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REVIEW

Plant-derived VLP: a worthy platform to produce vaccine against SARS-CoV-2

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Abstract After its emergence in late 2019 SARS-CoV-2 was declared a pandemic by the World Health Organization on 11 March 2020 and has claimed more than 2.8 million lives. There has been a massive global effort to develop vaccines against SARS-CoV-2 and the rapid and low cost production of large quantities of vaccine is urgently needed to ensure adequate supply to both developed and developing countries. Virus-like particles (VLPs) are composed of viral antigens that self-assemble into structures that mimic the structure of native viruses but lack the viral genome. Thus they are not only a safer alternative to attenuated or inactivated vaccines but are also able to induce potent cellular and humoral immune responses and can be manufactured recombinantly in expression systems that do not require viral replication. VLPs have successfully been produced in bacteria, yeast, insect and mammalian cell cultures, each production platform with its own advantages and limitations. Plants offer a number of advantages in one production platform, including proper eukaryotic protein modification and assembly, increased safety, low cost, high scalability as well as rapid production speed, a critical factor needed to control outbreaks of potential pandemics. Plant-based VLP-based viral vaccines currently in clinical trials include, amongst others, Hepatitis B virus, Influenza virus and SARS-CoV-2 vaccines. Here we discuss the importance of plants as a next generation expression system for the fast, scalable and low cost production of VLP-based vaccines.

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Introduction

Coronaviruses (CoVs) are a major group of viruses belonging to the family Coronaviridae that cause a wide spectrum of diseases. The early twenty-first century has experienced the unprecedented spread of previously unknown, deadly coronaviruses (Song et al. 2019). CoVs infect the gastrointestinal, respiratory, hepatic, and central nervous systems of humans, birds, livestock, mouse, bat, and many other wild animals (Chen and Guo 2016; Ge et al. 2013; Wang et al. 2006).

SARS-CoV emerged as a new human infection in South China in November 2002 and ended in July 2003. It infected 8096 people and caused 774 deaths with an overall mortality rate of about 9.6% (Drosten et al. 2003; Ksiazek et al. 2003). MERS-CoV, another highly pathogenic CoV, first emerged in Saudi Arabia, caused a total of 2494 laboratory-confirmed cases of infection and 858 deaths in 27 countries (mortality rate, 34.4%) since September 2012 (http://www.who.int/emergencies/mers-cov/en/) (Hemida 2020). These two highly pathogenic betacoronaviruses (β-CoVs) have posed a substantial threat to public health (Han et al. 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged from China in December 2019 and has since been reported all over the world with more than 124,000,000 confirmed cases and 2,730,000 deaths up to 22 March 2021 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/; https://www.worldometers.info/coronavirus/). (Hemmati et al. 2020). In comparison with the H1N1 Influenza virus that has a 0.02% mortality rate, SARS-CoV-2 has a mortality rate of 3% (Rosales-Mendoza 2020). A number of therapeutic options for the treatment of SARS-CoV-2 have been proposed (Li and Clercq 2020) however, vaccination remains one of the most effective strategies in the prevention of new coronavirus virus epidemics (He and Jiang 2005).

SARS-CoV-2 is an enveloped, positive-sense, single-stranded linear RNA betacoronavirus with a genome ranging from 26 to 32 kb in length (the largest viral RNA genome known) (Armbruster et al. 2019). Similar to SARS and MERS, the SARS-CoV-2 genome encodes structural proteins [e.g., spike glycoprotein (S)], non-structural proteins (e.g., 3-chymotrypsin-like protease, papain-like protease, helicase, and RNA-dependent RNA polymerase), and accessory proteins. These five proteins were recognized as attractive targets for developing vaccines and other antiviral agents against SARS and MERS (Fehr and Perlman 2015; Han et al. 2020; Li et al. 2020; Malik et al. 2020). The spike glycoprotein (S) forms homotrimers that are presented on the surface of the virion resulting in the typical coronavirus crown-like appearance. This S protein is indispensable for virus-cell receptor interactions during viral entry (Mahmood et al. 2021; Zumla et al. 2016) and is the major antigen capable of eliciting protective immune responses (Du et al. 2009; Gralinski and Menachery 2020). It is therefore not surprising that the S protein has been the most appealing target protein included in the 12 vaccines that have received authorization for use in at least one region (Funk et al. 2021). Among these are a lipid nanoparticle-encapsulated mRNA vaccine and recombinant adenovirus-vectored vaccine expressing the S protein (Folegatti et al. 2020; Mulligan et al. 2020). Subunit vaccines containing individual SARS-CoV-2 antigens as well as virus-like particle (VLP)-based vaccines presenting antigens in multiarrays on their surfaces are also being developed and are being tested in clinical trials (Capell et al. 2020; Ward et al. 2020; Palca 2020, Novavax.com,). The recent emergence of neutralization-resistance SARS-CoV-2 variants (Williams and Burgers 2021) necessitate the employment of vaccine expression systems that allow for the rapid production of relevant vaccines at a low-cost ensuring vaccine availability to even resource constrained developing countries (Fuenmayor et al. 2017; Giddings 2001; Loh et al. 2017; Twyman et al. 2003).

