An Extensive Review on the Development of Plant-Based Vaccines against SARS-Cov-2 and Respiratory Disorders Using Plant Biotechnology Platform

ABSTRACT

The COVID-19 pandemic is the most recent of numerous pathogenic viral pandemics that have posed risks to global health. Its exponentially expanding global transmission is placing pressure on the global health system. There are currently a large number of COVID-19 candidate vaccines on the market, and there is fierce worldwide competition to obtain the most vaccinations for each nation. The main objective among several nations during the exceptional COVID-19 epidemic has been to build herd immunity through the planning of large-scale vaccination efforts. However, the availability of vaccines has been a challenge for developing countries. However, there are strains in the world's vaccine manufacturing as a result of the high demand. This international effort aims to develop plant-based heterologous expression systems, virus-like particles (VLPs)-vaccines, antiviral drugs, a rapid supply of antigen-antibodies for detecting kits, and plant-origin bioactive compounds that boost immunity and provide tolerance to fight against virus infection, also includes the use of a plant biotechnology-based expression system for vaccine production. Due to possible benefits including low cost, high manufacturing volume, and physicochemical characteristics, the idea of a plant-based vaccination has gained more and more momentum in recent years. By utilising local commodities, we suggest plant-based vaccines as an appealing alternative to widespread and inexpensive vaccination methods against COVID-19 in this review. Additionally, we discussed the processes of action, necessary standards, and prospects for applying unique biotechnology tools in near future.

Keywords: SARS-CoV-2 virus; COVID-19 vaccine; plant-based vaccines; respiratory disorder; expression systems.

1. INTRODUCTION

A potentially catastrophic SARS-Cov-2 epidemic with fatal respiratory syndrome was reported in Wuhan, China, around the end of 2019. A pandemic has been sparked by this outbreak all over the globe. It has affected 223 nations and resulted in more than 175,306,598 infection cases and more than 3,792,777 fatalities as of June 2021, 16 months after its discovery (WHO) [1]. Over one million people worldwide pass away every year as a result of infectious diseases. Recent years have witnessed a significant number of novel or previously unidentified bacterial, fungal, viral, and parasitic diseases arise. However, many previously treated infections have simultaneously returned or developed resistance to antimicrobial treatments. New viral strains and resurgent infectious illnesses are endangering public health worldwide and are a constant threat [2]. Compared to the influenza A virus subtype H1N1 pandemic, which had a fatality rate of roughly 0.02%, COVID-19 has a greater mortality rate (2.2 %) and transmissibility. Single-stranded RNA viruses known as coronaviruses fall into one of four categories: α-CoVs, β-CoVs, γ-CoVs, and δ-CoVs. Governments are attempting to manage this outbreak by urgent testing and containment. These actions will decrease the spread of infections, lower mortality rates, and stop the healthcare system from failing. Additionally, it will give researchers enough time to create quick diagnostic kits, therapies that prevent infection, and a potential vaccine to immunize the general public.

This pandemic highlights the urgent need for a vaccination that can terminate the disease and, ideally, prohibit the virus from spreading [3]. Pharmaceutical formulations classified as vaccines aim to elicit an immune response against a specific disease [4]. They can be made from disease-causing organisms that have been

killed or rendered inactive, recombinant vectors, protein fragments, or DNA/RNA nucleic acids. The immune system is strengthened during the vaccination process, preparing the body to distinguish and fight against new infectious invaders [5,6]. By harnessing their expertise and platform to produce a solution as quickly as possible in comparison to months or years based on a cell-based platform, researchers working on plant-based vaccines can also play a significant role during this pivotal time [4]. An innovative vaccination idea that has recently undergone testing may be able to address the shortcomings of conventional vaccines. This novel idea refers to the bioengineering of antigens using genetically engineered plants [7]. Vaccines manufactured from plants are referred to as plant-based medicines. In recent years, plant-based vaccines have emerged as a new innovative technology that has drawn significant interest from both academia and business [8,9]. This intriguing method seeks to quickly trigger particular immune responses after oral administration and absorption of the plant-based vaccination. Given that plant diseases are not known to infect humans, one intriguing benefit of plant-based vaccinations is that the probability of contamination by plant pathogens is very negligible or even undetectable [10]. Plant-based production platforms are viewed as an excellent alternative to traditional vaccinations since they can be manufactured quickly and easily [11]. Plant-based vaccines are a good option that can open the door for mass production to satisfy market needs with shorter processing times in addition to the manufacturing process and cost-effectiveness [12]. These unique benefits make plant-based vaccines an appealing idea for quickly creating effective vaccinations for COVID-19, a disease that has suffered an unexpected and sudden outbreak throughout the entire world [13,14]. In this paper, we propose plant-based vaccines as a potential method for widespread immunisation against COVID-19 and talk about the benefits, drawbacks, and future potential of this potent biotechnological resource.

2. THE CONCEPT OF BIO-FARMING WITH PLANT-BASED-VACCINES

A genetically engineered transgenic plant offers the ideal manufacturing infrastructure for substantial biopharmaceutical production. These plants have been extensively utilised for the creation of biopharmaceuticals throughout the past three decades. A vast variety of biopharmaceuticals, including cytokines, growth factors, antibodies, and vaccines, have been created using this method [15]. According to Hiatt et al., transgenic tobacco plants produce antibodies [16]. “The first instance of bio-farming, where the goal is to recover and use only protein products as opposed to the entire plant, was this one. By overexpressing the human serum albumin gene, recombinant human serum albumin is synthesized in transgenic potato and tobacco plants” [17]. These ground-breaking studies let a wave of plant bio-farming in plants [3]. The key benefits of these plant-based viral expression methods comprise preventing the replication of human diseases, making complicated proteins easily, and using straightforward bioreactors [18].

