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Prospects of plant based edible vaccines in combating COVID-19 and other viral pandemics: A review

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ABSTRACT

Many pathogenic viruses have always posed serious threats and challenges to human existence by causing epidemic and pandemic outbreaks and losses of millions of lives every year. Recently, a novel corona virus, COVID-19 has been creating havoc with huge numbers of casualties the world over. Vaccination with a suitable vaccine is the hope for the survival of humans on earth. Vaccines are used as prophylactic as well as curative measures against viral diseases and are effective in eradicating deadly pathogens. Conventional vaccines though effective but have high production costs, involve tedious purification processes and have bio safety issues, requiring time-consuming tests and procedures for commercial production whereas plant-based vaccines offer several advantages over the conventional systems such as ease of production, suitable folding of foreign protein, target protein stability, higher yields, storage, and safety. Plant based edible vaccines can play a major role in immunization to control the viral diseases across the world, especially in poor and developing countries as these products can be easily scaled up for millions of doses within a limited period and can save millions of life who now die for lack of access to the traditional vaccine. The edible vaccine has the potential to solve the problem of bioterrorism by immunizing against a wide range of different dreaded viruses and can save the earth from any future epidemic and pandemic. It could become the best and cheapest alternative to conventional vaccines, for which there is a great need to strengthen research and development activities in this promising area so that in near future children may get immunized by foods instead of painful immunization through needles.

Key words: Bioreactors, COVID-19, human viral diseases, pandemic, plant edible vaccines

INTRODUCTION

The last ten decades has witnessed many deadly viral pandemics. The first and most severe pandemic known as Spanish influenza, occurred in 1918 and was caused by an H_1N_1 influenza A virus (IAV) strain (Jordan, 2019). Approximately 500 million people were infected, and 50 million people died during this pandemic. The second pandemic, known as "Asian influenza," occurred in 1957, was caused by an H_2N_2 IAV strain, and resulted in nearly 1.1 million deaths worldwide (Glezen, 1996). The third pandemic, known as "Hong Kong flu," occurred in 1968 and was caused by an H_3N_2 IAV strain, resulting in nearly one million deaths worldwide (Viboud, 2005). The last 40 years has been no exception, as the world witnessed the emergence and reemergence of viral

outbreaks, of which Human Immunodeficiency Virus (HIV) in 1981, Severe Acute Respiratory Syndrome Corona virus (SARS-CoV) in 2002, H_1N_1 influenza virus in 2009, Middle East Respiratory Syndrome Corona virus (MERS-CoV) in 2012, Ebola virus in 2013 and the Severe Acute Respiratory Syndrome Corona virus-2 (SARS-CoV-2) in 2019, are noteworthy (Zappa et al., 2009; Bloom and Cadarette, 2019; Grubaugh et al.,2019; Cascella et al., 2020; Roychoudhury et al.,2020). The emergence of deadly viruses and their global outbreaks pose threats to the public health and economy of the world. The COVID-19 pandemic is proving to be an unprecedented disaster, especially in terms of the health, social and economic aspects. Countries with both high and low-income groups are facing catastrophic consequences (Bong et al., 2020). Viral diseases like smallpox, polio, tetanus, measles, etc. are restricted by vaccination (Altindis et al., 2014). Vaccination is the process by which the body is made ready to face and fight off new infections by improving immunity for a long duration through antibody production so that future infections can be warded off (Malik et al., 2011). Thus, higher priority is given to vaccination for prevention and control of dreaded viral diseases. Traditional vaccines consist of inactivated or attenuated pathogens and are not entirely safe (Chan and Daniell, 2015). Live attenuated and killed viruses carry the risk of reverting to virulence, as well as other drawbacks in terms of antigenic variability between species, low levels of immunogenicity, and possible gene transfer to wild-type strains (Adeniji and Faleye, 2015; Burns et al., 2014). An outbreak of type 2 vaccine-derived polio in Nigeria was first detected in 2006, became endemic in Africa, and persists today (Famulare and Hu, 2015). This large poliomyelitis outbreak, caused by type 2 circulating vaccine-derived poliovirus (cVDPV2), began in 2005 in northern Nigeria (Burns et al., 2013). Additionally, they are inefficient in producing a protective response at mucosal surfaces such as the lungs and intestinal tract, the actual sites where disease agents enter the body. Therefore, new approaches are needed to improve current vaccines. The use of transgenic plants to produce subunit vaccine proteins has been developed as an alternative platform for the large-scale production and delivery of vaccines to induce protective immune responses via the mucosal immune system (Daniell et al., 2009). Plants offer several major advantages in vaccine generation, including low-cost production by eliminating expensive fermentation and purification systems, sterile delivery, and cold storage/transportation. Most importantly, oral vaccination using plant made antigens confers both mucosal (IgA) and systemic (IgG) immunity (Streatfield, 2006; Rybicki, 2010; Salyaev et al., 2010; Obembe et al., 2011). The rapid spread and frequent pandemic of severe contagious viral diseases such as HIV, SARS, Ebola, COVID-19, etc require extremely rapid development and comprehensive distribution of edible vaccines against potentially deadly, novel pathogenic viruses. The review describes the importance of plant-derived vaccines, various advantages, research and developments, challenges and future prospects in human immunity against viral diseases.

PRESENT GLOBAL CRISIS ON COVID PANDEMIC

About COVID-19

The 21st century witnessed a severe disease outbreak due to spread of a novel form of corona virus i.e. SARS CoV-2 that causes COVID-19. Corona viruses (subfamily Coronavirinae, order Nidovirales) are common human pathogens. They are enveloped, positive-sense, single-stranded RNA viruses that belong to the family Coronavirdiae, and are known to cause acute respiratory, hepatic and neurological diseases with varying severity in humans as well as animals (Denis et al., 2020 and Zumla et al., 2016). SARS-CoV-2 is transmitted via inhalation or direct contact with droplets from infected people. It has an incubation period ranging from 2 to ≥14 days.(Denis et al., 2020 and Zumla et al., 2016). On 11 March 2020, WHO characterized SARS-CoV-2 as a pandemic situation. In China, the total number of confirmed cases reported is 84,520 including 4645 deaths. The USA has reported the highest number of cases (1,525,186) and deaths (91,527) (mortality rate >12%). The spread of COVID-19 in India, which is a neighbouring

country to China, where the number of active cases (118,447) is increasing on a daily basis but with a low death rate i.e. ~3.0% (Sharma et al., 2020). It was first reported during December 2019 from Wuhan, China. Department of Health, Republic of China confirmed it on 23.01.2020 (WHO, 2019). Slowly, it spread to different other countries. Majority of population of Wuhan Province were affected and there were large number of casualties. China took strong steps and restricted flights from and to Wuhan and imposed lockdown and shutdown. Health examination guaranteed, affected people were quarantined, people were advised to use mask and maintain social distance. If no mask, harsh punishment was implemented. Stadiums were turned into quarantine and health service centres. General public accepted and cooperated with the new set of regulations and ensured quick immunization of its citizens. With the above set up measures, they were able to control the disease significantly. From late December, 2019 to early 2020, many people flew from China to Italy, Germany, Spain, America, Brazil, India and other countries becoming carriers and spreading the disease in rest of the world. As of now, around 195 countries of the world have been affected with this dreaded disease. Globally, as on 07 June 2021, near about 173 millions confirmed cases of COVID-19 including more than 3.72 million deaths have been reported (WHO, 2021). Later on, due to the severe impact of COVID-19 and subsequent reduction of body immunity with steroid led treatment, a fatal black fungus (Mucormycosis) disease reappeared and affected the people of some countries causing huge casualties. Its white and yellow variants have also been reported in various parts. Mucormycosis happens to be a fungal infection that causes blackening or discoloration over the nose, blurred or double vision, chest pain, difficulties in breathing and oozing of blood during coughing etc. With exposure to mucor mould, commonly found in soil and air, this black fungus occurs (Govt. of Odisha, 2021). Amphotericin B liposome injections are recommended against black fungus infection (ICMR, 2021). The COVID-19 infection rate and death cases of different regions of the world reflects the transmissibility and virulence nature of this virus. On 22 May 2020, there were 4,995,996 confirmed cases of SARS-CoV-2 including 327,821 deaths in 216 countries, and the number is increasing worldwide. The graphical presentation from WHO data is herewith presented to analyze the increase in global deaths (Fig.1). It's impact was such that as on June 2021 there were 179513309 confirmed cases, 3895661 confirm deaths. The vaccine doses administered were 2624733776 (WHO, 2021).

