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Review Paper

Exploring possible SARS-CoV2 vaccines using plant biotechnology

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Abstract

This decade began with an unprecedented crisis. The Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV2) global pandemic capsized our lives and completely overturned the global infrastructure where healthcare and international economics were affected the most. Due to a high transmissible and mutation rate coupled with a staggering mortality rate, this virus has outdone its closely related precursors – MERS/SARS-CoV in terms of lethality. Scientists and renowned pharmaceutical companies have come under immense pressure to mitigate this problem as soon as possible, for which they have been compelled to think out of the box, as well. The construction and mass production of an efficient and accurate vaccine is the global objective now. Scientists and academicians from all walks of science have come together in this joint venture. During this desperate time, plant science has recently been gaining the spotlight via its production of transgenic plants by stable/transient expression of recombinant proteins, which poses to be a ludicrous technology, primarily due to its high-cost effectiveness. Several established pharmaceutical companies have already started to make capital out of this technology. This review paper aims to highlight the plant system as a stable, upcoming, and efficient manufacturing and delivery system of vaccines.

Keywords: Plant vaccines; plant biotechnology; COVID-19.

Introduction

In late December 2019, some local health authorities reported clusters of patients with pneumonia of unknown cause, which was epidemiologically linked to a seafood market in Wuhan, Hubei Province, China. The local hospitals detected the pathogen via a surveillance technique – "Pneumonia of unknown etiology" that was developed during the SARS-COV outbreak of 2003, which allows timely identification of novel pathogens[1]. However, due to the rapid escalation of the virus, the World Health Organization (WHO) on 11th March 2020 declared the novel corona virus as a pandemic[2]. By 1st June 2020, the World Health Organization had already reported more than 6 million confirmed cases and 371 thousand deaths globally[3].

Corona viruses (CoVs) are a large family, belonging to the family *Coronaviridae* and order *Nirovales*, of enveloped, single-stranded, positive sense RNA viruses which can infect animals and humans, causing respiratory, gastrointestinal, hepatic, and neurologic diseases. These viruses generally have some of the largest genomic sizes, ranging from 27 to 32 kb, amongst RNA viruses. To date, the viruses could be classified into – *Alphacorona viruses*, *Betacorona viruses*, *Gamma corona viruses*, and *Deltacorona viruses*, wherein the *Alphacorona viruses*, *Betacorona viruses*, and *Deltacorona viruses* infect both mammalian and avian species, *Gamma corona viruses* infect avian species. Human Corona virus NL63 (HCoV-NL63), Porcine transmissible gastroenteritis corona virus (TGEV), Porcine epidemic diarrhoea virus (PEDV), Porcine respiratory corona virus all belongs to *Alphacorona viruses.* SARS-CoV, MERS-CoV, Bat corona virus HKU4, Mouse hepatitis corona virus (MHV), and Bovine corona virus (BCoV) and Human corona virus (OC43) all belongs to *Betacorona viruses.* Avian infectious bronchitis corona virus (IBV) belongs to *Gammacorona viruses and* Porcine Deltacorona virus belongs to *Deltacorona viruses*[4-10]. The novel corona virus, which is the causative agent of the ongoing global pandemic, belongs to β corona viruses. All human betacorona viruses are unique from one another; however, they share a certain degree of genetic and structural homology. For example, SARS-CoV-2 genome sequence homology with SARS-CoV and MERS-CoV is 77% and 50%, respectively. A helical capsid, made of nucleocapsid protein (N), encloses the genomic content of the virus. An envelope further encloses the helical capsid[3]. The viral envelope could be associated with three viral proteins – Membrane protein (M), Envelope protein (E), and Spike protein (S). The membrane protein (M) and the envelope proteins (E) are involved in viral assembly functions, while the Spike protein (S) is the pathway through which the virus infects the host cells. Corona virus (*Corona* in Latin means crown) was aptly given due to the crown-like appearance by the protrusions from the viral surface produced by the spike protein**.**