“Measles, cholera, foot and mouth illnesses, hepatitis B, C, and E are only a few of the target diseases for which plant-based vaccine technology has been used” [19]. “Several plant species have been utilised extensively to express foreign antigens in their plant-based sections, including Arabidopsis, alfalfa, potato, soybean, lupine, lettuce, tomato, wheat, cowpea, apple, rice, black-eyed bean, corn, banana, canola, carrot, clover, papaya, peanut, spinach, and tobacco. A few of these have progressed to more advanced preclinical and clinical evaluation stages (e.g., potato, spinach, and lettuce have reached phase 1, tobacco and maize reached phase 2, while carrot cell suspension has reached phase 3)” [20]. The primary mode of action of plant-based vaccines is the expression of a transgene in a chosen plant cell, which stimulates the systemic and mucosal immune systems to combat a targeted foreign pathogen. The transgene does not always require to be integrated into the plant host genome, though. It is possible to express genes rapidly and at high levels using plant viruses, such as the Tobacco Mosaic Virus (TMV), temporary expression by the invasion of tissues with *Agrobacterium*, or direct gene delivery techniques without the use of a carrier [4]. The composition of a plant-based vaccine is meant to act as a source of recombinant antigen produced in the host, biomass, or purified fractions which are meant to act as inducers of protective immunity during administration by various means. This technology has promise for the creation of mucosal-delivered vaccines, especially oral vaccines that require little preparation for processing and administering raw plant material. Many pathogenic agents enter the body primarily through the mucosa after entering through the respiratory, vaginal, or digestive tracts. This causes a secretory immunoglobulin A (IgA) response, which acts as the body's initial line of defence against pathogens. An organised set of

lymphoid tissue structures known as mucosa-associated lymphoid tissue (MALT) structures is coupled to mucosal immune cells [21]. The capacity of mucosal and systemic immune responses to be stimulated, offering two important arms for immunoprotection, is one of the key characteristics of mucosal vaccines. With regard to this, mucosal IgA secretion has been seen in response to several plant-based vaccines given orally, and these antibodies have been detected in both the mucosal site of antigen presentation and additional mucosal sites. Additional proof for the development of mucosal responses comes from the identification of antibody-secreting cells in peripheral blood following oral administration of plant-based vaccinations [22,23].

When given orally, the main technical challenges for subunit vaccines, including plant-based vaccines, are to endure gut digestion, cross the mucus barrier, interact with epithelial and professional sampling cells (e.g., M-cells), and then be absorbed in a way that triggers an immune response and confers protection. Different techniques have been used to shield antigens from digesting enzymes. The use of bacterial strains that have been attenuated and the encapsulation of the antigen in a barrier are the two primary categories. The benefit of attenuated strains is that they direct the antigen to the surface of the colon, where the mucosal immune system can absorb it [23]. However, this strategy could have the same safety drawback as any vaccination based on an attenuated strain. Encapsulation, which can be performed with biodegradable polymers, liposomes, proteosomes, or a product made from transgenic plants expressing the antigen, is a naturally safer means of antigen protection. Protecting these antigens through bioencapsulation in seeds is inexpensive in comparison to other antigen encapsulation techniques, which demand additional processing steps. Plant-based antigens are now produced from genetically modified plants using two different techniques: stable transformation and transient expression transformation systems (Fig. 1). The approach chosen will depend on where the transgene was put into the cells. Given that the desired transgene is inserted into the host's genome,