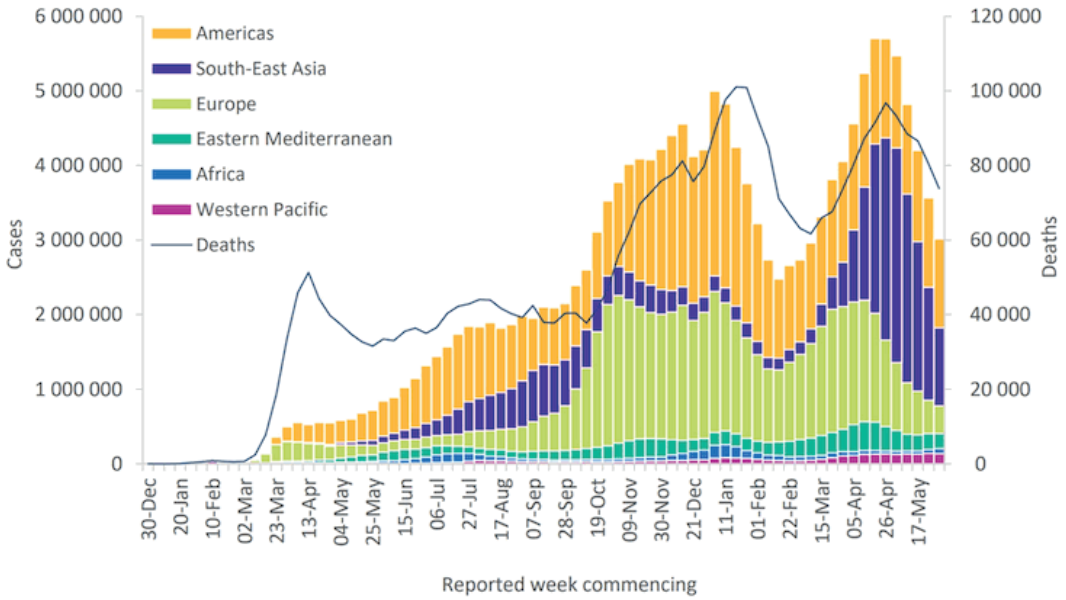

Fig. 1. COVID-19 cases and deaths in different world regions

The WHO, in collaboration with national authorities, institutions and researchers, routinely assesses if variants of SARS-CoV-2 result in changes in transmissibility, clinical presentation and severity, or if they result in changes in the implementation of public health and social measures (PHSM) by national health authorities. Systems have been established to detect "signals" of potential Variants of Concern (VOCs) or Variants of Interest (VOIs) and assess these based on the risk posed to global public health. Table 1 lists currently designated global VOIs and VOCs.

The second wave in India during April-May, 2021 was an emerging lineage of SARS-CoV-2 variants B.1.617, particularly its sub-lineage B.1.617.2 (a.k.a. delta variant). B.1.617 variant which was first reported in India in October 2020, contained mutations in the key spike protein regions involved in interactions with the host and induction of neutralizing antibodies and the strain evolved to three more sub-lineages B.1.617.1-3. Recent studies of the Delta variant in the United Kingdom of Great Britain and Northern Ireland suggest a possible increased risk of severe disease, and support previous observations of increased transmissibility. An analysis comparing Delta and Alpha variant confirmed cases in the United Kingdom from 29 March to 20 May 2021 showed the Delta variant was associated with a possible increased risk of hospitalization (hazard ratio 2.61, 95%CI 1.56-4.36), and an increased risk of emergency care attendance or hospitalization (hazard ratio 1.67, 1.25-2.23) within 14 days of specimen collection, as compared to the Alpha variant (WHO, 2021).

More than thirty million children throughout the world do not receive even the most basic immunizations each year. As a result, a large proportion of these children die from viral diseases that are fully vaccine-preventable. To save these children, the idea of large scale production of edible vaccines for various diseases can provide the opportunity of best solution.Virus evolution is expected, and the more SARS-CoV-2 circulates, the more opportunities it has to evolve (WHO, 2021).

Table 1 : SARS-CoV-2 Variants of Concern (VOCs) and Variants of Interest (VOIs)

WHO label	Pango lineage	GISAID clade	Next strain clade	Earliest documented samples	Date of designation
Variants of Concern (VOCs)					
Alpha	B.1.1.7	GRY (formerly GR/501Y.V1)	20I/501Y.V1	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351	GH/501Y.V2	20H/501Y.V2	South Africa, May-2020	18-Dec-2020
Gamma	P.1	GR/501Y.V3	20J/501Y.V3	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2	G/452R.V3	21A/S:478K	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021
Variants of Interest (VOIs)					
Epsilon	B.1.427/ B.1.429	GH/452R.V1	20C/S.452R	United States of America, Mar-2020	5-Mar-2021
Zeta	P.2	GR	20B/S.484K	Brazil, Apr-2020	17-Mar-2021
Eta	B.1.525	G/484K.V3	20A/S484K	Multiple countries, Dec-2020	17-Mar-2021
Theta	P.3	GR	20B/S:265C	Philippines, Jan-2021	24-Mar-2021
Iota	B.1.526	GH	20C/S:484K	United States of America, Nov-2020	24-Mar-2021
Kappa	B.1.617.1	G/452R.V3	21A/S:154K	India, Oct-2020	4-Apr-2021

Monoclonal antibodies, a recent advancement

In India, compared to 2020, the daily infections being reported this year are arising in alarming rate; over 50 lakhs patients across India in first fortnight of May 2021 indicating the gravity of 2nd wave of infections. Unlike the characteristics of the infections, reported in 2020, this year doctors and experts have pointed out that the younger population and more members in one family are being infected. Amidst the sudden rise of cases, uncertainties over the behavior of the virus and with only least research, experts advise people to get themselves vaccinated immediately and urging the government to expand the vaccine coverage across all age groups (Ranjan et al., 2021). Pharmaceutical company like CIPLA has recently produced the mono-clonal antibodies, a synthetic protein against treatment of present COVID variant. It is basically cocktailing of 12 mg Casirivimab mixed with 12 mg of Imdevimab. It is a single shot drug given through intra-veinous transfusion. After the injection, the patients are advised for 1 hour hospital observation. It is reported that if this drug is given within 24 hours of onset of symptoms of COVID-19, then there is every possibility for the patient to recover. If the patients are already hospitalized, and the patients' lungs and vital organs are already affected significantly by COVID virus attack, then this injection does not have any role to play and not recommended to be administered (FDA, 2020).

2 DG, the anti-COVID drug

Recently DRDO, Government of India has developed a drug i.e. 2DG (2-deoxy-D-glucose), an analogue of glucose, which is available as ORS pouch and is easily consumed through water. This drug is recommended to the Corona affected patients (moderate to severe), mostly those who are hospitalized. All patients beyond 18 years age group can take this except patients with high blood pressure and high blood sugar. This drug is said to work wonders in such patients and they are recovering fast with reduced days of hospitalization (DRDO, 2021).

Prophylactic measures against COVID-19

In post pandemic corona devastation, many countries have started developing vaccine to protect their citizens. China state owned company Sinopharm jab develop by the Beijing Institute of Biological Product which got approval from WHO. The WHO had previously only approved vaccine made by Pfizer, Astra Zeneca, Johnson & Johnson and Moderna. Then another vaccine at China was developed by Sinovac. In the meanwhile, in other countries Pfizer Inc., an American multinational Pharma's vaccine against covid-19 was started in use. Then Russia produced Sputnik (Ranjan et al., 2021). In India, Serum Institute of India produced Covishield and later Bharat Bio-tech produced Covaxin which were of 2 doses recommendations. The guidelines for 2nd dose covishield injection has been notified by Health and FW Department (Govt. of Odisha, 2021).

Keeping in view of this, this particular review is undertaken to explore the possibilities of plant derived vaccines for easy and economic usage to protect and safeguard the human life against corona viral pandemic in the present context. The authors urge upon the scientific community of the world to give a serious thought to expand the research outlets to reach into some kind of conclusive recommendations in these areas.

ADVANTAGES OF PLANT BASED EDIBLE VACCINE

Conventional vaccines produced from attenuated pathogens involve the synthesis of antigenic proteins via mammalian cell culture which is easily prone to contamination with harmful pathogens. Vaccine production through microbial system invites the possibility of endotoxin contamination. When cell culture and transgenic animals are used to make vaccines, the contamination possibly arises with viruses, prions, and oncogenic DNA. Hence, biosecurity and biosafety issues need to be addressed to cultivate these pathogenic agents (Mishra et al., 2008). Vaccine construction in several cases has been hampered because of varying or mutating strains of the pathogen, antigen drift, antigenic shift, and other unrevealed mechanisms. Such regulations make it difficult to select the actual peptide sequence to prime the immune system since the peptide sequences of the individual strains will all

be different. Even if successfully produced, these commercial vaccines have a specific expiry period and need refrigeration facilities, thereby enhancing control, storage, transportation, and distribution costs. Vaccine degradation after acid digestion in the stomach is another concern (Khan et al., 2019). Most of the available vaccines are associated with several problems including safety issues due to vaccine-associated side effects in human subjects. Thus, the World Health Organization in 1990 presented the task of finding cheap methods of oral vaccine production that must be safer and do not need refrigeration requirements. Dr. Charles Arntzen of Arizona State University first put forward the idea of edible vaccines which pose an interesting alternative in overcoming some of the constraints of traditional vaccines. They offer cost-effective, easy to administer, storable, widely-acceptable, and bio-friendly vaccine delivery system, particularly in developing countries. Edible vaccines are generally antigen-expressing plants, where the edible part of a plant is genetically modified to express the antigens, thereby eliciting an immune response upon consumption, and serving as a factory for vaccine manufacturing (Doshi et. al., 2013). The various advantages of plant derived edible vaccine in comparison to the conventional vaccine are given in Table 2.