The spike protein also plays a very crucial role in determining the viral host range and in the induction of major immunological

responses. It is composed of three segments – a large ectodomain, a single-pass transmembrane anchor, and an intracellular tail. There is a receptor-binding subunit S1 and a membrane-fusion subunit S2. Recent electron microscopic studies have revealed that the spike protein is actually a clove-shaped trimeric structure with three S1 heads along with a trimeric S2 stalk. During infection, the receptor-binding S1 subunit binds to receptor human Angiotensin-Converting Enzyme 2 (hACE2) while the membrane-fusion subunit S2 fuses the membranes of the virus and the host cell facilitating the entry of the viral genome into the host cell[11-16]. Due to the relative ease with which they can adapt to new environments through genomic mutations and recombinations, now corona viruses are frequently found over a wide geographical distribution[10]. SARS-COV-2, like SARS-COV and MERS-COV, have likely originated from bats, although much more scientific analysis is yet to be done to gather this data[1]. Recent findings suggest that the virus is 96% similar, at the whole genome level, to the bat corona viruses, which likely indicates that bats might have been a possible host of SARS-CoV-2[17,18]. Another study by Lam *et al*. suggests that bats and minks could be potential hosts to the virus, with minks being the intermediate host, and have also revealed that pangolins might be another plausible organism acting as an intermediate host to the virus[19]. These findings suggest that multiple organisms could be host to this virus, indicating a zoonotic transmission of the disease.

The massive surge in the number of cases brought about an unprecedented crisis to humanity globally. As of 11thJune 2021, 174,061,995 confirmed cases of SARS-CoV-2 have been reported with 3,758,560 deaths[20]. With the rampant loss of lives, healthcare infrastructure being already collapsed or on the verge of a collapse, and big economies being severely hampered, there has been an urgent need, now more than ever before, to look for fast and efficient testing and therapeutic approaches to mitigate this problem as soon as possible. This created substantial global pressure on scientists and academicians, all of whom converged on a particular solution, mass vaccination. The availability of the genomic and structural information of the COVID-19 virus made in record time, already existing advanced bioinformatics predictions, epitope mapping, previous knowledge from vaccine candidates of SARS/MERS, and top of that, the visionary approach made by the Coalition for Epidemic Preparedness Innovation (CEPI) enabled to push the fast forward button designing and manufacturing of the vaccine for a "short list of the pathogen with pandemic potential"[20-32]. Yet, the process in itself is not free of hurdles such as – vaccine designing, manufacturing, global distribution, cold chain requirements, and logistics, all of which pose a great barrier to the efficacy of the vaccine manufacturing process and also to timely outreach to the common public.