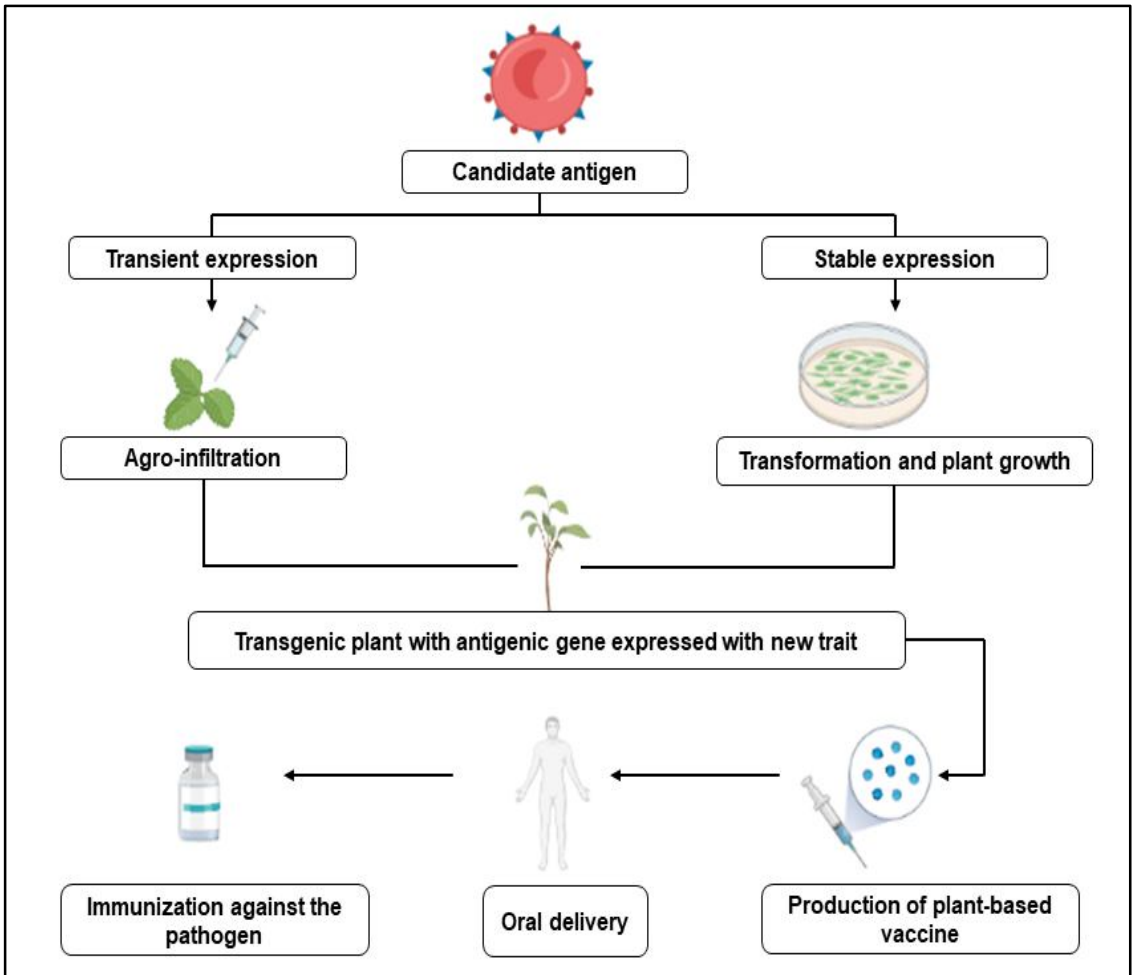

Fig. 1. Concept of plant-based vaccine with stepwise elaboration
(Created with BioRender https://biorender.com/)

the stable transformation system, also known as nuclear and plastid transformation, is defined by its capacity to generate persistent mutations within the genetic material of the recipient cell.

The stable transformation system is the most widely used method for expressing heterologous proteins in plants. However, the lengthy time required to produce changed plant lines has drawbacks. Transient expression systems are the favoured option for producing large volumes of recombinant proteins quickly. These systems can either rely on infecting plants with a modified plant virus, invading plants with Agrobacterium, or combining the two techniques. The temporary expression system is faster in all forms than steady transformation [24]. By using vacuum infiltration technology, *Agrobacteria* (transgenic vector system carriers) can be injected into all aerial regions of the plant and can convert their T-DNA into plant cells [25]. Strong yields, simple scaling, and the availability of industrial procedures are the hallmarks of the *Agrobacterium* delivery-based technique. By using these criteria, it is possible to avoid the difficulties with steady integration [26].

3. THE STATUS OF PLANT-BASED VACCINES FOR RESPIRATORY DISEASE IN MODERN TIMES

"For diseases including Bursal disease virus, influenza, Respiratory syncytial virus, *Streptococcus pneumoniae*, *Bacillus anthracis*, *Mycobacterium tuberculosis*, and asthma, there are numerous plant-based vaccine candidates available" [7]. These vaccines can be produced affordably and safely utilising low-cost bioreactors. It may be taken orally, therefore there is no need to purify the antigen, which reduces production costs significantly.

"Transient expression of VP2 was employed to create a plant-based vaccination against the infectious Bursal disease virus in *Nicotiana benthamiana"* [27]. "Haemagglutinin, a surface glycoprotein involved in influenza virus infection, and M1 protein were employed in a plant-based influenza vaccine (most abundant structural matrix protein in the viral core)" [28, 29]. "D'Aoust et al. reported the generation of enveloped influenza VLPs in the plant in a ground-breaking study" (30). It paved the way for the mass manufacturing of a plant-based H5N1 influenza vaccine based on VLP with a yield that might reach up to 1500 doses per kilogramme of infiltrated leaves [31]. "The expression of HAs from the strains A/Indonesia/5/05 (H5N1) or A/New Caledonia/7/2009 resulted in the creation of VLPs, according to another study (H1N1). They appeared briefly in N. benthamiana" [32].

"Both adults and children might get sick from the lower respiratory tract due to the respiratory syncytial virus. The RSV fusion (F) protein gene was recently expressed in transgenic tomato plants to create a fruit-based consumable subunit vaccination" [33–35]. "The fruit-specific E8 promoter regulated the expression of the F-gene in ripening tomato fruit. Oral administration of mature transgenic tomato fruit to mice caused the development of mucosal and serum RSV-F specific antibodies" [36].

Nearly two million children under the age of five per year die from an infection brought on by Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae (the pneumococcus). Despite the widespread use of pneumococcal vaccinations, S. pneumoniae-related disease is still prevalent. It is mostly caused by the vaccine's lack of serotypes [37]. According to a new study, plants can be modified to produce bacterial polysaccharides, which can act as a protective immunity. Additionally, they illustrated this idea using S. pneumoniae serotype 3 capsular polysaccharides, which are frequently isolated from disease cases (Table 1).

Another condition for which plant-based vaccinations worked well was anthrax. Anthrax is brought on by the Gram-positive bacteria Bacillus anthracis [38]. Even in the harsh climate, its spores can survive for generations. These spores create three anthrax poisons inside the host cells: oedema factor (EF), lethal factor (LF), and protective antigen (PA) [39]. "Spore inhalation spreads B. anthracis along the respiratory tract, resulting in severe respiratory distress, cyanosis, shock, and eventual death. Numerous studies on the use of heterologous expression systems for vaccines, such as bacterial, viral, or plant systems, have been published" [40]. "Plant-based vaccines enhance the immune response in the gut system by gradually releasing the antigen due to their natural bio-encapsulation protection from digestive enzymes. The primary virulence factor that causes anthrax is PA" [41]. Through intraperitoneal immunisation, PA expression in tobacco and tomato induces deadly toxin-neutralizing antibodies in a mouse model. *Agrobacterium*-mediated transformation has recently been exploited to express PA in mustard, which is frequently used as a vegetable for the stems and leaves as well as cattle feed in many different parts of the world [42]. A particular mucosal immune response was seen in those that were orally inoculated (Table 1).