Table 2. Comparison between the conventional and plant derived edible vaccines

Conventional/Traditional vaccines	Plant based edible vaccines	References
Comprised of weakened, live attenuated or killed pathogen	Comprises of plasmid / vector carrier system or metal particles containing small segment of target DNA sequence.	Mercenier et al., 2001; Taylor and Fauquet, 2002.
Injected intramuscularly or Subcutaneously thus painful immunization procedures	Given orally i.e. needle-less vaccination thus attractive and easier for children	Streatfield ,2005; Mishra et al., 2008.
Inefficient in producing a protective response at mucosal surfaces	Efficient in producing a protective response at mucosal surface.	Yuki and Kiono, 2003; Streatfield, 2006.
Possess residual virulence	No residual virulence	Streatfield, 2006; Lal et al., 2007; Mishra et al., 2008.
Need extensive safety precaution	Have a wide of safety.	Daniell et al., 2001; Altindis et al.2014; Chen,2015; Concha et al. 2017.
Production difficulty and larger cost	Relative ease of production and cost effective	Giddings et al.2000; Streatfield, 2005; Nochi et al., 2007; Govea-Alonso et al., 2014.

MODE OF ACTION OF EDIBLE VACCINE

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein, that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response. Most pathogens enter through the mucosal surface lining the digestive, respiratory, and urino-reproductive tracts which are collectively the largest immunologically active tissues in the body. The mucosal immune system (MIS) is the first-line defense as it is the area where human pathogens initiate their infection and the most effective site for vaccination against pathogens (Tacket and Mason, 1999). Hence, the success of an edible vaccine requires the induction of the mucosal immune system. Induction of a mucosal immune response starts with the recognition of an antigen by specialized cells called M-cells. These cells are localized in the mucosal membranes of lymphoid tissues such as Peyer's patches within the small intestines. The M-cells channel the antigen to underlying tissues where antigen-presenting cells

internalize and process the antigen. The resulting antigenic epitopes are presented on the APC surface, and with the assistance of helper T cells activate B cells. The activated B cells migrate to the mesenteric lymph nodes where they mature into plasma cells and migrate to mucosal membranes to secrete immunoglobulin-A (IgA). Upon passing through the mucosal epithelial layer towards the lumen, the IgA molecules complex with membrane-bound secretory components to form secretory IgA (sIgA). Transported into the lumen, the sIgA interacts with specific antigenic epitopes and neutralize the invading pathogen (Walmsley and Arntzen, 2000). Orally administered vaccines are particularly efficient at stimulating local mucosal immune responses at the intestinal surface, and the integrated nature of the mucosal immune system also allows other mucosal sites to be primed (Ruedl and Wolf, 1995)

RESEARCH AND DEVELOPMENTS IN EDIBLE VACCINES FOR HUMAN USE

The first plant-made vaccines (PMVs) were described by Curtiss and Cardineau in 1990 (Kirk et al., 2005). The first demonstration of expression of a vaccine antigen in plants was the *Streptococcus mutans* surface protein antigen A (SpaA) in tobacco in 1990. This research resulted in the first patent (US patent No. 5,654,184) related to the plant-based vaccine technology (Curtiss and Cardineau, 1997). Chimeric plant viruses were proven effective as carrier proteins for vaccinogens in 1994 after rabbits raised an immune response against purified chimeric CPMV particles expressing epitopes derived from human rhinovirus 14 (HRV-14) and HIV-1 (Porta et. al., 1994). Antigenicity of the resulting vaccinogen was demonstrated through mice feeding trials using the native gene for comparison. The synthetic gene increased antigen accumulation in leaves and tubers by 3-14-fold in comparison to wild-type transgene expression (Mason et al., 1998). Modelska et al. (1998) were the first to detect a mucosal immune response after oral induction with a plant virus-derived vaccinogen. It is well documented that delivery of a plant-derived vaccine to a mucosal tissue induces both a mucosal and a systemic immune response (Haq et al., 1995, Mason et al., 1996, Arakawa et al., 1998, Koo et al., 1999). In 1998, the first human trial of the edible vaccine was carried out with raw potato expressing a part of *Escherichia coli* toxin that causes diarrhoea. It was for the first time proved by the National Institute of Allergy and Infectious Diseases (NIAID) that significant immunogenicity can be induced safely by an edible vaccine. Brennan et al., (1999) further characterized the immune response induced by mucosal delivery of a plant-derived vaccine. During 2000, antigens of the Norwalk virus (that causes diarrhoea) were expressed in potato (Tacket et al., 2000). First-generation plant-based vaccines were produced against the influenza virus, human papillomavirus, and norovirus by modifying PVX or TMV. In second-generation plant-based vaccines, deconstructed viral vectors devoid of different viral elements needed for its replication and infectivity were used. Recombinant viral vectors with heterologous coat protein, with cell surface presentation of foreign antigen in the viral coat protein and sub-genomic promoters etc. were more stable, environmentally safe, and provided high yield.

In humans, plant-based edible vaccines have been found protective for human respiratory syncytial virus (F protein), hepatitis B (HBsAg), measles (H protein), Japanese encephalitis virus (envelope protein E), Norwalk viral gastroenteritis (virus capsid protein), anthrax [protective antigen domain IV, PA(dIV)], human papillomaviruses (HPV 16 E7 protein, HPV major capsid protein L1), tetanus for both human and animals (antigen TetC), rabies in farm, wild animals and humans (glycoprotein and nucleoprotein epitopes), human immunodeficiency virus (HIV), influenza etc. (Streatfield, 2006; Mishra et al., 2008; Daniell et al., 2009; Buonaguro et al., 2010; Lugade et al., 2010; Rybicki, 2010; Salyaev et al., 2010; Obembe et al., 2011; Rukavtsova et al., 2011; Shoji et al. 2015; Hayden et al., 2012).

The concept of using engineered or transgenic plants to produce and deliver subunit vaccines was introduced by Charles Arntzen (Mason et al., 1992). Potato and tobacco were used as model organisms initially in the development of

plant-based vaccines. Tobacco plants are a good model for evaluating recombinant proteins and can be harvested several times in a year and their leaves which are major sources of biomass. However, due to the composition of a high level of toxic alkaloids, it causes more toxicity (Tiwari et al., 2009; Doshi et al., 2013). The potato was used as a model plant in edible vaccine production since it was easy and efficient to transform, tuber-specific promoters could be used to express transgene/antigen gene, the out crossing risk was low, clonal propagation to produce stable transgenics was possible, tubers could be eaten (cooked), were used in the food industry, affordable and tubers could be stored for long periods without refrigeration. The major limitation is that, it needs cooking which can denature antigen and decrease immunogenicity (Arakawa et al., 1998; Hafiz and Eyob, 2015). Later, many different crops i.e. tomato, maize, rice, soybean, spinach, lupin, lettuce, etc are selected for research and development of an edible vaccine (Table 3).

Table 3. Developments of edible vaccines in different plants against human viral diseases

Sl. No.	Vaccine/ Antigen	Pathogen/ Viral diseases	Crop	Reference
1	Hepatitis B surface antigen	Hepatitis B	Tobacco	Mason et al.1992; Valdes et al. 2003; Huang et al.,2007, 2008
			Potato	Thanavala et al., 1995; Domansky et al., 1995; Richter et al., 2000; Youm et al. 2010
			Lettuce	Hayden et al.2012; Czyz et al. 2014
			Maize	Arakawa,1998;
			Tomato	Richter et al. 2000; Lou et al., 2007; Wang and Li, 2008
			Carrot	Zhao et al. 2002
			Lupin leaves	Pniewski et al., 2006,2011; Waghulkar, 2010
			Rice	Qian et al. 2008
			Banana	Guan et al.2010
			Cherry tomatillo	Gao et al. 2003
2.	Rabies virus glycoprotein and nucleo protein	Rabies	Tobacco	Hooper et al.1994; Arango2008; van Dolleweerd et. al.,2014
			Tomato	McGarvey et al., 1995
			Spinach	Modelska et al., 1998
			Potato	Yusibov, 2002
			Maize	Loza-Rubio et al,2008
3.	Norwalk virus capsid protein	Norwalk virus causing Gasteroenteritis	Tobacco	Mason et al. 1996
			Potato	Mason et al. 1996; Tacket et al, 2000; Yusibev et al. 2002
			Banana	Carter et al. 2002
			Tomato	Zhang et al., 2006,
4	Herps	Herps	Soybean	Zeitlin et al. 1998
5	Respiratory Syncytial viral G and F protein	Respiratory Syncytial virus	Tobacco	Belanger et al.2000
			Tomato	Sandhu et al. 2000

6	Paramyxovirus surface protein hemagglutinin	Measles	Tobacco	Huang et al 2001
			Tobacco, potato, rice and lettuce	Webster et al., 2005
7	Rotavirus VP6 protein	Rotavirus	Potato	Yu and Langridge, 2001; Yu-Zang et al., 2003; Wu et al., 2003, Li et al., 2006
			Tobacco	Yang et al., 2011; Pera et al., 2015
8	HIV antigen	AIDS	Tobacco	Marusic et al., 2001; Zhang et al. 2002, Meyers et al., 2008; Gonzalez-padabe et al. 2011; Rubio-infante et al. 2015
			Potato	Horn et al., 2003
			Spinach	Karasev., 2005
			Tomato	Shchelkunov et al., 2006
			Lettuce	Govea-Alorso et al., 2013
			Carrot	Lindh et al., 2014
9	Human papillomavirus E7 and L1 proteins	Cervical cancer	Potato	Franconi et al., 2002; Biemelt et al., 2003; Warzecha et al., 2003
			Tobacco	Liu et al., 2013
			Tomato	Monray-gracia et al., 2014
10.	Recombinant vaccinia virus B5	Smallpox	Spinach	Yusibev et al., 2002
			Tobacco and collard	Golovkin, 2007
11	SARC-CoV 2 Protein	SARS	Tomato and tobacco	Pogrebnyak et al., 2005
12.	Dengue virus glycoprotein	Dengue	Tobacco	Kim, 2009 and 2015
13.	Ebola immune complex	Ebola virus	Tobacco	Phoolcharoen, 2011; Fulton, 2015
14.	Influenza vaccine	Influenza	Tobacco	D'Aoust et al., 2010; Shoji et al., 2015; Ward et al., 2014; Pillet et al., 2015