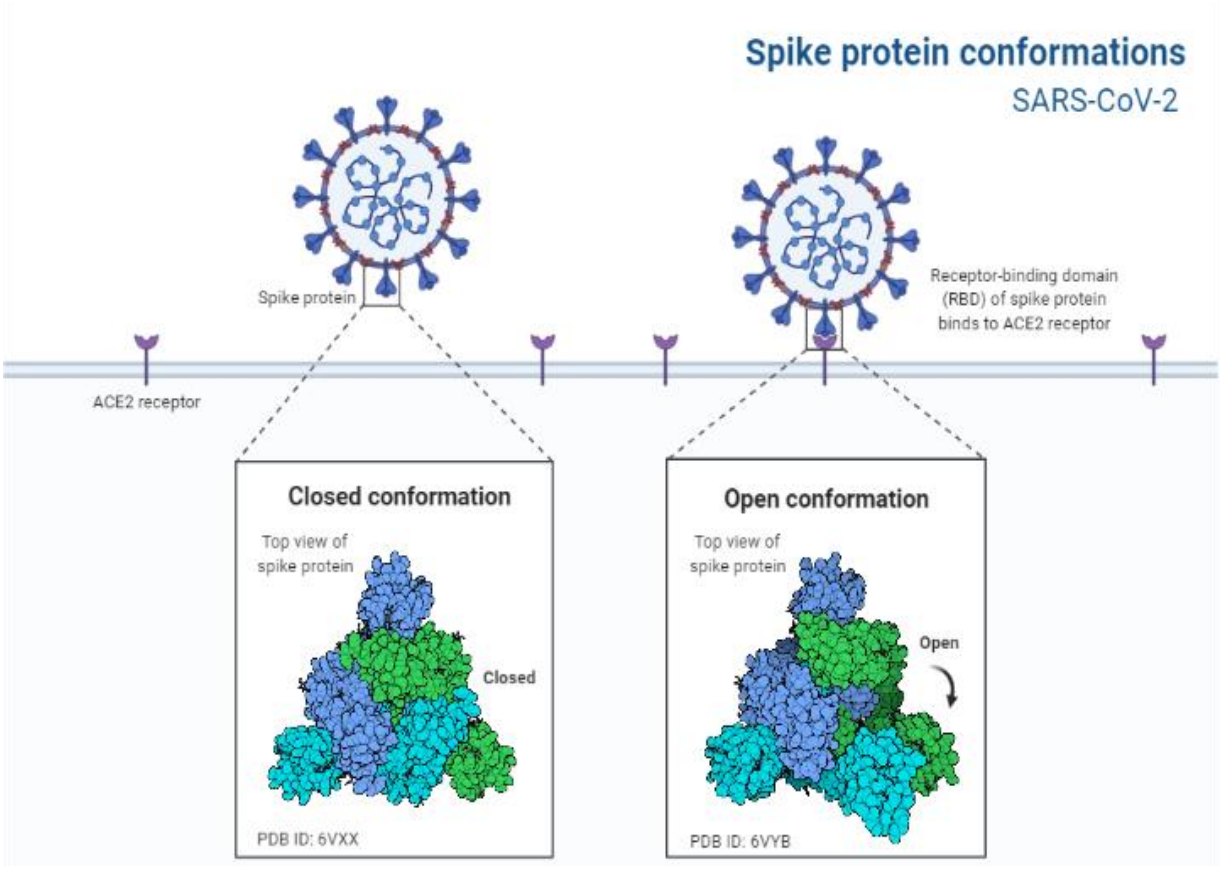

Figure-1: Closed and open conformation of the spike protein of SARS-CoV2.

During vaccine designing, the key characteristics that need to be identified are – the antigen (a foreign material that is capable of inducing an immune response in the body), the adjuvant (an agent which is capable of boosting the immunogenic response towards the antigen delivered), the manufacturing system and the delivery strategy[33]. During this need of the hour, when the demand is excessively higher than supply, we need to venture out and look for alternative strategies, with maybe even more excellent efficacy rates, to meet the demand in time. After the first successful expression of recombinant antibodies in plants, plants were started to be used as biopharmaceutical platforms to manufacture diagnostic reagents and therapeutic proteins[34]. Few specific enzymes and reagents have been commercialized, using a plant as manufacturing systems, such as – Tobacco has been used to manufacture human type I collagen which can self-assemble into fine homogenous fibrils, Tryp Zean by Sigma Aldrich used maize to express the Bovine Trypsin, Human lysozyme and Lactoferrin could be expressed and manufactured in rice. An Israel-based company, Protalix, generated plant-based pharmaceuticals in cultured transgenic carrot or tobacco. In 2012, along with its partner company, Pfizer received approval from the United States Food and Drug Administration (USFDA) for taliglucerase alfa for Gaucher's disease[35]. To date, the only plant-made vaccine that has been approved by the United States Department of Agriculture (USDA) is the vaccine against Newcastle Disease in poultry which is produced using suspension-cultured tobacco cells[35].

The only other plant-based product which was approved is the plant-made single-chain variable monoclonal antibody (scFv mAb) against the recombinant Hepatitis B virus (HBV) in Cuba[36,37]. As a manufacturing platform, plants do have the upper hand over the other media which are based on recombinant mammalian cell cultures such as – Using the transient expression technique via agroinfiltration or viral vectors[38,39], the desired engineered proteins could be produced in transgenic plants, after receiving the required protein sequence[40], as less as within two months. The efficacy of this platform was quite well demonstrated in the case of antibody cocktail production against the *Ebola* virus[41]. In plant-based bioreactors, the recombinant proteins which are produced can be protected from any animal or human pathogens, as these pathogens can't infect plants and can also be stored without refrigeration at a low cost[35]. Plant systems are much more cost-effective than mammalian systems, which require high-priced culture media.

The cultivation cost of transgenic tobacco plants is near about 0.0024$ per liter[42], and the cultivation cost of mammalian systems is near about 59$ per liter[43]. From controlled environmental conditions such as Greenhouse cultivation, Vertical farming to large-scale land farming, the production of the recombinant protein could be accelerated multifold[44-48]. Plants have a eukaryotic endomembrane system, which is quite similar to the mammalian system, and so can facilitate post-translational modifications of proteins, including glycosylation and the assembly of multi-subunit proteins[49].

Understanding conceivable plant vaccine ideas for the prevention of COVID-19

The last two decades have seen a significant shift to plant-based biopharmaceuticals and bioreactor systems, compared to the conventional techniques, because of their ability to produce proteins with such varying complexity, efficacy along with the complete elimination of any mutation or contamination by any pathogens such that they are increasingly becoming our best bet. Plant-derived vaccines are subunit vaccines in which the antigen genomic or protein sequence is expressed in tissues of the desired plant system.

