these four protein units, Spike (S) protein is the most efficient antigen for vaccine production because of its capacity to bind to the angiotensin-converting enzyme 2 (ACE2) receptors on human cell surface during infection and amino acid sequence diversity among coronaviruses, thus eliciting specific and strong immune response[9]. The genetic sequence of the gene of interest eliciting antigenic responses such as S protein is then integrated into the nuclear genome of a plant.

TECHNIQUES OF INTRODUCTION OF A GENE INTO THE PLANT AND ITS AMPLIFICATION

***Agrobacterium*-mediated nucleus transformation**

The genetic sequence of the gene causing antigenic reactions is integrated into the nuclear genome or chloroplast genome of a plant using a plant transformation vector such as *Agrobacterium tumefaciens* or microprojectile bombardment techniques, causing long-term genetic alterations in recipient cells[10,11.] As a result, a transgenic line producing inheritable antigens is established and seed banks can be created out of it. However, this technology has limitations of inserting the gene randomly and incorrectly. This might cause disruption and silencing of essential genes, and unpredictable levels of gene expression in obtained transgenic plants. However, these constraints can now be resolved by site-directed insertion using several innovative techniques such as genome editing with zinc finger nucleases, TALENs, and the CRISPR/Cas9 system[12.]

According to the recent research, SARS-CoV-1 spike protein has been successfully synthesised in tomato and tobacco plants with low nicotine concentrations[13]. In mice, this plant-based antigen induced the production of immunoglobulin A (IgA) and immunoglobulin G (IgG)[13]. The technology could be utilized to produce Covid 19 vaccine.

Virus Like Particles

Virus-like particles (VLPs) are virus-derived entities made up of one or more different molecules that can self-assemble[14]. They emulate the shape and size of a virus particle but lack the genetic material. They are, therefore, non-infectious, and harmless to the host but stimulate the immune response[14]. Because of this characteristic, VLPs are a viable choice for antigen for vaccine development including Covid-19 coronavirus.

The technology involved inserting specific sequences of viral DNA which code for the coronavirus' outer structure proteins are inserted into the *Agrobacterium tumefaciens* genome. This genetically modified *A. tumefaciens* are then infiltrated into the leaf tissue of *N. benthamiana*. Once inside the plant's cells, the genetically modified *Agrobacterium* delivers the inserted viral DNA to the plant genome where it is copied and translated to produce the virus-like particles. VLP's are extracted and purified to form the material for vaccine. The vaccine can easily be maintained between 2–8 °C, enabling cold chain management with existing infrastructure. This ensures that the vaccine is distributed equally over the world. It's a viable alternative to the currently available vaccinations that require ultracold storage temperatures[15.] According to studies, continued innovation, and enhancement in VLP technology may enable the development of vaccinations with advantages over traditional vaccines, such as fast antigen reproduction and potential room temperature stability.

***In Silico* Approach**

The in silico approach is a rapidly developing science that focuses on locating, evaluating, and combining chemical, biological, and medical data utilising software or internet databases and archives [16]. Antibody epitope reactivity in the immune response is one of the most important aspects of *in silico* vaccine design. The technology enables for a more efficient and faster vaccine manufacturing process by utilising immunoinformatic and computational techniques to anticipate B- and T-cell epitopes (antigen determinants) from SARS-CoV-2's extremely antigenic structural proteins, Samad et al. (2020) created an effective multiepitope vaccine[17]. SARS-CoV-2 structural proteins (surface glycoprotein, envelope protein, and membrane glycoprotein) were chosen from GenBank by the research team. Such bioinformatic and computational genetic engineering technologies could also be utilized to ascertain potential antigens that could then be incorporated into plant genome to produce plant-based vaccines.

Status of Plant-Based Vaccines

To combat the pandemic, several pharmaceutical companies and research groups around the world are working to produce a plant-based COVID-19 vaccine. *Covifenz*, a plant-based COVID-19 vaccine created utilising VLP technology was recently approved in Canada[18]. The vaccine has been developed by Medicago-a privately owned biotechnology company. Kentucky

Bio Processing (KBP) a US. Biotech arm of British American Tobacco company is second in the race to develop a SARS-CoV-2 vaccine candidate (CoVRBD121-NP) using plant-based production and a Tobacco Mosaic Virus-like nanoparticle[19,20]. The vaccine candidate is comprised of the SARS-CoV-2 receptor-binding domain (RBD) of the spike glycoprotein (S) fused to a human IgG1 Fc domain (CoV-RBD121) and conjugated to a modified tobacco mosaic virus (TMV) nanoparticle. This vaccine's pilot batch was made in just 28 days, and it has been given the go ahead to move forward with human testing[19,20].

Newcotiana, a European Union-funded initiative, used innovative plant breeding techniques like CRISPR/Cas9 to modify the genetic sequence of the *N. benthamiana* plant with desirable biochemical components, including antigens required for the COVID-19 vaccine20. The technique promises to generate vaccines at a faster rate in a shorter time[21,22].

Maria Cocaa, researcher of the Spanish Research Council (CSIC) at the Centre for Research in Agricultural Genomics (CRAG) succeeded in engineering a plant virus that produces antifungal proteins inside plant leaves in 2019[23]. This similar technique is now being utilised to manufacture SARS-CoV-2 antigens in lettuce plants as well as *Nicotiana benthamiana*[24]. According to Maria Coca, antigen generation in lettuce could allow for oral immunisation testing.

Daniel Garza, a biotechnologist from the Institute of Biotechnology of the Autonomous University of Nuevo Leon in Mexico, has proposed generating an edible COVID-19 vaccine using genetically modified tomato plants. Bioinformatic and computational genetic engineering technologies will be employed to determine potential antigens that could be produced in genetically modified crops as part of the vaccine development process. The research is at the analysis and selection stage[25].

CONCLUSION AND FUTURE PERSPECTIVE OF PLANT-BASED VACCINES

Plants as a transient/stable expression system could be used to develop vaccines, antivirals, and medicines to combat a variety of critical and emerging infections, such as COVID-19. New plant engineering strategies for efficient expression of certain genes of interest in plants have been developed in recent years by scientists. The new methods not only enable the plants to function as bioreactors or factories, producing therapeutically relevant biological products such as vaccines, monoclonal antibodies, immunomodulatory proteins, and medicines on a large scale in a matter of weeks for various infections including COVID-19, but also ensure product safety by eliminating pathogens found in both plants and animals. However, plant-based vaccines, like any other technique, face hurdles such as dose consistency, antigen selection, plant host selection, manufacturing issues, and unclear immunological correlates in COVID-19 patients.Plant systems, despite these difficulties, are favourable in terms of cost effectiveness and ability to deliver high outputs. Several plant-based COVID-19 vaccines now under research have showed promising results in preclinical and clinical trials, indicating the possibility of a successful vaccine development. *Covifenz,* Medicago's plant-based COVID-19 vaccine produced with VLP technology, has been approved in Canada, and other companies are vying for approval. However, *Covifenz* is unlikely to be listed for emergency use by the World Health Organization.

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