Tomato plant grows quickly, cultivated broadly, heat-stable, and high vitamin A composition may boost immune response. Antigen-containing powders can be filled into capsules without having any requirement of special facilities for storage and transportation. However, it has demerit as spoils easily; hence cannot be stored for over a long period (Renuga et al., 2014). Bananas are sterile so the genes do not pass from one banana to another which is the main reason why bananas are a good choice for an edible vaccine. The tropical climate is suitable for growing bananas. It does not need cooking. Proteins are not destroyed even if cooked and they can be eaten as raw. It is inexpensive, can be grown widely in developing countries, grow quickly, and have a high vitamin A content which boosts immune response. The disadvantages

are that the trees take 2-3 years to mature. A transformed tree takes about 12 months to bear fruits, spoils rapidly after-ripening (William, 2002). Maize plants generate a protein that is used to develop the hepatitis B virus vaccine. It is cheaper and does not need to be refrigerated. A major disadvantage of this vaccine is to be cooked for the use which causes degradation of proteins (Arakawa et al. 1998). Rice plant has been reported that a strain of rice can serve as a vaccine and last for more than a year and a half at normal room temperature. It is used as pediatric food because of the low level of allergenic potential but grows slowly and requires specialized glass house conditions (Hafiz and Eyob, 2015).

Generally, vaccine development has six-phases according to the Center for Disease Control and Prevention (CDC), USA. These are exploratory, preclinical, clinical development, regulatory review and approval, and finally manufacturing and quality control (Baylor, 2016). In the exploratory phase, research and development on synthetic or natural antigens or weakened strains of the pathogenic virus are carried out to treat or prevent disease. In the pre-clinical phase, tissue culture or cell cultures and animal testing are undertaken to verify the effectiveness of the edible vaccine to provide immunity. In the third phase i.e. in clinical development, a proposal describing the research findings required to be submitted by the vaccine manufacturing firm to the sanctioning authority conducting clinical trials. After getting the approval to conduct clinical trials, human testing or trials are conducted in 3 stages i.e. in phase-I, the candidate vaccine is administered to a small group of people (<100) to know the safety. Phase-II involves a larger group in many hundreds to know about safety immunogenicity, immunization schedule, dosage, etc. A larger subject group of thousands are covered in Phase III trials where side-effects, safety, and effectiveness of the candidate vaccine is assessed. This is followed by regulatory review and approval where an application for a license for manufacturing by the firm is scrutinized for approval. The next step is manufacturing the vaccines and finally quality control to monitor the performance, safety, and effectiveness of the vaccine.

Plant-derived vaccines have been developed for many human diseases such as hepatitis B, Human Immunodeficiency Virus (HIV), rabies, etc. More than 25 vaccines are licensed for use in humans and many more in the development pipeline (Yusibov and Rabindran, 2008). In March, 2018, Medicago Inc. conducted phase III clinical trials to develop a flu vaccine in tobacco and is expected to be launched in the market by 2020-2021. In 2018, researchers from the University of Nottingham, Malaysia, launched a project to develop a plant-based vaccine against dengue fever, caused by Aedes mosquitoes. Researchers from Arizona State University's (ASU) Bio-design Institute developed a norovirus vaccine from the tobacco plant in 2018. Other than tobacco, many edible crop plants are also used such as the dengue virus vaccine produced in lettuce through chloroplast transformation in 2016. Medicago Inc., Prodigene, Rubicon lab, Agr Genet, Rhone-Merieux, iBio Inc., Icon Genetics-GmbH, Creative Biolabs, etc. are involved in plant-based vaccine development (Mishra et al., 2008). Some of the edible vaccine patents are also described in Table 4.

The Queensland University of Technology, Australia plans to use the genome sequence information of *Nicotiana benthamiana* to use the plant as a bio-factory to produce antibodies, vaccines, and therapeutics, to develop protein-based diagnostic products in bulk quantities at a low cost against COVID-19 or similar viruses or pathogens. The Canada-based biopharmaceutical company Medicago Inc. is experimenting on the transient expression of SARS-CoV-2 virus S protein, using a virus-like particle (VLP) grown in *Nicotiana benthamiana* to develop a potential vaccine against the coronavirus disease that has now reached a global pandemic level. Universities and institutes from several countries including the US, Germany, UK, South Africa, South Korea, Mexico, and Thailand are working in the molecular farming field, investing efforts and establishing partnerships and collaborations for production of vaccine/ treatment for COVID-19.

Table 4. Patents on edible vaccine technologies for human viral disease

Sl. No.	Patent holder	Claims
1.	University of Texas	Hepatitis B core antigen recombinant vaccine
2.	Prodigene	Production and transfer of recombinant antigen to plant cells through the plasmid-vector system; production of vaccine for hepatitis and transmissible gastroenteritis virus
3.	University of Philadelphia	Rabies vaccine expressed in tomato plant
4.	Biochem; Rhone-merieux	Transgenic plant used to produce rabies vaccine
5.	Biosource	Plant viral vector with potential as anti-HIV vaccine
6.	Rubicon Lab	Retrovirus expressed in animal or plant cells useful as virus and cancer vaccines
7.	Agr genet	Edible vaccine for human rahino virus

Transient transformation approaches are rapid (expression within a week) while the regeneration of a stably transformed plant takes up to 3 months, which is not suitable for addressing a fatal, exponentially growing pandemic such as COVID-19. Though, concept and methodology are concise, there is only a limited number of edible vaccines that are approved, tested and commercialized. (Parvathy, 2020).

FUTURE CHALLENGES

The major challenges of edible vaccine production are: (i) the selection of antigen and plant expression host, which will ensure the safety of the vaccine produced and its thermo-stability, (ii) consistency of dosage, and (iii) manufacturing of vaccines according to Good Manufacturing Practice (GMP) procedures (Laere et al., 2016). Therefore, the challenges facing plant-based-vaccine development include technical, regulatory, economic aspects, and public perception. Among technical challenges to be considered, the crop should provide ample biomass for the accumulation of a sufficient quantity of the antigenic protein.

The main regulatory concern is the expression of antigens in plants. Targeting transgene expression via a tissue-specific promoter may reduce regulatory concerns. Not all the vaccine candidate proteins are highly immunogenic in plant tissues. The secondary metabolites found in plants may compromise the ability of the candidate vaccine protein to induce immunity. Among regulatory challenges, issues relevant to any genetically modified crop that have to gain regulatory approval from the USDA, FDA, and/or EPA apply equally to vaccines generated in edible plant parts. Allergenic reactions to plant protein glycans and other plant antigens are challenging issues. It has been suggested that plant-derived recombinant proteins or antibodies may have increased immunogenicity or allergenicity, as compared to mammalian counterparts along with few side effects such as toxicity on the central nervous system, cytokine-induced sickness, and autoimmune diseases. Another problem is that the glycosylation pattern of plant and human protein is different which may affect or alter the functions of vaccines (Streatfield, 2005).

There are chances of contamination of recombinant proteins by pesticides, herbicides, mycotoxins, or potentially toxic factors because some plant species contain numerous toxic alkaloids and other secondary metabolites. All these factors invite regulatory constraints and uncertainties for approval as a human drug. The stability of vaccines in fruits is uncharacterized. Moreover, there may be weakening of the medicinal property and denaturation of vaccine proteins in the case of cooked foods. Evaluation of dosage requirement and proper maintenance of dosages are difficult, since the consistency of dosage may vary within the plants of the same species, from fruit to fruit and from generation to generation due to the size and ripeness of the fruits or plants

(Sharma and Sood, 2011). Fruits like tomato and banana do not appear in the market in fixed or standard sizes so that it is quite difficult to optimize the dosage. It is also quite difficult to evaluate the required dosage for every patient. To determine the right dosage, one needs to consider the person's weight, age; fruit/plant size, ripeness, and protein content. The amount to be taken is critical, especially in infants, who might spit it after taking, may eat a part of it, or may take it and throw it up later. The levels of innate and adaptive immune responses generated in different individuals may vary, based on the types of antigens being exposed in the body. Between two patients with different body weight as well as their age, the dosage of plant-derived vaccine required will be different. If this issue is not monitored carefully, an immunological tolerance will be induced when the patient is overdosed, while too low a dose would fail to induce antibodies. The development of immune-tolerance to the vaccine protein or peptide is therefore a major concern (Concha et al., 2017). Edible vaccines are dependent on plant stability as certain foods cannot be eaten raw (e.g. potato) and needs cooking that cause denaturation or weaken the protein present in it (Moss et al., 1999). Variable conditions for the edible vaccine are also a major problem. The potato-containing vaccine to be stored at 4°C could be stored for a longer time, while a tomato does not last long. Thus, these vaccines need to be properly stored to avoid infection through microbial spoilage (Merlin et al. 2017). Another challenge would be in dealing with diseases caused by multiple serotypes (dengue) or by rapidly mutating organisms (HIV, influenza). Though, the plant edible vaccines are a lucrative option in the field of vaccination, several potential issues need to be addressed via exhaustive research and development to use this area of health care for greater benefits (Raychoudary, 2020).