To use plants as platforms for designing and producing vaccines, the pathogenic antigen sequence should have a high level of expression, and the plant system should also be capable of designing quickly and producing new pathogen types in response to the pathogen subtypes[35]. The range of plants and plant tissues that can be used for vaccine production includes[50] – i. Leaf, stem tissues of tobacco of various species and varieties, ii. *Arabidopsis thaliana*, *iii. Medicago sativa* (Alfalfa), iv. Aquatic weeds – *Lemna spp.* (Duckweed), v. Seeds of rice, beans, maize, tobacco, vi. Fruits like tomatoes, strawberries, vii. Root vegetables like carrots, viii. Single-cell cultures of the algae *Chlorella* and *Chlamydomonas*, ix. Suspension cell cultures of tobacco and other plants, x. Hairy root cultures derived from various plants via *Agrobacterium rhizogenes* transformation, xi. Transformed chloroplasts of a variety of plant species.

Various recombinant technologies are followed while using plants as bioreactor systems[35]**–** In stable expression systems, the gene/sequence of interest is incorporated into nuclear or plastid genome using biolistic or via *Agrobacterium*.

***Agrobacterium* based nucleus transformation:** This process entails the introduction of genes into the nuclear genome of the plants with the help of the soil-borne gram-negative bacteria *Agrobacterium tumefaciens*. This bacterium has an extra chromosomal DNA known as the Ti Plasmid, which has been developed as a binary vector in *Escherichia* coli, consisting of a specific site in its genome sequence, which is known as the T DNA and this T DNA can be replaced with pathogen's antigenic protein sequence or the desired genomic sequence. The infection of *Agrobacterium* in plants is mediated by phenolic exudates from a wound site on the plant, which is sensed by the bacterium. These exudates activate the bacterial virulence genes (*vir*) that produce the Vir proteins. The T DNA of the Ti plasmid links with these Vir proteins to make the T DNA complex. When introduced into plants, this T DNA gets transcribed, induces abnormal production of plant hormones which leads to tumor causing Crown Gall disease. After the production of the transgenic line stably expressing the pathogenic antigen protein or the desired genomic sequence, it can be used as a stable source of vaccine and a master seed bank can also be created.

A massive benefit of *Agrobacterium* or biolistic processes based on nuclear genome transformation is the post-transcriptional modification carried out by plants. Plants have particular sugar modifying enzymes that are specific to only them. The genes encoding these plant-specific enzymes could be replaced with mammalian enzyme complexes synthesizing mammalian sugars, enzymes[51]. Yet, there are certain disadvantages to this system – gene silencing, positional effect, low expression levels, and risk of transgene contamination via pollen or seeds, which limits the commercial expansion of recombinant vaccines produced by this strategy[52].

Plastid transformation

Vaccines produced by the transformation of plant chloroplastids could mitigate some of the technical hurdles faced by nuclear transformation. Chloroplast has its genomic sequence, which is smaller than the nuclear genomic sequence. Chloroplastid transformation is generally carried out by biolistic processes (a vector independent direct gene delivery method which is also known as gene gun or microprojectile bombardment method which involves the use of Gold and Tungsten, as a microcarrier, to coat the pathogenic antigen sequence. The DNA is loaded onto a macrocarrier, inserted into a gene gun, and then subjected to high pressure of Helium gas[53-55] or by polyethylene glycol treatment of protoplasts. While integrating the transgene, the antigenic protein sequence along with a selectable marker gene is positioned between the two flanking sequences of the chloroplastid genome so that homologous recombination takes place between the vector and the chloroplastid genome. Compared to injected vaccines, plastid transformed vaccines are much more cost-effective as they can be delivered orally along with their low purification costs[56,57]. As chloroplast follows maternal inheritance, so plants can stably produce proteins without any need to create a generation of transgenic plants via pollination, and also collecting the vegetative leaf tissues prior to flowering also removes the possibility of escape via pollen. The expression of foreign genes is generally high, as there are 10 000 copies of the chloroplast genome in each leaf cell [82]. These vaccines trigger a mucosal response when given orally or injected. A number of chloroplastid transformed vaccines against diseases are already in use (Table-1).

Transient expression with plant virus expression vectors: In transient expression, the epitope of the antigen, i.e., the specific part of the antigen which evokes an immunogenic response upon entry into the host cell, is integrated within a plant virus vector (generally within the coat protein gene). When the desired plant is infected by this viral vector, there is an intracellular production and accumulation of the antigenic epitope, but the epitope and the sequence of the virus never get integrated into the plant genomic sequence. Various plant viruses have already been exploited, such as - tobacco mosaic virus (TMV), cowpea mosaic virus (CPMV), potato virus (PVX), alfalfa mosaic virus, and plum pox virus. The viruses have a higher replicative ability leading to higher yields in vaccine production. Some vaccines against human antigens have already been produced using this technology – Human Papilloma Virus (HPV)[58,59]; influenza virus[60,61]; norovirus. There are several infiltration techniques that are followed for transient expression of genes.