The infectious disease tuberculosis is also prevented by vaccinations based on plant biotechnology. Tuberculosis is brought on by *Mycobacterium tuberculosis*. Droplets released into the air by an infected person can spread it from person to person [43]. "In terms of mortality, TB was even more deadly than HIV, making it a more serious epidemic than was anticipated. Seven oral plant biotechnology-based TB vaccines have so far undergone thorough evaluations in preclinical, experimental, and phase I clinical studies. Ag85B, ESAT-6, MPT64, and MPT83 antigens are all expressed in potatoes" [44,45]. "Acr and Ag85B antigens are expressed in tobacco; LTB and ESAT-6 antigens are expressed in *Arabidopsis thaliana*; CFP10 and ESAT-6 antigens are expressed in carrot, and Mtb72F (Mtb32/Mtb39) and ESAT-6 fused to CTB and its antigens are expressed in the chloroplast of tobacco and lettuce, respectively" [46,47].

The above-mentioned plant-based vaccination study can have a big impact on SARS-CoV2 because it is also a respiratory disease. Medicago Inc. has previously submitted various applications employing the same virus-like particle technology that it employed in the study noted earlier for a plant-based H5N1 influenza vaccine. Although their development has been slower than anticipated, vaccinations based on plant biotechnology are now a reality. It is especially true of oral vaccinations, which include

Table 1. List of a few plant-based vaccines used for respiratory disorders

Candidate vaccine	Plant	Antigen	Animal study	Route of delivery	Effects	References
Streptococcus pneumoniae	Tobacco	Serotype 3 capsular polysaccharide/extracted	MF1 female mice	Intraperitoneal	Anti-pneumococcal polysaccharide serum antibody levels were noticeably higher in immunised mice.	[37]
Bacillus anthracis	Tobacco, Tomato, and Mustard	Protective antigen (PA)/extracted	BALB/c mice	Intraperitoneal/Protein extracted from tomato leaves	A specific mucosal immune response was observed	[42]
Mycobacterium tuberculosis	Potato, Tobacco, Carrot, Arabidopsis, and Lettuce	Ag85B, ESAT-6, MPT64, MPT83, Acr, Ag85B, ESAT-6 fused to LTB, CFP10, ESAT-6, Mtb72F, and ESAT-6 fused to CTB/extracted	C57BL/6 mice, BALB/c mice, Female ICR mice, Seryi velikan strain rabbits.	Orally, intranasal, intraperitoneal	Antigens expression	[44,45]
Respiratory syncytial virus	Tomato	F-gene/extracted	BALB/c mice	Oral immunization/each	Mice were given ripe transgenic tomatoes, which increased mucosal and serum RSV-F specific antibodies.	[36]
Bursal disease virus	Tobacco	VP2/extracted	Embryonated eggs of White Leghorn chickens	Intramuscular	Plant-produced VP2 can stimulate an adequate immunological response in chickens.	[27]
Asthma	Lupin	SSA-lupin/extracted	BALB/c mice	Intraperitoneal	Through a CD4+CD45RBlow T Cell and IFN- γ -dependent mechanism, SSA-lupin consumption increased an Ag-specific IgG2a Ab response.	[48]
Bronchial hyper-responsiveness	Rice	Der p 1/purified	BALB/c mice	Orally vaccinated by feeding 6–8-week-old female BALB/c mice were orally vaccinated	Mice given Tg rice seeds orally experience decreased IgE responses and T helper 2 (Th2) cytokine production in response to allergens (IL-4, IL-5, and IL-13)	[49]