Some of the techniques to overcome these limitations are (i) optimization of the coding sequence of viral genes for expression as plant nuclear genes, (ii) expression in plastids, (Ruf et al., 2001) (iii) plant viruses expressing foreign genes (Nemchinov et al. 2000) (iv) coat-protein fusions, (Modelska et al.,1998) (v) viral-assisted expression in transgenic plants(Mor et al., 2003) and (vi) promoter elements of bean yellow dwarf virus with reporter genes GUS (β-glucuronidase) and GFP (green fluorescent protein), substituted later with target antigen genes. Antigen genes may be linked with regulatory elements which switch on the genes more readily, or do so only at selected times (after the plant is nearly fully grown), or only in its edible regions. Exposure to some outside activator molecules may also be tried. Although edible vaccines fall under genetically modified plants, it is presumed that these vaccines will avoid controversy, as they are intended to save lives.

Currently, it has not been studied that all the approved vaccines will work against the newly mutated variant of COVID-19 that are continuously evolving. The SARS-CoV 2 Spike protein can be used to develop a plant derived edible vaccine against the COVID-19 as previous work on SARS-CoV vaccines have shown that the Spike glycoprotein (S) is the main inducer of neutralizing antibodies in respiratory diseases. The Spike (S) protein gene or subunit of Spike like S1 subunit can be cloned into a plant expression vector and the desired plant like tomato, cucumber or lettuce can be transformed. The developed transgenic plants can be used as salad and easily delivered orally and immunized the human against the newly emerged virus and can develop herd immunity against the COVID-19 as well as other respiratory pathogenic viruses of the same order/family.

CONCLUSION

Plant-derived edible vaccines are safer with numerous advantages over the conventional vaccines such as easy to administer, readily acceptable vaccine delivery system, easy to store and are cost-effective. These can play a major role in immunization control of the viral diseases across the world, especially in poor and developing countries. These products can be easily scaled up for millions of users within a limited period. The development of a suitable edible vaccine is of great importance to prevent and control the spread of the virus. The edible vaccine has the potential to solve the problem of bioterrorism by immunizing against a wide range of different dreaded viruses and can save the earth from any future epidemic and pandemic. Currently, it appears that almost every country all over the World urgently feels the need of designing new vaccines against COVID-19 and other dreaded viruses as fast as possible to save human

life. Hence, there is a lot of scope for research and development in the field of the edible vaccine in the forthcoming era. The day is not far off when children may be immunized by foods instead of painful immunization through needles and agricultural food vaccines might save millions of life who now die for lack of access to the traditional vaccine. "Let food be thy medicine"-Hippocrates. The conclusive remark: Till now, although hundreds of years have been passed, research in entire world could not be able to eradicate the viral deaths and address the pandemic issues in right earnest and hence, the future children's health and safety are always at risk. Thus, urgently we should focus on developing edible vaccine to create a broad spectrum resistance continuously and naturally through food.

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REFERENCES

Adeniji, J.A. and Faleye, T.O. 2015. Enterovirus C strains circulating in Nigeria and their contribution to the emergence of recombinant circulating vaccine-derived polioviruses. *Arch. Virol.* **160** (3): 675-683.

Altindis, E., Iz, S.G., Ozen, M.O., Nartop, P., Gurhan, I.D. and Gurel, A. 2014. *Plant Derived Edible Vaccines and Therapeutics.* In: Atta-ur-Rahman, Ed., Frontiers in Clinical Drug Research: Anti -Infectives, Bentham Science Publishers, Sharjah, 200-236.

Arakawa, T., Chong, D.K.X. and Langridge, W.H.R. 1998. Efficacy of a food plant based oral cholera toxin B subunit vaccine. *Nat. Biotechnol.* **16**:292-297

Arango, I.P., Rubio, E.L., Anaya, E.R., Flores, T.O., delaVara, L.G. and Lim, M.A.G. 2008. Expression of the rabies virus nucleoprotein in plants at high-levels and evaluation of immune responses in mice. *Plant Cell Rep.* **27**: 677-685.

Baylor, N.W. 2016. The Regulatory Evaluation of Vaccines for Human Use Methods. *Mol. Biol.*, 1404, 773-787.

Belanger, H., Fleysh, N., Cox, S., Bartman, G., Deka, D., Trudel, M., Koprowski, H. and Yusibov, V. 2000. Human respiratory syncytial virus vaccine antigen produced in plants. *FASEB J.* **14**, 2323-2328.

Biemelt, S., Sonnewald, U., Galmbacher, P., Willmitzer, L. and Müller, M. 2003. Production of human papillomavirus type 16 virus-like particles in transgenic plants. *J. Virol.*, **77**, 9211-9220.

Bloom, D.E. and Cadarette, D. 2019. Infectious disease threats in the twenty-first century: Strengthening the global response. *Front. Immunol*., **10**: 549.

Bong, C.L., Brasher, C., Chikumba, E., McDougall, R., Mellin-Olsen, J. and Enright, A. 2020. The COVID-19 pandemic: Effects on low- and middle-income countries. *Anesth. Anal*., **131**: 86-92.

Brennan, F.R., Bellaby, T., Helliwell, S.M., Jones, T.D., Kamstrup, S., Dalsgaard, K., Flock, J.I. and Hamilton, W.D.O. 1999. Chimeric plant virus particles administered nasally or orally induce systemic and mucosal immune responses in mice. *J. Virol.* **73**: 930-938.

Buonaguro, F.M. and Ransohoff J.E.B. 2010. Pharma Plant: the new frontier in vaccines. *Expert Rev. Vaccines*. **9**: 805-807.

Burns, C.C., Diop, O.M., Sutter, R.W. and Kew, O.M. 2014. Vaccine-derived polioviruses. *J. Infect. Dis*. **210** (Suppl. 1): S283-S293

Burns, C.C., Shaw, J., Jorba, J., Bukbuk, D., Adu, F., Gumede, N., Pate, M.A., Abanida, E.A., Gasasira, 2013. Multiple independent emergences of Type 2 vaccine derived polioviruses during a large outbreak in northern Nigeria. *J. Virol*. **87**: 4907-4922.

Carter, J.E. and Langridge, W.H.R. 2002.Plant-based vaccines for protection against infectious and autoimmune diseases. *Crit. Rev. Plant Sci.* **21**:93-109.

Cascella, M., Rajnik, M., Cuomo, A., Dulebohn, S.C., and Napoli, R.D. 2020. *Features, evaluation and treatment coronavirus* (COVID-19). Stat Pearls Publishing: Treasure Island, FL, USA; pp. 1-16.

Chan, Hui-Ting and Daniell, H. 2015. Plant-made oral vaccines against human infectious diseases-Are we there yet? *Plant Biotechnol J.* **13**(8): 1056-1070.

Chen, Q. 2015 Plant-Made Vaccines against West Nile Virus Are Potent, Safe, and Economically Feasible. *Biotechnol. J.* **10**: 671-680

Concha, C., Canas, R., Macuer, J., Torres, M.J., Herrada, A.A., Jamett, F. and Ibanez, C. 2017. Disease Prevention: An opportunity to expand edible plant-based vaccines? *Vaccines*. **5**, pii: E14.

Curtiss, R. and Cardineau, G.A. 1997. Oral Immunization by Transgenic Plants. United States Patent No.-5654184.

Daniell, H., Singh, N.D., Mason, H., Streatfield, S.J. 2009. Plant-made vaccine antigens and biopharmaceuticals. *Trends Plant Sci.* **14**:669–679.

Daniell, H., Streatfield, S.J. and Wycoff, K. 2001. Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci*. **6**: 219-226.

Denis M, Vandeweerd V, Verbeke R, Van der Vliet D., 2020 COVID-19 overview of information available to support the development of medical countermeasures and interventions against COVID-19 Published on 20 April 2020. *Transdisciplinary Insights - Living Papers*. pp. 1-173.

D'Aoust, M. A., Couture, M. M. J., Charland, N., Trépanier, S., Landry, N., Ors, F. and Vézina, L.-P. 2010. The production of hemagglutinin-based virus-like particles in plants: a rapid, efficient and safe response to pandemic influenza. *Plant Biotechnol*. J. **8**(5), 607-619.

Domansky, N. 1995. Organ-Specific Expression of Hepatitis B Surface Antigen in Potato. *Biotechnol. Lett.* **17**:863-866.

Doshi, V., Rawal, H. and Mukherjee, S. 2013. Edible vaccines from GM crops:Current status and future scope. *J. Pharm. Sci. Innov*. **2**: 1-6.

DRDO. 2021. Guidelines realised by DRDO, Published on 01.06.2021. https://www.india.com

Famulare, M. and Hu, H. 2015. Extracting transmission networks from phylogeographic data for epidemic and endemic diseases: Ebola virus in Sierra Leone, 2009 H1N1 pandemic influenza and polio in Nigeria. *Int. Health.* **7**: 130-138.