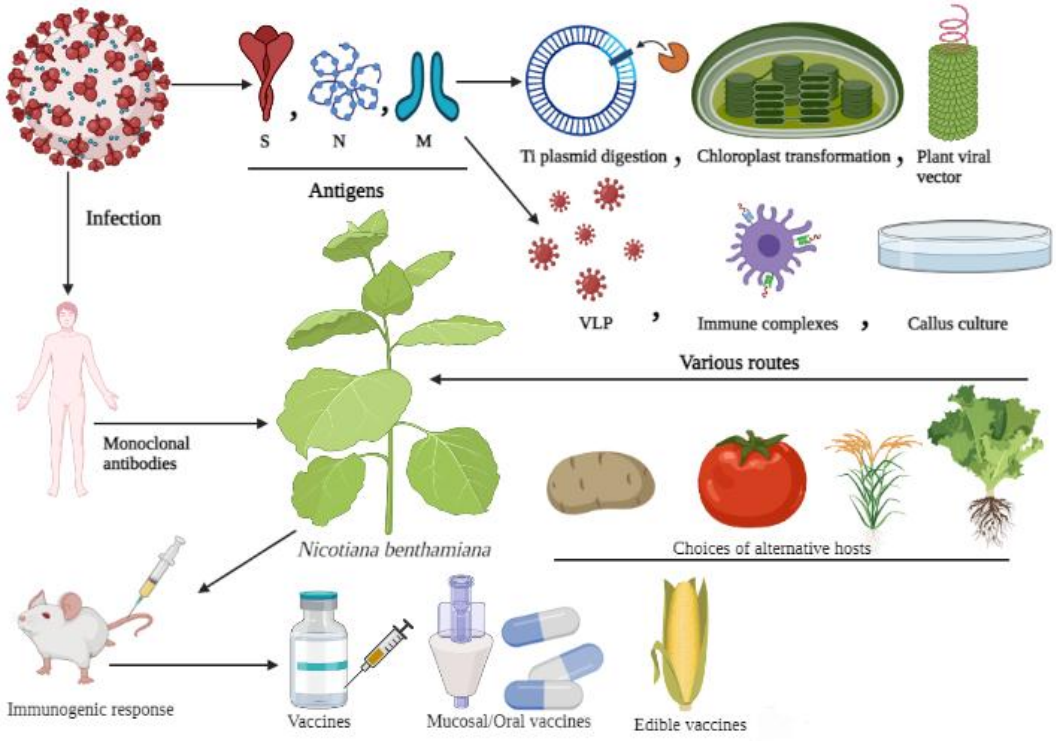

Figure-2: Schematic representation of various routes for plant-based vaccine production and the outcomes.

Popularly known as agroinfiltration, includes the injection or vacuum infiltration of specific plant parts with a bacterial suspension containing the antigenic epitope. This technique was spearheaded by a Canada-based biotechnology company called Medicago, where they developed Virus like Particles (VLP) vaccines, which are molecules that imitates the virus but are not infectious, against influenza HA antigens[62]. In the wake of the recent pandemic, the company developed a plant-based VLP vaccine candidate against COVID-19, named CoVLP, using transient transfection of *Nicotiana benthamiana* and *Agrobacterium tumefaciens* as a vector[63]. As agroinfiltration uses suspension cultures of *A. tumefaciens*, it is much faster compared to the stable expression systems[64].

Another is called Magnifection, which was developed as an enhancement over agroinfiltration for tackling the safety concerns, use of an intact viral vector, and likely transgene loss during systematic spreading. It merges agroinfiltration with the delivery of cDNA encoding for a "deconstructed" TMV-based vector wherein there is a significant amplification of the mRNA. This technique is limited to *Nicotiana benthamiana* by Icon Genetics, a Germany based company[65], named MagnICON^{TM}, which has been used to make vaccines against antigens such as – Hepatitis B surface antigens (HBsAg), norovirus capsid proteins[66,67] and non-Hodgkin Lymphoma vaccines.

Fraunhofer USA. Center for Medical Biotechnology (CMB) had developed a "launch vector," which is an up to date combination of the TMV vector and the *A.tumefaciens* binary plasmid named as pBID4 comprises of the 35S promoter from cauliflower mosaic virus (35S CaMV) that drives transcription of the viral genome, the nopaline synthase (nos) terminator, genes for virus replication and cell-to-cell movement proteins, and the target gene cloned under the transcriptional control of the coat protein subgenomic mRNA promoter. After infiltration, the primary transcripts are transported from the nucleus to the cytoplasm[76].

pEAQ system, based on full-length or trimmed versions of CPMV (Cow Pea Mosaic Virus) RNA-2, is a series of plasmids that permits rapid protein production with substantial efficacy and also without viral replication[68,69]. In this system, a series of binary vectors, such as – 35S CaMV (Cauliflower Mosaic Virus) promoter, nos (nopaline synthase) terminator, P19 sequence coding for a suppressor of silencing, 5' and 3' UTRs' from CPMV (Cow Pea Mosaic virus) RNA-2, wherein the pathogenic antigen sequence is integrated within the UTR sequences.