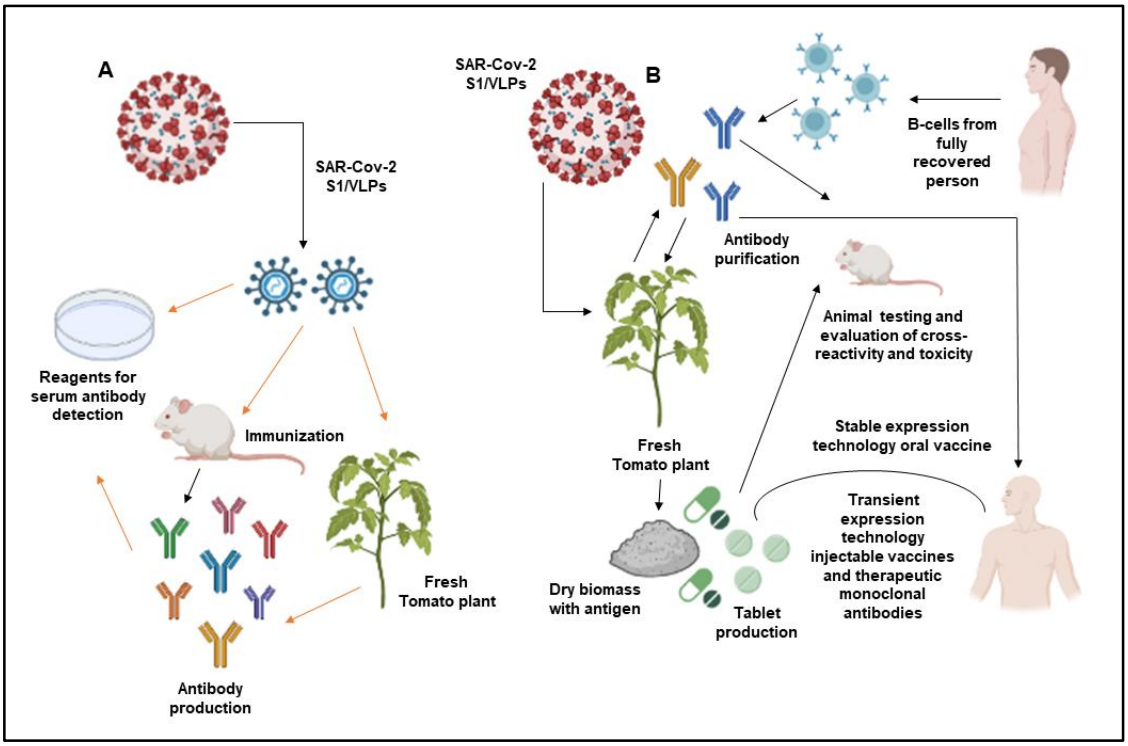

Fig. 2. Applications of plant-based biotechnology toward the development of SARS-CoV2 vaccine candidates and assessment techniques. (A) Plant-based production of diagnostic reagents is indicated by red arrows. (B) Plant-based production of vaccine candidates against the SARS-CoV2 indicated by black arrows

This figure is made using BioRender, (https://biorender.com/)

bioavailability, poor repeatability, and antigen stability as potential downsides [3,50]. Plant biotechnology enables the expression of foreign proteins in plants and suggests a near-term strategy for a viable SARS-CoV-2 vaccine candidate. The type of the targeted antigen will determine the expression strategy. We have explored employing a plant biotechnology platform as a potential strategy for developing a SARS-CoV-2 vaccine in the section that follows (Fig. 2).

As a large-scale manufacturing platform, a tomato plant is displayed as a model plant for both transient expression and stably transformed transgenic plants (Fig. 2). Using genetic engineering techniques, target antigens can be expressed either stably or momentarily, allowing researchers to employ various immunisation strategies. High antigen protein yields in the transformed plants can be purified using the temporary transformation process to create therapeutic monoclonal antibodies or injectable vaccinations. The edible plant species can produce oral vaccination formulations, such as capsules or tablets containing antigens from freeze-dried leaves, using a reliable genetic transformation process. They can also be used as an enhancer.

4. APPLICATION OF PLANT BIOTECHNOLOGY FOR SARS-COV-2 VACCINE DEVELOPMENT:

The protein structures known as virus-like particles (VLPs) and nanoparticles (NPs) resemble native viruses but lack their viral genomes and infectious properties, making them a safer platform for vaccine candidates [51]. Both NPs and VLPs are self-assembling proteins that have the target epitope more densely distributed on their surface. Antigenic epitopes must be repeated in nanoparticles for the innate humoral immune system and B cells to be activated [52,53]. The development of numerous platforms for NPs/VLPs design in the 21st century includes the use of viral core proteins, site-specific ligations-driven covalent links of individual folded

Table 2. Plant-based vaccination candidates for COVID-19 are now recognised by the WHO as being in the trial stage

Vaccine	Vaccine platform	Developers	Transformation method	Expression system	Status	References
COVID-19 VPL Vaccine (CoVLP)	Virus-like particle (VLP)/Spike protein	Medicago Inc. (Québec, Canada)	VLPExpress™ system (Agro-infiltration)	*Nicotiana benthamiana*	Phase 2/3	[54]
COVID-19 Subunit Vaccine (KBP-201)	Protein Subunit	Kentucky BioProcessing, Inc. (KBP)	Agro-infiltration	*Nicotiana benthamiana*	Phase 2	[55]
COVID-19 Subunit Vaccine (IBIO-201)	Protein Subunit/Spike protein	iBio, Inc. (NY, USA)	FastPharming™ system (Agro-infiltration)	*Arabidopsis thaliana*	Pre-clinical	[56]
COVID-19 Subunit Vaccine	S1 and S2 (Spike) and nucleocapsid subunits-based recombinant protein vaccines are being developed using a plant expression vector.	Akdeniz University (Turkey)	Agro-infiltration	*Nicotiana benthamiana*	Pre-clinical	[57]
COVID-19 Subunit Vaccine	Plant-based subunit (RBD-Fc + Adjuvant)/Spike protein	Baiya Phytopharm/Chula Vaccine Research Center (Thailand)	Agro-infiltration	*Nicotiana benthamiana*	Pre-clinical	[9]
SARS-Cov-2	S1 Protein	-	-	Transgenic tomato	Preclinical	[55]
COVID-19 VLP	Virus-like particle/Spike protein	Shiraz University (Iran)	Agro-infiltration	*Nicotiana benthamiana*	Pre-clinical	[57]

proteins, and non-covalent intramolecular formation of de novo protein nanostructure through intermolecular interactions. Self-assembled protein NPs and VLPs both provide highly stable, organised, and monodisperse vaccine formulations as well as advanced bio agricultural production. The most investigated and promising molecular carriers for the creation of the new vaccine are now NPs/VLPs [51]. The phase 2/3 clinical trials for the COVID-19 vaccine candidate from Medicago and the pandemic adjuvant from GlaxoSmithKline (GSK) have begun. Utilizing Coronavirus-Like-Particle (Co-VLP) technology, Medicago's plant-derived vaccine candidate against COVID-19 is made of recombinant spike (S) glycoprotein and expressed as virus-like particles (VLPs). The following table (Table 2) suggests a brief overview of the plant-based vaccines developed for fighting against coronavirus.