Virus-like particles (VLPs) as vaccine candidates

Virus-like particles (VLPs) are self-assembled structures from viral antigens that mimic the three-dimensional, morphological structure of virions but lack the viral genome (Grgacic and Anderson 2006; Kushnir et al. 2012). Due to their similarity with the native

virion in terms of size, shape, and the repetitive array of immunogenic epitopes displayed on the VLP surface, VLPs, like native virions, can induce potent cellular and humoral immune responses without adjuvant (Bachmann and Jennings 2010; Chackerian 2007; Ge et al. 2013; López-Macías 2012; Roldao et al. 2010). Due to particulate nature and size, VLPs interact and are taken up by dendritic cells (DC's) and which then process and present the thousands of epitopes a single VLP contains on MHC class I/II molecules inducing a potent T-cell mediated immune responses (Chackerian 2007; Roldao et al. 2010). The high density display of epitopes in repetitive arrays on the surface of VLPs are also able to elicit high titre and durable B-cell immune responses in the absence of adjuvants (Bachmann et al. 1993; Fehr et al. 1998).

VLPs also present a safer alternative to the current generation of live, attenuated or inactivated vaccines due to their lack of viral nucleic acid and are thus non-infectious (Marsian and Lomonossoff 2016). In addition, antigens being presented in their native conformation on the surface of a VLP are more stable than in a subunit form resulting in smaller and less frequent doses of the antigen required to elicit a protective immune response (Chen and Lai 2013).

The intrinsic characteristics of VLPs have made them into one of the most successful recombinant vaccine platforms. VLP-based vaccines that have been approved by regulatory agencies and are available on the commercial market include those against human papillomavirus virus (HPV) (Cervarix™ by GlaxoSmithKline and Gardasil® and Gardasil9® by Merck), composed of the HPV major capsid protein L1, and against hepatitis B virus (HBV) (Energix® GlaxoSmithKline and Recombivax® by Merck) composed of hepatitis B surface antigen (HBsAg). These vaccines have been shown to be highly effective and safe in humans and capable of inducing long-lasting humoral immune responses These vaccines can induce long-lasting immune responses due to the induction of antibodies that are highly effective and safety profiles in humans (Chackerian 2007; Ge et al. 2013). These successes have encouraged the development of VLP-based vaccines against a wide variety of other diseases. However, the high costs associated with the yeast and insect cell expression systems that produce the HPV and HBV vaccines preclude the widescale use of these vaccines in developing countries (Waheed et al. 2012).