Virus Like Particles (VLP.): These are macromolecules that imitate a virus but are not infectious as they don't contain the genome of the virus. This bypasses the route for designing vaccines by incorporating dead or attenuated pathogens. VLP platform has been used for a whole range of human antigens, such as – Influenza virus, HPV (Human Papillomavirus), HIV, Foot and mouth disease, Norwalk virus, Rift valley fever virus, and Hepatitis virus[70].

Multiepitopic vaccines: This approach entails producing vaccines by selecting epitopes that can induce strong immunogenic responses in the host. A critical factor that determines the efficacy of vaccines produced via this strategy is genetic variability. The SARS-CoV2 has been observed to evolve into two types – L and S, where the former (near about 70%) predominates the latter (near about 30%) and is also much more virulent[71]. So, while designing multiepitopic vaccines, the epitopic selection must be conserved amongst the viral variants, which must also have the ability to induce a neutralizing humoral response.

Immune complexes: Immune complexes/Antigen-Antibody complexes are macromolecular entities where antigens are bound to their respective antibodies. These complexes are recognized and captured by the antigen-presenting cells[72] which induces a strong immunogenic response, both humoral and cellular[73,74]. IC-based on Tetanus toxin C fused to a monoclonal antibody was produced in transgenic tobaccos, which were shown to induce an immunogenic response when administered subcutaneously in mice[75]. The main disadvantage is that for forming this complex, the antibodies should be obtained in their purest form, which is still not available for the case of SARS-CoV2.

Cell suspension cultures: These are individual cells or assemblage of cells from derivatives or whole callus tissues used to generate a stable cell suspension. Transgenic explants or clusters of cells or even a single callus cell can produce recombinant pathogenic antigens, which can be doubled up in a fermenter.

One of the crucial objectives of vaccine production is the attainment of the desired expression levels of the pathogenic antigen sequence/gene of interest/gene of interest, and for that, many techniques are applied, such as – codon optimization[79] (enhancement in gene expression by increasing the translational efficiency of the pathogenic antigen sequence/gene of interest/ gene of interest/gene of interest by incorporating codon bias, wherein there is an increased tendency of a particular codon to occur more frequently, in the host organism), using strong plant promoters, untranslated leader sequences, signal peptide sequences like KDEL sequence, intron introduction, co-expression of a suppressor for silencing.

Ways to administer a vaccine: Vaccines administered by injections are the most common ways of inoculation. The standard routes are intradermal, intramuscular, and subcutaneous, can induce a strong immunogenic response by preferentially inducing IgG production, and are most suitable against pathogens that attack via the systemic or the respiratory route. This mode of vaccine administration is also known as parenteral administration. The efficacy of this method depends on the route vaccines are administered. These types of vaccines are often produced using tobacco plants as platforms for transient expression.

Vaccines can also be administered via the mucosal method, where there are two routes for administration – orally, nasally. Ideally, oral or nasal vaccines are the ideal type of vaccines as the vaccine antigens can be quickly and directly transported to the circulatory system. Their manufacturing process is simple, and they don't require any extra medical equipment for its administration. There is a major drawback in oral vaccines, and that is the digestion of the antigen in the stomach. Plant cell wall-derived oral and nasal vaccines have effectively addressed this drawback. The cell wall protects the vaccine antigens from the acidic environment of the stomach till it reaches the gut, where the cell wall is digested by the commensal microbes which then release the antigen into the gut lumen. The gut epithelium uptakes the antigens via the specific tags, which are fused for delivery to the particular immune cells. CTB (Cholera Toxin B subunit), LTB (Heat labile enterotoxin B), DC (Dendritic cells) act as carriers for the antigens to the specific delivery cells. The biological properties also get retained in the gastrointestinal tract due to the natural bioencapsulation within plant cell organelles. Plant systems that have been utilized for the development of oral vaccines include – Rice, Maize, Potato, Lettuce, Carrot.