We have the ideal platform for creating a vaccine against SARS-CoV-2 thanks to our prior experience creating VLPs for heterologous generated MERS and SARS-CoV-1 antigens in recombinant systems. According to a study, generated VLPs resembled SARS-CoV-1 virions in terms of morphology [58]. According to another study, membrane and envelope proteins (E and M, respectively) are necessary for the effective production of virus-like particles and may be seen using electron microscopy. Immature dendritic cells (DCs) were activated by VLPs made of membrane proteins from various sources, which increased the production of co-stimulatory molecules and cytokine release [59,60].

5. CONCLUSION AND FUTURE PROSPECTS

A global health emergency caused by the COVID-19 outbreak necessitates the development of new vaccinations to combat this pandemic. Candidates for a vaccine based on plant biotechnology present an attractive strategy for controlling this pathogen. The expression platform that is currently accessible provides pertinent guidelines for creating a COVID-19 candidate vaccine. One of the alternate methods for producing vaccines is a transient expression system for deconstructing viral vectors. Tobacco is used as the host plant, which enables quick utilisation of plants as effective, large-scale biofactories for injectable vaccine candidates. The probable loss of exogenous genes and subsequent loss of systemic infectivity are significant drawbacks of this method. Using a subgenomic promoter produced from a different virus can stop this, though. Heterologous genetic recombination will result from it. Another possibility is the VLPs vaccine, which offers an alluring method for creating effective and safe vaccinations that maintain antigenic determinants and have good immunogenicity. The fact that VLPs-based vaccinations cannot be employed for all virus types could be a significant disadvantage for this technique. The VLPs vaccine has a lot of potentials, though, when you consider its benefit. The VLPs platform has a history of success with the previous SARS-CoV-1. Therefore, developing VLPs based on several SARS-CoV-2 structural proteins is a great strategy against COVID-19. An alternative strategy is to create oral vaccinations based on edible plant species that undergo nuclear transformation. Immunotherapy for the mucosa will be administered. The possibility of a plant-based anti-COVID-19 vaccine is encouraged by the existence of currently licenced plant-based influenza vaccines. According to the Coalition for Epidemic Preparedness Innovations (CEPI), the ability of the world's vaccine factories to produce 2-4 billion doses of vaccinations yearly will be insufficient to meet demand by 2023–2024. Along with various restrictions, this capability may also be product-specific. For instance, biosafety level 3-capable facilities are required for the production of whole-inactivated viral vaccines. The regulatory licencing, technology transfer and scale-up of vaccine production, purification, or formulation processes may take time in addition to this administrative procedure, and meeting these needs promptly will continue to be difficult. The shortfall can be filled and the supply/demand balance maintained via a platform of plant-based vaccines. To fully assess the potential of a plant-based vaccination for COVID-19 or any future pandemic, the upcoming years will be critical.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020 Mar;63(3):457–60.
2. Kaufmann SHE, Dorhoi A, Hotchkiss RS, Bartenschlager R. Host-directed therapies

for bacterial and viral infections. Nat Rev Drug Discov. 2018 Jan;17(1):35–56.
3. Rosales-Mendoza S, Márquez-Escobar VA, González-Ortega O, Nieto-Gómez R, Arévalo-Villalobos JI. What does plant-based vaccine technology offer to the fight against COVID-19? Vaccines. 2020 Apr 14;8(2):183.
4. Kurup VM, Thomas J. Edible vaccines: Promises and challenges. Mol Biotechnol. 2020 Feb;62(2):79–90.
5. Govea-Alonso DO, Rybicki E, Rosales-Mendoza S. Plant-based vaccines as a global vaccination approach: Current perspectives. In: Rosales-Mendoza S, editor. Genetically Engineered Plants as a Source of Vaccines against Wide Spread Diseases [Internet]. New York, NY: Springer New York; 2014 [cited 2022 Aug 7]:265–80. Available:http://link.springer.com/10.1007/978-1-4939-0850-9_13
6. D'Amico C, Fontana F, Cheng R, Santos HA. Development of vaccine formulations: past, present, and future. Drug Deliv and Transl Res. 2021 Apr;11(2):353–72.
7. Márquez-Escobar VA, Rosales-Mendoza S, Beltrán-López JI, González-Ortega O. Plant-based vaccines against respiratory diseases: current status and future prospects. Expert Review of Vaccines. 2017 Feb 1;16(2):137–49.
8. Sohrab SS, Suhail Mohd, Kamal MA, Husen A, Azhar EI. Recent development and future prospects of plant-based vaccines. CDM [Internet]. 2017 Dec 26 [cited 2022 Aug 7];18(9). Available:http://www.eurekaselect.com/154118/article
9. Shakoor S, Rao AQ, Shahid N, Yaqoob A, Samiullah TR, Shakoor S, et al. Role of oral vaccines as an edible tool to prevent infectious diseases. av. 2019;63(03):245–52.
10. Ulmer JB, Valley U, Rappuoli R. Vaccine manufacturing: challenges and solutions. Nat Biotechnol. 2006 Nov;24(11):1377–83.
11. Gunasekaran B, Gothandam KM. A review on edible vaccines and their prospects. Braz J Med Biol Res. 2020;53(2):e8749.
12. Dhama K, Natesan S, Iqbal Yatoo Mohd, Patel SK, Tiwari R, Saxena SK, et al. Plant-based vaccines and antibodies to combat COVID-19: current status and prospects. Human Vaccines & Immunotherapeutics. 2020 Dec 1;16(12):2913–20.
13. Esqueda A, Chen Q. Development and expression of against viruses in. In: Lucas AR, editor. Viruses as Therapeutics [Internet]. New York, NY: Springer US. 2021 [cited 2022 Aug 7]:25–38. (Methods in Molecular Biology; vol. 2225). Available:https://link.springer.com/10.1007/978-1-0716-1012-1_2
14. LeBlanc Z, Waterhouse P, Bally J. Plant-based vaccines: The way ahead? Viruses. 2020 Dec 22;13(1):5.
15. Fischer R, Buyel JF. Molecular farming – The slope of enlightenment. Biotechnology Advances. 2020 May;40:107519.
16. Hiatt A, Caffferkey R, Bowdish K. Production of antibodies in transgenic plants. Nature. 1989 Nov;342(6245):76–8.
17. Stoger E, Fischer R, Moloney M, Ma JKC. Plant molecular pharming for the treatment of chronic and infectious diseases. Annu Rev Plant Biol. 2014 Apr 29;65(1):743–68.
18. Salazar-González JA, Bañuelos-Hernández B, Rosales-Mendoza S. Current status of viral expression systems in plants and perspectives for oral vaccines development. Plant Mol Biol. 2015 Feb; 87(3):203–17.
19. De Muynck B, Navarre C, Boutry M. Production of antibodies in plants: status after twenty years: Antibodies in plants: twenty years later. Plant Biotechnology Journal. 2010 Jun;8(5):529–63.
20. Korban SS. Targeting and expression of antigenic proteins in transgenic plants for production of edible oral vaccines. In Vitro Cell Dev Biol - Plant. 2002 May;38(3):231–6.
21. Govea-Alonso DO, Gómez-Cardona EE, Rubio-Infante N, García-Hernández AL, Varona-Santos JT, Salgado-Bustamante M, et al. Production of an antigenic C4(V3)6 multiepitopic HIV protein in bacterial and plant systems. Plant Cell Tiss Organ Cult. 2013 Apr;113(1):73–9.
22. Streatfield SJ. Delivery of plant-derived vaccines. Expert Opinion on Drug Delivery. 2005 Jul;2(4):719–28.
23. Streatfield SJ, Howard JA. Plant production systems for vaccines. Expert Review of Vaccines. 2003 Dec;2(6):763–75.
24. Redkiewicz P, Sirko A, Kamel KA, Góra-Sochacka A. Plant expression systems for production of hemagglutinin as a vaccine against influenza virus. Acta Biochim Pol [Internet]. 2014 Sep 9 [cited 2022 Aug 7];61(3). Available:https://ojs.ptbioch.edu.pl/index.php/abp/article/view/1877
25. Wirz H, Sauer-Budge AF, Briggs J, Sharpe A, Shu S, Sharon A. Automated production