FDA. 2020. US Food and Drug Administration Fact Sheet for Health Care Providers: Emergency Use Authorization (EUA) of Casirivimab and Imdevimab. https://www.fda.gov

Franconi, R., Di Bonito, P., Dibello, F., Accardi, L., Muller, A., and Cirilli, A., 2002. Plant-derived human papillomavirus 16 E7 oncoprotein induces immune response and specific tumor protection. *Cancer Res*. **62**: 3654-3658.

Fulton, A., Lai, H., Chen, Q. and Zhang, C. 2016. Corrigendum to "Purification of monoclonal antibody against Ebola GP1 protein expressed in Nicotiana benthamiana". *J. Chromatogr. A* 1389: 128-132. *J. Chromatogr*. *A*., **1448**: 127.

Gao, Y., Ma, Y., Li, M., Cheng, T., Li, S.-W., Zhang, J. and Xia N.-S. 2003. Oral immunization of animals with transgenic cherry tomatillo expressing HBsAg. *World J. Gastroenterol.* **9**: 996-1002.

Giddings, G., Allison, G., Brooks, D. and Carter, A. 2000. Transgenic plants as factories for biopharmaceuticals. *Nat. Biotechnol.* **18**: 1151- 1155.

Glezen, W.P. 1996. Emerging infections: pandemic influenza. *Epidemiol. Rev.* **18**: 64-76.

Golovkin, M., Spitsin, S., Andrianov, V., Smirnov, Y., Xiao, Y., Pogrebnyak, N., Markley, K., Brodzik, R., Gleba, Y., Isaacs, S. N. and Koprowski, H. 2007. Smallpox subunit vaccine produced in Planta confers protection in mice. *Proc. Natl. Acad. Sci. U.S.A*. **104**(16): 6864-6869.

Gonzalez-Rabade, N., McGowan, E.G., Zhou, F., McCabe, M.S., Bock, R., Dix, P.J., Gray, J.C. and Ma, J.K. 2011. Immunogenicity of chloroplast-derived HIV-1 p24 and a p24-Nef fusion protein following subcutaneous and oral administration in mice. *Plant Biotechnol. J.* **9**: 629-638.

Govea-Alonso, D.O., Cardineau, G.A. and Rosales-Mendoza, S. 2014. Principles of plant-based vaccines against wide-spread diseases. In: Rosales-Mendoza, S., Ed., *Genetically engineered plants as a source of vaccines*, Springer, New York. pp. 1-13.

Govea-Alonso, D.O., Rubio-Infante, N., Garcıa-Hernandez, A.L., Varona-Santos, J.T., Korban, S.S., Moreno-Fierros, L. and Rosales-Mendoza, S. 2013. Immunogenic properties of a lettuce-derived C4(V3)6 multiepitopic HIV protein. *Planta*. **238**, 785-792.

Govt. of Odisha. 2021. Guidelines issued by H & FW Deptt., G.O.; Notification of Health and Family Welfare Department, Govt. of Odisha. No. 15058/ Dt. 18.05.2021.

Govt. of Odisha. 2021. Management protocol for post COVID Mucormycosis (Black fungus), diagnosis, management and prevention during COVID-19; Notification of Health and Family Welfare Department, Govt. of Odisha. Notification No. 15297/ Dt. 20.05.2021.

Grubaugh, N.D., Lander, J.T., Lemey, P., Pybus, O.G., Rambaut, A., Holmes, E.C. and Andersen, K.G. 2019. Tracking virus outbreaks in twenty-first century. *Nat. Microbiol*., **4**: 10-19.

Guan, Z. J., Guo, B., Huo, Y. L., Guan, Z. P. and Wei, Y. H. 2010. Overview of expression of hepatitis B surface antigen in transgenic plants. *Vaccine*, **28**: 7351-7362.

Hafiz, E. and Eyob, H. 2015. Review on edible vaccine. *Acad. J. Nutr.* **4**: 40-49.

Haq, T.A., Mason, H.S., Clements, J.D. and Arntzen, C.J. 1995. Oral immunization with a recombinant bacterial antigen produced in transgenic plants. *Science*. **268**: 714-716.

Hayden, C.A., Streatfield, S.J., Lamphear, B.J., Fake, G.M., Keener, T.K., Walker, J.H., Clements, J.D., Turner, D.D., Tizard, I.R. and Howard, J.A. 2012. Bioencapsulation of the hepatitis B surface antigen and its use as an effective oral immunogen. *Vaccine*, **30**: 2937-2942.

Hooper, D.C., Pierrard, L., Modelska, A., Otvos, L., Fu, Z., Koprowski, H. and Dietzschold, B. 1994. Rabies nucleocapsid as an oral immunogen immunological enhancer. *Proc. Natl. Acad. Sci.* USA. **91**: 10908-10911

Horn, M. E., Pappu, K. M., Bailey, M. R., Clough, R. C., Barker, M., Jilka, J. M., Howard, J. A. and Streatfield, S. J. 2003. Advantageous features of plant-based systems for the development of HIV vaccines. *J. Drug. Target.***11**: 539-545.

Huang, Z., I., Dry., Webster, D., Strugnell, R. and Wesselingh. S.2001. Plant-derived measles virus hemagglutinin protein induces neutralizing antibodies in mice. *Vaccine*, **19**: 2163–2171.

Huang, Z., LePore, K., Elkin, G., Thanaval, Y. and Mason, H.S. 2008. High-yield rapid production of hepatitis B surface antigen in plant leaf by a viral expression system. *Plant Biotechnol. J.* **6**: 202-209.

ICMR, GoI. 2021. Indian Council for Medical Research Report to Govt. of India. 04.06.2021

Jordan, D. 2019. The Deadliest Flu: The Complete Story of the Discovery and Reconstruction of the 1918 Pandemic Virus. Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD). Available online at: https://www.cdc.gov/flu/pandemic-resources/1918-pandemic-h1n1.html

Karasev, A. V., Foulke, S., Wellens, C., Rich, A., Shon, K. J., Zwierzynski, I., Hone, D., Koprowski, H. and Reitz, M. 2005. Plant based HIV-1 vaccine candidate: Tat protein produced in spinach. *Vaccine*. **23**(15): 1875-1880.

Khan, A., Khan, A., Khan, I., Shehzad, M.A., Ali, W. and Muhammad, A. 2019. A review on natural way of vaccination: plant derived edible vaccines. *Int. J. Biosci*. **15**(4): 95-100.

Kim, M.Y., Reljic, R., Kilbourne, J., Ceballos-Olvera, I., Yang, M.S., Reyes-del Valle, J. and Mason, H.S. 2015. Novel vaccination approach for dengue infection based on recombinant immune complex universal platform. *Vaccine*. **33**:1830-1838.

Kim, M.Y., Yang, M. S. and Kim, T. G. 2009. Expression of Dengue Virus e Glycoprotein Domain III in Non-Nicotine Transgenic Tobacco Plants. *Biotechnol. Bioprocess Eng.* **14**: 725-730.

Kirk, D.D., McIntosh, K.M., Walmsley, A.M. and Peterson, R.K.D. 2005. Risk Analysis for Plant-Made Vaccines. *Transgenic Res*. **14**: 449-462.

Koo, M., Bendahmane, M., Lettieri, G.A., Paoletti, A.D., Lane, T.E., Fitchen, J.H., Buchmeier M.J. and Beachy, R.N.1999. Protective immunity against murine hepatitis virus (MHV) induced by intranasal or subcutaneous administration of hybrids of tobacco mosaic virus that carries an MHV epitope. *Proc Natl Acad Sci USA*, **96**: 7774-7779.

Laere, E., Ling, A.P.K., Wong, Y.P., Koh, R.Y., Lila, M.A.M. and Hussein S. 2016. Plant-Based Vaccines: Production and Challenges. *J. Bot.* 2016; 1-11

Lal, P., Ramachandran, V.G., Goyal, R. and Sharma, R.2007. Edible vaccines: current status and future. *Indian J. Med. Microbiol*. **25**: 93-102.

Li, J.T., Fei, L., Mou, Z.R., Wei, J., Tang, Y., He, H.Y.,Wang, L. and Wu, Y.Z. 2006. Immunogenicity of a plant-derived edible rotavirus subunit vaccine transformed over fifty generations. *Virology*. **356**: 171-178.

Lindh, I., Brave, A., Hallengₒard, D., Hadad, R., Kalbina, I., Strid, A. and Andersson, S. 2014. Oral delivery of plant-derived HIV-1 p24 antigen in low doses shows a superior priming effect in mice compared to high doses. *Vaccine*. **32**: 2288-2293.

Liu, H., Li, X., Lei, T., Li, W., Si, L. and Zheng, J. 2013. Transgenic tobacco expressed HPV16-L1 and LT-B combined immunization induces strong mucosal and systemic immune responses in mice. *Hum. Vaccin. Immunother*. **9**:83-89.

Lou, X.M., Yao, Q.H., Zhang, Z., Peng, R.H., Xiong, A.S. and Wang, H.K. 2007. Expression of the human hepatitis B virus large surface antigen gene in transgenic tomato plants. *Clin. Vaccine Immunol*.**14**: 464-469.