VLP production platforms, including plants

Current VLP expression platforms include bacterial, yeast, insect, mammalian cell culture and more recently, plants, with the yeast, insect and mammalian systems used for commercial production (Chen and Lai 2013). Each system has its unique advantages and limitations and the choice of platform is usually dependent on the structure and function of the VLPs produced, the scalability and cost of the production process (Gecchele et al. 2015). The use of Escherichia coli bacteria is widespread due its rapid growth rate and simplicity, which allows recombinant proteins to be produced in less than a day. However, the inability to perform glycosylation and other post-translational protein modifications which are critical in the correct protein folding and assembly of VLPs preclude the use of bacterial expression for VLPs (Edman et al. 1981; Ma et al. 2005; Yao et al. 2015). The presence of bacterial endotoxins that require removal post production is also a major drawback. In yeast cells glycosylation is mostly limited to inconsistent high mannose glycoforms (Wildt and Gerngross 2005) which is not optimal for the assembly of many VLPs. The simple post translational modifications (high mannose glycosylation), as well as coproduction of baculovirus particles, are limitations of the insect cell expression system (Chen and Lai 2013; Demain and Vaishnav 2009; Loh et al. 2017). The most optimal environment for authentic post-translational protein modifications and correct VLP assembly are mammalian cell cultures. However, significant capital investment to set up a manufacturing facility, significantly higher production costs and well as contaminating adventitious human pathogens in the cultures are significant drawbacks to this production system (Chen 2008). Indeed, all cell-culture-based production systems require the building of new facilities and fermentation tanks for large scale production creating challenges in scalability. Plants, however, are inexpensive to grow on a large scale in greenhouses or bioreactors, and have been investigated for the last 20 years for the production of therapeutics for humans and animals (Lico et al. 2008; Ma et al. 2003; Twyman et al. 2003).

Current plant expression systems enable the production of large quantities of recombinant protein with post-translational modifications allowing for VLP assembly, at low cost and at low risk of introducing

adventitious human pathogens (Faye and Gomord 2010). Early attempts at VLP production in plants had several drawbacks including low yields and a very slow production process which involved the production of stable transgenic plants (Davies 2010). The recent development of plant virus-based transient expression systems, depicted in Fig. 1, have greatly increased VLP production speed and yield (Marsian and Lomonossoff 2016).

Deconstructed viral vectors, based on Tobacco Mosaic virus (TMV), the Cowpea Mosaic virus (CPMV), Potato virus X and Bean yellow dwarf geminivirus, are delivered into plants by Agrobacterium allowing for the fast expression of recombinant proteins at high yields and allowing for highly scalable, GMP-complying industrial processes (Salazar-González et al. 2015). The TMV RNA replicon (the Magnicon) system (Huang et al. 2009), CPMV RNA replicon (the pEAQ) system (Sainsbury and Lomonossoff 2008) and the geminiviral DNA replicon system based on bean yellow dwarf virus (BeYDV) (Santi et al. 2008) are popular transient expression systems that could be used for the rapid expression and assembly of VLP vaccines against viruses that mutate their surface antigens rapidly, like coronaviruses.

N-glycosylation of proteins in plants is similar to that in mammalian cells however, plant-specific β-1,2-xylose and core α-1,3-fucose residues are added to complex N-linked glycans and no terminal β1,4-gal and N-acetylneuraminic acid (Neu5Ac) residues are added (Gomord et al. 2010). These minor differences between plant and mammalian cell protein glycosylation were previously thought to be capable of reducing efficacy of plant-produced vaccines and/or eliciting allergic adverse side-effects in immunized individuals (Chen and Lai 2013). Recent studies in 14,000 people immunized with plant-derived influenza vaccine candidates indicate no allergic-type reaction to these plant-produced influenza VLPs presenting plant glycosylation (Ward et al. 2014). Plant-specific glycosylation thus does not interfere with the proper folding and assembly of the influenza VLPs which are also immunogenic. 'Humanized' plant lines, which have been genetically modified to eliminate plant-specific glycans and/or introduce mammalian-specific glycosylation, produce proteins with specific mammalian glycoforms including high mannose, GnGn, G0–G2 galactose, bisected GlcNAc, fucosylated and non-fucosylated and terminal sialic acid addition with a high degree of glycan uniformity (Castilho et al. 2011, 2008, 2010; Cox et al. 2006; Gomord et al. 2010; Schähs et al. 2007; Strasser et al. 2009, 2008).