of plant-based vaccines and pharmaceuticals. SLAS Technology. 2012 Dec;17(6):449–57.
26. Laere E, Ling APK, Wong YP, Koh RY, Mohd Lila MA, Hussein S. Plant-based vaccines: Production and challenges. Journal of Botany. 2016 Apr 13;2016:1–11.
27. Gómez E, Lucero MS, Chimeno Zoth S, Carballeda JM, Gravisaco MJ, Berinstein A. Transient expression of VP2 in *Nicotiana benthamiana* and its use as a plant-based vaccine against Infectious Bursal Disease Virus. Vaccine. 2013 May;31(23):2623–7.
28. Rybicki EP. Plant-based vaccines against viruses. Virol J. 2014 Dec;11(1):205.
29. Musiychuk K, Stephenson N, Bi H, Farrance CE, Orozovic G, Brodelius M, et al. A launch vector for the production of vaccine antigens in plants: Plant-produced vaccines. Influenza and Other Respiratory Viruses. 2007 Jan 19;1(1):19–25.
30. D'Aoust MA, Lavoie PO, Couture MMJ, Trépanier S, Guay JM, Dargis M, et al. Influenza virus-like particles produced by transient expression in *Nicotiana benthamiana* induce a protective immune response against a lethal viral challenge in mice. Plant Biotechnology Journal. 2008 Dec;6(9):930–40.
31. Landry N, Ward BJ, Trépanier S, Montomoli E, Dargis M, Lapini G, et al. Preclinical and clinical development of plant-made virus-like particle vaccine against avian H5N1 influenza. Fouchier RAM, editor. PLoS ONE. 2010 Dec 22;5(12):e15559.
32. Le Mauff F, Mercier G, Chan P, Burel C, Vaudry D, Bardor M, et al. Biochemical composition of haemagglutinin-based influenza virus-like particle vaccine produced by transient expression in tobacco plants. Plant Biotechnol J. 2015 Jun;13(5):717–25.
33. Foley DA, Phuong LK, Englund JA. Respiratory syncytial virus immunisation overview. J Paediatr Child Health. 2020 Dec;56(12):1865–7.
34. Meng J, Stobart CC, Hotard AL, Moore ML. An Overview of Respiratory Syncytial Virus. Racaniello V, editor. PLoS Pathog. 2014 Apr 24;10(4):e1004016.
35. Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. The Lancet. 2017 Sep;390(10098):946–58.
36. Sandhu JS, Krasnyanski SF, Domier LL, Korban SS, Osadjan MD, Buetow DE. [No title found]. Transgenic Research. 2000; 9(2):127–35.
37. Smith CM, Fry SC, Gough KC, Patel AJF, Glenn S, Goldrick M, et al. Recombinant plants provide a new approach to the production of bacterial polysaccharide for vaccines. Miyaji EN, editor. PLoS ONE. 2014 Feb 3;9(2):e88144.
38. Abrami L, Reig N, van der Goot FG. Anthrax toxin: the long and winding road that leads to the kill. Trends in Microbiology. 2005 Feb;13(2):72–8.
39. Guichard A, Nizet V, Bier E. New insights into the biological effects of anthrax toxins: linking cellular to organismal responses. Microbes and Infection. 2012 Feb; 14(2):97–118.
40. Baillie LWJ, Rodriguez AL, Moore S, Atkins HS, Feng C, Nataro JP, et al. Towards a human oral vaccine for anthrax: The utility of a *Salmonella typhi* Ty21a-based prime-boost immunization strategy. Vaccine. 2008 Nov;26(48):6083–91.
41. Twyman RM, Schillberg S, Fischer R. Transgenic plants in the biopharmaceutical market. Expert Opinion on Emerging Drugs. 2005 Feb;10(1):185–218.
42. Aziz MohdA, Sikriwal D, Singh S, Jarugula S, Anand Kumar P, Bhatnagar R. Transformation of an edible crop with the *pagA* gene of *Bacillus anthracis*. FASEB j. 2005 Sep;19(11):1501–3.
43. Zenteno-Cuevas R. Successes and failures in human tuberculosis vaccine development. Expert Opinion on Biological Therapy. 2017 Dec 2;17(12):1481–91.
44. Zhang Y, Chen S, Li J, Liu Y, Hu Y, Cai H. Oral immunogenicity of potato-derived antigens to < italic & gt;Mycobacterium tuberculosis</ italic> in mice. ABBS. 2012 Oct 1;44(10):823–30.
45. Pepponi I, Diogo GR, Stylianou E, van Dolleweerd CJ, Drake PMW, Paul MJ, et al. Plant-derived recombinant immune complexes as self-adjuvanting TB immunogens for mucosal boosting of BCG. Plant Biotechnol J. 2014 Sep;12(7):840–50.
46. Rigano MM, Dreitz S, Kipnis AP, Izzo AA, Walmsley AM. Oral immunogenicity of a plant-made, subunit, tuberculosis vaccine. Vaccine. 2006 Jan;24(5):691–5.
47. Uvarova EA, Belavin PA, Permyakova NV, Zagorskaya AA, Nosareva OV, Kakimzhanova AA, et al. Oral