Loza-Rubio, E., Rojas, A.E., Gómez, N.L., Olivera, F.M.T.J. and Gómez-Lim, M. 2008. Development of an edible rabies vaccine in Maize using the Vnukovo strain. *Dev Biol* (Karger). **131**: 477-482

Lugade, A.A., Kalathil, S., Heald, J.L. and Thanavala, Y. 2010. Transgenic plant-based oral vaccines. *Immunol. Invest.* **39**: 468-482.

Malik, A., Vashisht, V.K., Rashid, R., Sharma, S. and Singh. J. 2011. "Edible" vaccine vegetables as alternative to needles. *Int. J. Curr. Res*. **3**:18-26.

Marusic, C., Rizza, P., Lattanzi, L., Mancini, C., Spada, M., Belardelli, F., Benvenuto, E. and Capone, I. 2001. Chimeric plant virus particles as immunogens for inducing murine and human immune responses against HIV type 1. *J. Virol*.**75**: 8434-8439.

Mason, H.S., Ball, J.M., Shi, J.J., Jiang, X., Estes, M.K. and Arntzen, C.J. 1996. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. *Proc. Natl. Acad. Sci. USA*. **93**(11): 5335-5340.

Mason, H.S., Haq, T.A. and Clements, J.D.1998. Edible vaccine protects mice against *Escherichia coli* heat-labile enterotoxin (LT): potatoes expressing a synthetic LT-B gene. *Vaccine*, **16**:1336-1343.

Mason, H.S., Lam, D.M. and Arntzen, C.J. 1992. Expression of Hepatitis B Surface Antigen in Transgenic Plants. *Proc. Natl. Acad. Sci. USA*. **89**: 11745-11749.

Mc Garvey, P. B., Hammond, J., Dienelt, M. M., Hooper, D. C., Fu, Z. F., Dietzschold, B., Koprowski, H. and Michaels, F. H. 1995. Expression of the rabies virus glycoprotein in transgenic tomatoes. *Biotechnology*. (N. Y.) **13**(13): 1484-1487.

Mercenir, A., Wiedermann, U. and Breiteneder, H. 2001. Edible genetically modified microorganisms and plants for improved health. *Curr. Opinion Biotechnology.* **12**(5): 510-515.

Merlin, M , Pezzotti, M. and Avesani, L. 2017. Edible plants for oral delivery of biopharmaceuticals. *Br. J. Clin Pharmacol.* **83**: 71-81.

Meyers, A., Chakauya, E., Shephard, E., Tanzer, F. L., Maclean, J., Lynch, A., Williamson, A.-L. and Rybicki, E. P. 2008. Expression of HIV-1 antigens in plants as potential subunit vaccines. *BMC. Biotechnol.* **8**: 53.

Mishra, N., Gupta, P.N., Khatri, K., Goyal, A., Vyas, K. and Suresh, P. 2008. Edible vaccines: A new approach to oral immunization. *Indian J. Biotechnol.* **7**: 283-294.

Modelska, A., Dietzschold, B., Sleysh, N., Fu, Z. F., Steplewski, K., Hooper, D. C., Koprowski, H. and Yusibov, V. 1998. Immunization against rabies with plant-derived antigen. *Proc. Natl. Acad. Sci. U.S.A*. **95**(5): 2481-2485.

Monroy-Garcıa, A., Gomez-Lim, M.A., Weiss-Steider, B., Hern andez-Montes, J., Huerta-Yepez, S., Rangel-Santiago, J.F., Santiago-Osorio, E. and de Mora Garcıa, M.L. 2014. Immunization with an HPV-16 L1-based chimeric viruslike particle containing HPV-16 E6 and E7 epitopes elicits long-lasting prophylactic and therapeutic efficacy in an HPV-16 tumor mice model. *Arch. Virol.* **159**: 291-305.

Mor, T.S., Moon, Y.S., Palmer, K.E. and Mason, H.S. 2003. Geminivirus vectors for high level expression of foreign proteins in plant cells. *Biotechnol. Bioeng*. **81**: 430-437.

Moss, W.J., Cutts, F. and Griffin, D.E. 1999. Implications of the human immunodeficiency virus epidemic for control and eradication of measles. *Clin. Infect. Dis.* **29**: 106-112.

Nemchinov, L.G., Liang, T.J., Rifaat, M.M., Mazyad, H.M., Hadidi, A and Keith J.M. 2000. Development of a plant-derived subunit vaccine candidate against hepatitis C virus. *Arch Virol.* **145**: 2557-73.

Nochi, T., Takagi, H., Yuki, Y., Yang, L., Masumura, T., Mejima, M., Nakanishi, U., Matsumura, A., Uozumi, A., Hiroi, T., Morita, s., Tanaka,K., Takaiwa, F. and Hiroshi Kiyono. 2007. Rice-based mucosal vaccine as a global strategy for cold-chain-and needle-free vaccination. *Proc. Natl. Acad. Sci. USA,* **104**:10986-10991.

Obembe, O.O., Popoola, J.O., Leelavathi, S. and Reddy, S.V. 2011. Advances in plant molecular farming. *Biotechnol. Adv*. **29**: 210-222.

Parvathy S. T. 2020. Engineering Plants as Platforms for Production of Vaccines. *Ame. J. Plant Sci.* **11**: 707-735.

Pêra, F. F. P. G., Mutepfa, D. L. R., Khan, A. M., Els, J. H., Mbewana, S., van Dijk, A. A. A., Rybicki, E. P. and Hitzeroth, I. I. 2015. Engineering and expression of a human rotavirus candidate vaccine in *Nicotiana benthamiana. Virol. J*. **12**: 205.

Phoolcharoen, W., Dye, J.M., Kilbourne, J., Piensook, K., Pratt, W.D., Arntzen, C.J., Chen, Q., Mason, H.S. and Kralovetz, M.M. 2011. A nonreplicating subunit vaccine protects mice against lethal Ebola virus challenge. *Proc. Natl. Acad. Sci. USA.* **108**: 20695-20700.

Pillet, S., Racine, T., Nfon, C., Di Lenardo, T. Z., Babiuk, S., Ward, B. J., Kobinger, G. P. and Landry, N. 2015. Plant-derived H7 VLP vaccine elicits protective immune response against H7N9 influenza virus in mice and ferrets. *Vaccine*. **33**(46): 6282-6289.

Pniewski, T., Kapusta, J. and Płucienniczak, A. 2006. *Agrobacterium tumefaciens* -mediated transformation of yellow lupin to generate callus tissue producing surface antigen of HBV in a long-term culture. *J. Appl. Genet.* **47**: 309-318.

Pogrebnyak, N., Golovkin, M., Andrianov, V., Spitsin, S., Smirnov, Y., Egolf, R. and Koprowski, H. 2005. Severe acute respiratory syndrome (SARS) S protein production in plants: Development of recombinant vaccine. *Proc. Natl. Acad. Sci. USA*. **102**: 9062-9067.

Porta, C., Spall, V.E., Loveland, J., Johnson, J.E., Barker, P.J. and Lomonossoff, G.P. 1994. Development of cowpea mosaic virus as a high-yielding system for the presentation of foreign peptides. *Virology*. **202**: 949-955.

Qian, B., Shen, H., Liang, W., Guo, X., Zhang, C,. Wang, Y., Li, G., Wu A., Cao, K. and Zhang, D.2008. Immunogenicity of recombinant hepatitis B virus surface antigen fused with preS1 epitopes expressed in rice seeds. *Transgenic Res.* **17**: 621-631.

Ranjan, R., Sharma, A. and Verma, M.K. 2021. Characterization of the 2nd wave of COVID- 19 in India. 20 April, 2021. pp. 1-26. http://www.current science.ac.in

Renuga, G., Tandipani, A.B. and Arur, A.K. 2014. Transgenic banana callus derived recombinant cholera toxin B subunit as potential vaccine. *Int J Curr. Sci.* **10**: 61-68.

Richter, L.J., Thanavala, Y., Arntzen, C.J. and Mason, H.S. 2000. Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nat. Biotechnol.* **18**: 1167-1171.

Roychoudhury, A. 2020. Edible Vaccines and Oral Immunization against Viruses: Prospects, Promises and Pitfalls, *SF J. Med. Res.* **3**(1):1-6.

Roychoudhury, S., Das, A., Sengupta, P., Dutta , S., Roychoudhury , S. Choudhury, A. P., Ahmed A. B. F., Bhattacharjee, S. and Slama, P. 2020. Viral Pandemics of the Last Four Decades: Pathophysiology, Health Impacts and Perspectives. *Int. J. Environ. Res. Public Health.* **17**; 9411

Rubio-Infante, N., Govea-Alonso, D.O., Romero-Maldonado, A., Garcıa Hernandez, A.L., Ilhuicatzi-Alvarado, D., Salazar-Gonzalez, J.A., Korban, S.S., Rosales-Mendoza, S. and Moreno-Fierros, L. 2015. A plant-derived Multi-HIV antigen induces broad immune responses in orally immunized mice. *Mol. Biotechnol.* **57**: 662-674.

Ruedl, C. and Wolf, H. 1995. Features of oral immunization. *Int. Arch. Allergy Immunol.* **108**: 334-339.

Ruf, S., Hermann, M., Berger, I.J., Carrer, H. and Bock R. 2001. Stable genetic transformation of tomato plastids and expression of a foreign protein in fruit. *Nat Biotechnol.* **19**: 870-75.