Strategies for development of plant vaccines against SARS-COV2: To develop plant-based vaccines against this novel virus, we need to have a clear understanding of the host-pathogen interaction that goes on when SARS-CoV2 infects. An essential precursor for this field is the already existing vaccine candidates for SARS-CoV-1 and MERS, closely related to SARS-CoV-2. The vaccines which employ individual proteins as antigens with suitable adjuvants in a prime-boost schedule or VLPs with multiple viral antigens could be used against SARS-CoV2. The structural proteins of the virus – Nucleocapsid protein (N), Membrane protein (M), and Spike protein (S) have been reported to evoke neutralizing antibodies and $CD4^+$/$CD8^+$ T cell responses. The spike protein is the most researched one and is the crucial target for the majority of the vaccines being developed. While designing vaccines, the efficacy of producing natural antibodies by the antigens should be considered before the antigen selection. The antigenic mapping of the spike protein mediated by Bioinformatics-based epitope prediction has revealed crucial immunogenic proteins that can be utilized in vaccine production[76,77]. Spike protein-based vaccines could be manufactured in such a way to evoke Antibody-Dependent Cell Cytotoxicity (ADCC) and cross-presentation for obtaining highly efficient cell-mediated immunogenic response[78].

Table-1: List of plant derived vaccines against human antigens[35,56,57,79-96].

Pathogen/ Disease	Antigen	Plant used	Expression	Administration	Clinical phase/Dosage	Observations
Enterotoxigenic *E.coli*	LTB	Potato Maize	Transgenic	Oral	Phase I/ Dosage – Transgenic potato tubers (0.4 – 1.1 mg) were given to volunteers on days 0, 7 and 21. Maize derived LTB (controlled group)-2.1 g of either transgenic or wild type maize germ meal suspended in water on days 0,7 and 21	LTB specific IgA cells were detected in peripheral blood after one week of vaccination. The serological survey indicated that the volunteers had a 91% increase in LTB specific IgG and a 20% increase in IgA
Norovirus	Capsid protein	Potato	Transgenic	Oral	Phase I/Dosage –500 µg of recombinant VP1, of norovirus, on days 0, 7 and 21/days 0 and 21.	20% of volunteers developed norovirus specific IgG
Hepatitis B virus	Viral major surface protein	Lettuce Potato	Transgenic	Oral	Phase I/Dosage–HBsAg transgenic lettuce leaves (0.1–0.5µg HBsAg per 100g leaf tissue) were given to volunteers (primarily 200g subsequently 150g within two months). Volunteers for transgenic potato tubers had received HBV vaccine within 15 years. The control group was given non-transgenic potato	Two out of three volunteers showed transient levels of protection comprising HBsAg specific IgG within two weeks after vaccination. 52.9% of two test group volunteers and 62.5% of third test group volunteers showed high serum

					tubers, and the test groups were given 100g of transgenic tubers (850 ± 210 µg of antigen) on days 0, 28. A third test group was vaccinated with the same dose on days 0, 14, and 28.	levels of HBsAg antibodies over the 70 day follow-up period after the first vaccination.
Rabies	Glycoprotein and Nucleoprotein (chimeric fusion peptide – G5-24-31D)	Spinach	Viral vector (Fusion chimeric protein fused with Alfalfa mosaic virus coat protein and integrated into TMV lacking its coat protein)	Oral	Phase I	Phase I: Three volunteers, who had previously received the already existing rabies vaccine, showed a spike in rabies specific IgG antibodies after having three doses of Spinach (20 g – 84 µg of chimeric rabies peptide antigen each) within two weeks of vaccination
Influenza virus (H5N1)	HA (Influenza Hemagglutinin)	*Nicotiana benthamiana*	Launch vector	Intramuscular	Phase I	
Influenza virus (H1N1; 2009 Pandemic)	HA	*Nicotiana benthamiana*	Launch vector	Intramuscular	Phase I	
Influenza virus (H5N1)	HA (H5; VLP.)	*Nicotiana benthamiana*	*Agrobacterial* binary vector	Intramuscular	Phase I/Phase II/Dosage – In Phase I trial, 5/10/20 µg of H5-VLP was administered subcutaneously with alum as adjuvant. Phase II clinical trial of H5-VLP was conducted as a randomized, a placebo-controlled, dose-ranging study that used 20, 30, or 45 µg of H5-VLP	The vaccine induced Hemagglutinin inhibition Titer at all tested doses. After six months of vaccination with H5-VLP, the volunteer group showed cross-protective $CD4^+$ vT-cell responses, which were not observed in the placebo group, indicating strong Induction of long-term cell-mediated immunity by plant-made H5-VLP.
Influenza virus (H7N9)	HA (H7; VLP.)	*Nicotiana benthamiana*	*Agrobacterial* binary vector	Intramuscular	Phase I	
Cholera	CTB.	Rice	Transgenic	Oral	Phase I	
Anthrax (*Bacillus anthracis*)	PA (Protective antigen)	Tobacco	Transplastomic			Immunized mice produced high-titer IgG antibodies