Immunogenicity of Plant-Made *Mycobacterium tuberculosis* ESAT6 and CFP10. BioMed Research International. 2013;2013:1–8.
48. Smart V, Foster PS, Rothenberg ME, Higgins TJV, Hogan SP. A plant-based allergy vaccine suppresses experimental asthma via an IFN-γ and $CD4^{+}$ $CD45RB^{low}$ T cell-dependent mechanism. J Immunol. 2003 Aug 15;171(4):2116–26.
49. Suzuki K, Kaminuma O, Yang L, Takai T, Mori A, Umezu-Goto M, et al. Prevention of allergic asthma by vaccination with transgenic rice seed expressing mite allergen: induction of allergen-specific oral tolerance without bystander suppression: Rice-based oral vaccine against HDM allergy. Plant Biotechnology Journal. 2011 Dec;9(9):982–90.
50. Lee MF, Chiang CH, Li YL, Wang NM, Song PP, Lin SJ, et al. Oral edible plant vaccine containing hypoallergen of American cockroach major allergen Per a 2 prevents roach-allergic asthma in a murine model. Lai HC, editor. PLoS ONE. 2018 Jul 30;13(7):e0201281.
51. Perotti M, Perez L. Virus-like particles and nanoparticles for vaccine development against HCMV. Viruses. 2019 Dec 28;12(1):35.
52. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol. 2010 Nov;10(11):787–96.
53. Chabeda A, van Zyl AR, Rybicki EP, Hitzeroth II. Substitution of human papillomavirus type 16 L2 neutralizing epitopes Into L1 surface loops: The effect on virus-like particle assembly and immunogenicity. Front Plant Sci. 2019 Jun 20;10:779.
54. Ward BJ, Gobeil P, Séguin A, Atkins J, Boulay I, Charbonneau PY, et al. Phase 1 trial of a candidate recombinant virus-like particle vaccine for covid-19 disease produced in plants [Internet]. Infectious Diseases (except HIV/AIDS); 2020 Nov [cited 2022 Aug 8]. Available:http://medrxiv.org/lookup/doi/10.1101/2020.11.04.20226282
55. Pogrebnyak N, Golovkin M, Andrianov V, Spitsin S, Smirnov Y, Egolf R, et al. Severe acute respiratory syndrome (SARS) S protein production in plants: Development of recombinant vaccine. Proc Natl Acad Sci USA. 2005 Jun 21;102(25):9062–7.
56. Sainsbury F, Thuenemann EC, Lomonossoff GP. pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants. Plant Biotechnology Journal. 2009 Sep;7(7):682–93.
57. Mamedov T, Yuksel D, Ilgın M, Gürbüzaslan İ, Gulec B, Mammadova G, et al. Engineering, production and characterization of Spike and Nucleocapsid structural proteins of SARS–CoV-2 in *Nicotiana benthamiana* as vaccine candidates against COVID-19 [Internet]. Bioengineering; 2020 Dec [cited 2022 Aug 8]. Available:http://biorxiv.org/lookup/doi/10.1101/2020.12.29.424779
58. Lu B, Huang Y, Huang L, Li B, Zheng Z, Chen Z, et al. Effect of mucosal and systemic immunization with virus-like particles of severe acute respiratory syndrome coronavirus in mice: Mucosal immune responses of SARS-CoV VLPs in mice. Immunology. 2010 Apr 6; 130(2):254–61.
59. Satija N, Lal SK. The molecular biology of SARS coronavirus. Annals of the New York Academy of Sciences. 2007 Apr;1102(1):26–38.
60. Balke I, Zeltins A. Recent advances in the use of plant virus-like particles as vaccines. Viruses. 2020 Feb 28;12(3):270.

© 2022 Ekwempu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.