Rukavtsova, E.B., Chebotareva, E.,N., Rudenko N.V. and Buryanov Y.I. 2011. Immunogenicity of biologically safe potato tubers synthesizing hepatitis B surface antigen. *Doklady Biol. Sci.* **437**: 110-112.

Rybicki, E.P. 2010. Plant-made vaccines for humans and animals. *Plant Biotechnol. J*. **8**: 620-637.

Salyaev, R.K., Rigano, M.M. and Rekoslavskaya, N.I. 2010. Development of plant-based mucosal vaccines against widespread infectious diseases. *Expert Rev. Vaccines.* 9: 937-946.

Sandhu, J.S., Krasnyanski, S.F., Domier, L.L., Korban, S.S, Osadjan, M.D and Buetow, D.E. 2000. Oral immunization of mice with transgenic tomato fruit expressing respiratory syncytial virus-F protein induces a systemic immune response. *Transgenic.Res.* **9**(2): 127-135.

Sharma, A., Tiwari, S., Deb, M.K., and Marty, J.L., 2020. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies. *Int J Antimicrob Agents* **56**(2): 106054.

Sharma, M. and Sood, B. 2011. A banana or a syringe: journey to edible vaccines. *World J. Microbiol. Biotechnol.* **27**: 471-477.

Shchelkunov, S.N., Salyaev, R.K., Pozdnyakov, S.G., Rekoslavskaya, N.I., Nesterov, A.E., Ryzhova, T.S., Sumtsova, V.M., Pakova, N.V., Mishutina, U.O. and Kopytina, T.V. 2006. Immunogenicity of a novel, bivalent, plant-based oral vaccine against hepatitis B and human immunodeficiency viruses. *Biotechnol. Lett.* **28**: 959-967.

Shoji, Y., Prokhnevsky, A., Leffet, B., Vetter, N., Tottey, S., Satinover, S., Musiychuk, K., Shamloul, M., Norikane, J., Jones, R. M., Chichester, J. A., Green, B. J., Streatfield, S. J. and Yusibov, V. 2015. Immunogenicity of H1N1 influenza virus-like particles produced in *Nicotiana benthamiana. Hum. Vaccin. Immunother.***11**(1): 118-123.

Streatfield, S.J. 2005. Regulatory Issues for Plant-Made Pharmaceuticals and Vaccines. *Expert Rev. Vaccines,* **4**: 591-601.

Streatfield, S.J. 2006 Mucosal immunization using recombinant plant-based oral vaccines. *Methods*. **38**: 150-157.

Tacket, C.O. and Mason, H.S. 1999. A review of oral vaccination with transgenic vegetables. *Microbes Infect*. **1**(10): 777-783.

Tacket, C.O., Mason, H.S., Losonsky, G., Estes, M.K., Levine, M.M. and Arntzen, C.J. 2000. Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *J. Infect. Dis.* **182**: 302-305.

Taylor, N.J. and Fauquet, C.M. 2002. Microparticle bombardment as a tool in plant science and agricultural biotechnology. *DNA Cell Biol.* **21**: 963-977.

Thanavala, Y., Yang, Y., Lyons, P., Mason, H.S. and Arntzen, C. 1995. Immunogenicity of Transgenic Plant-derived Hepatitis B Surface Antigen. *Proc. Natl. Acad. Sci. USA*. **92**: 3358-3361.

Tiwari, S., Verma, P.C., Singh, P.K. and Tuli, R. 2009. Plants as bioreactors for the production of vaccine antigens. *Biotechnol Adv.* **27**:449-67.

Valdes, R., Gomez, L., Padilla, S., Brito, J., Reyes, B. and Alvarez, T. 2003. Large scale purification of an antibody directed against hepatitis B surface antigen from transgenic tobacco plants. *Biochem. Biophys. Res. Commun.* **308**: 94-100.

Van Dolleweerd, C.J., Teh, A.Y.H., Banyard, A.C., Both, L., Lotter-Stark, H.C.T. and Tsekoa, T. 2014. Engineering, expression in transgenic plants and characterisation of E559, a rabies virus-neutralising monoclonal antibody. *J. Infect. Dis.* **210**(2): 200-208.

Viboud, C., Grais, R.F., Lafont, B.A., Miller, M.A., and Simonsen, L. 2005. Multinational impact of the 1968 Hong Kong influenza pandemic: evidence for a smoldering pandemic. *J. Infect. Dis.* **192**: 233-48.

Waghulkar, V.M. 2010. Fruit Derived edible vaccines: Natural way for the vaccination. *Int. J. Pharm. Tech. Res.* **2**(3): 2124-2127.

Walmsley, A.M. and Arntzen, C.J. 2000. Plants for delivery of edible vaccines, *Curr. Opinion Biotechnol.* **11**: 126-129.

Wang, Y.Q. and Li, T. 2008. Transformation of HBsAg into tomato and production of transgenic tomato plants. *J. Southwest Univ* (Nat Sci Ed). **30**: 78-83

Ward, B. J., Landry, N., Trepanier, S., Mercier, G., Dargis, M., Couture, M., D'Aoust, M. A. and Vezina L.P. 2014. Human antibody response to N-glycans present on plant-made influenza virus-like particle (VLP) vaccines. *Vaccine*, **32**: 6098-6106

Warzecha, H., Mason, H.S., Lane, C., Tryggvesson, A., Rybicki, R., Williamson, A., Clements, J.D. and Rose, R.C. 2003. Oral immunogenicity of human Papillomavirus-like particles expressed in *potato. J. Virol.* **77**(16): 8702-8711.

Webster, D.E., Thomas, M.C., Huang, Z. and Wesselingh, S. L. 2005. The development of a plant-based vaccine for measles. *Vaccine,* **23**(15): 1859-1865.

William, S. 2002. A review of the progression of transgenic plants used to produce plant bodies for human usage. *J Young Invest.* **4**:56-61.

Wu, Y.Z., Li, J.T., Mou, Z.R., Fei, L., Ni, B., Geng, M., Jia, Z.C., Zhou, W., Zou, L.Y. and Tang Y. 2003. Oral immunization with rotavirus VP7 expressed in transgenic potatoes induced high titers of mucosal neutralizing IgA. *Virology*, **31**: 337- 342

Yang, Y.M., Lia, X., Yang, H., Qian, Y., Zhang, Y., Fang, R.X., Chen, X.Y. 2011. Immunogenicity and viruslike particle formation of rotavirus capsid proteins produced in transgenic plants. *Sci. China Life Sci.* **54**(1): 82-89

Youm, J.W., Won, Y.S., Jeon, J.H., Moon, K.B., Kim, H.C., Shin, K.S., Joung, H. and Kim, H.S. 2010. Antibody responses in mice stimulated by various doses of the potato-derived major surface antigen of hepatitis B virus. *Clin. Vaccine Immunol.* **17**: 2029-2032.

Yu, J. and Langridge W. 2003. Expression of rotavirus capsid protein VP6 in transgenic potato and its oral immunogenicity in mice. *Transgenic Res.* **12**: 163-169.

Yuki, Y. and Kiyono, H. 2003. New generation of mucosal adjuvant for the induction of protective immunity. *Rev. Med. Virol.* **13**(5): 293-310

Yusibov, V. and Rabindran, S. 2008. Recent progress in the development of plant derived vaccines. *Expert. Rev. Vaccines*.**7**(8): 1173- 1183.

Yusibov, V., Hooper, D.C., Spitsin, S.V., Fleysh, N., Kean, R.B. and Mikheva, T. 2002. Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine*. **20**: 3155- 3164

Yu-Zhang, W., Jin-Tao, L., Zhi-Rong, M., Lei, F., Bing, N., Miao, G., Zheng-Cai, Jia., Wei Zhou, Li-Yun, Z. and Yan, T. 2003. Oral immunization with rotavirus VP7 expressed in transgenic potatoes induced high titers of mucosal neutralizing IgA. *Virology*. **313**(2): 337-342.

Zappa, A., Amendola, A., Romano, L. and Zanetti, A. 2009. Emerging and re-emerging viruses in the era of globalization. *Blood Transfus*., **7**: 167-171.

Zeitlin, L., Olmsted, S. S., Monech, T. R., Co, M. S., Martinell, B. J., Paradkar, V. M., Russell D. R., Queen, C., Cone, R.A. and Whaley, K.J. 1998. A humanized monoclonal antibody in plants using a plant virus vector. *J. Immunol. Methods.* **220**: 69-75.

Zhang, G. G., Rodrigues, L., Rovinski, B. and White, K. A. 2002. Production of HIV-1 p24 protein in transgenic tobacco plants. *Mol. Biotechnol*. **20**(2): 131-136.

Zhang, X., Buehner, N. A., Hutson, A. M., Estes, M. K. and Mason, H. S. 2006. Tomato is a highly effective vehicle for expression and oral immunization with Norwalk virus capsid protein. *Plant Biotechnol. J.* **4**(4): 419-432.

Zhao, X.Y., Zhang, X. D. and Zhang, T. 2002. Cloning and expression of hepatitis B virus surface antigen in carrots. *Progress Microbiol. Immunol.*, **30**, 1:1

Zumla, A., Chan, J.F., Azhar, E.I., Hui, D.S. and Yuen, K.Y. 2016. Corona viruses-drug discovery and therapeutic options. *Nat Rev Drug Discov.* **15**: 327–347.