						against anthrax almost 1:320000; 100% protection was observed in immunized mice after challenge with a lethal dose of Bacillus anthracis
Plague	F1-V	Tobacco Lettuce Carrot	Transplastomic Transgenic Transgenic	Oral		Orally immunized mice produced high-titer IgG1, Ig G 2a, IgA, and 88% of mice were protected after a lethal dose of *Y. pestis* challenge. Immunized mice had higher IgG1 and IgG2 levels.
Human Papilloma virus			V.L.P. expression			
H.I.V.	p24 capsid protein SIV major surface glycoprotein[94] gp 41 derived novel molecule integrated into C terminal of CTB was used as an adjuvant [95-96] Tat monomer[97]	Tobacco Maize *Nicotiana Benthamiana* Spinach	Transgenic Agroinfiltration Recombinant TMV	Oral		Produced mucosal and serum antimembrane-proximal region (MPR) antibodies in mice after mucosal prime-systemic boost immunization.
Tetanus toxin	*tet* C bacterial and synthetic	Tobacco	Transplastomic			
Amoebiasis	lec A	Tobacco	Transplastomic			
Rotavirus	VP6/7	Potato tubers	Transgenic	Oral		
Lyme disease	OspA OspA-T	*Nicotiana tobacum*	Suspension cell culture transient expression			

Table-2: List of ongoing plant derived vaccines against SARS-CoV2[70,97-105].

Company/University	Antigen	Plant used	Expression	Clinical phase/Dosage	Observations
Medicago partnership with GlaxoSmithKline (GSK.)	Spike protein sequence in the form of VLP.	*Nicotiana benthamiana*	Transgenic (*Agrobacterium* mediated)	Phase I completed Phase II ongoing	Vaccine candidate developed antibody responses in the clinical trial volunteers after two doses with mild side effects.

British American tobacco and its US biotech subsidiary Kentucky Bioprocessing (KBP)	Genomic sequence of SARS-CoV2	*Nicotiana benthamiana*	Transgenic (*Agrobacterium* mediated)	Pre-clinical trials	
Department of Nanoengineering - University of California, San Diego - Researchers of Nicole Steinmetz's lab	B and T cell epitopes from the spike protein of SARS-CoV2		Transient expression with plant viral vector – Cow pea mosaic virus (CPMV) like particles		
Toronto, Canada	Viral deubiquitinase bound with the synthetic peptide. The ORF1a of the Corona virus has a protease with a deubiquitinating activity that protects the virus from the host immunity. A synthetic peptide, consisting of 80 amino acids, known as Ubiquitin variant (UbV) created by phage display library design could tightly bind with the DUB site, thus blocking its activity.		Transient expression with plant viral vector – N terminus of the coat protein of Papaya mosaic potexvirus. The UbV: CP (Ubiquitin variant:Coat protein) can assemble into a VLP.		
Baiya Phytopharm /Chula Vaccine Research Center	RBD-Fc+adjuvant			Pre-clinical stage	
iBio, Texas; Candidate – iBio 200 and iBio 201		*Nicotiana benthamiana*	V.L.P. - agroinfiltration	Pre-clinical stage	IBIO-201 showed a noticeably higher titer of the anti-spike neutralizing antibodies compared to IBIO-200. So IBIO-201 was selected as the leading candidate vaccine

Conclusion

As desperate times need desperate measures, scientists and big pharma companies have been forced to step out of the ordinary and think otherwise. Plant-based platforms has it's own bottlenecks as well, just like every other alternative out there – dosage inconsistencies, selection of antigen, and host plant system, unknown immune complications which the virus might trigger. Despite these challenges, plant-based vaccine development is gradually gaining the spotlight because of advantages in some fundamental regions such as – low cost of production, mass-scale production with high efficiency, minimal requirement of cold storage, complete elimination of pathogenic contamination, and also the fact that plants, also being eukaryotic systems, can function on a similar molecular level at par with mammalian systems, i.e., post-translational modifications are also carried out in plants. The manipulated crop plants can be grown in diverse environments, ensuring the continuous supply of vaccines using the existing infrastructures for agricultural production and distribution chain without the need for cold storage chains. Several plant-based COVID-19 vaccines currently being developed have shown promising results in their pre-clinical and clinical phases, indicating a potential for the successful development of an effective vaccine.

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