Advantages of the current plant transient expression systems are thus appropriate glycosylation of the expressed proteins resulting in proper folding and assembly of VLPs, rapid rate of protein production, high protein yields, high scalability, low cost and increased safety due to the low risk of adventitious

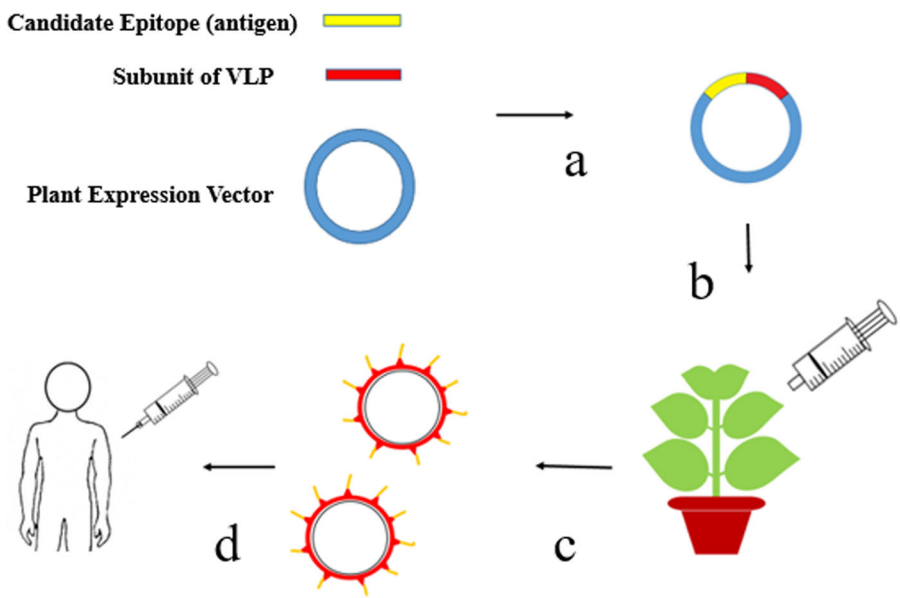

Fig. 1 Schematic review of recombinant protein expression in plants. *VLP* Virus-like particle, **a** Recombinant vector construction, **b** Agroinfiltration in plants, **c** 3–7 days post-infiltration recombinant proteins are expressed in the leaves, and VLP subunits assembled into VLPs displaying epitope on the surface, and **d** VLPs are purified and used as a vaccine to induce immune responses

human pathogens (Chen and Lai 2013). Unlike other expression systems, a plant-based system does not require expensive equipment for start-up and operation so that the cost of production based on the plant system has reduced to 0.1% of the mammalian cell culture system and 2–10% of microbial systems (Yao et al. 2015). Plants are photoautotrophs, easy to grow, and require no advanced equipment, thus they can be easily cultivated and produced in large numbers. Moreover, plants can make complex proteins with structures that are naturally similar to spider silk, collagen, and secretory immunoglobulin A (Tschofen et al. 2016). Moreover, plants are free of endotoxins, oncogenes, and mammalian pathogens, therefore, they are safe and exempt from the costs of refinement and product screening. More than 25 plant-expressed pharmaceuticals have been developed and evaluated in the clinical trials (Loh et al. 2017). It is thus not surprising that commercial companies have successfully developed a number of plant-expressed pharmaceutical vaccines and therapeutics (Table 1).

Plant-produced VLPs as vaccine candidates

Several VLPs have been developed as vaccine candidates in plants since plant expression systems were introduced. Hepatitis B virus (HBV), human papillomavirus (HPV), influenza virus, SARS-CoV-2, Norwalk virus, human immunodeficiency virus, foot-and-mouth disease virus, Rotaviruses, Bluetongue virus, and hepatitis C virus VLPs have all been assembled as vaccines in plants (C Thuenemann et al. 2013; D'Aoust et al. 2008; Mason et al. 1996; Meyers et al. 2008; Pan et al. 2008; Saldaña et al. 2006; Santos et al. 2005; Scotti et al. 2009; Ward et al. 2021). In this review we will discuss in detail the plant-produced vaccines against HBV, HPV, Influenza and SARS-CoV-2.

HBV

HBV VLPs have been widely studied in the last two decades. HBV VLPs were first assembled in yeast in 1984 and induced a good immune response as a vaccine candidate (McAleer et al. 1992). Two commercial vaccines, Recombivax® and Energix®, based on HBV VLPs, are produced in yeast expression systems. Arntzen's group first demonstrated the possibility of producing VLPs in plants in 1992. They expressed the hepatitis B core antigen (HBcAg) in tobacco and assembled 22-nm HBV VLPs capable of inducing an immune response in mice (Mason et al. 1992; Thanavala et al. 1995). More recently, HBV VLPs have been generated through the expression of HBcAg in transient expression systems mediated by expression vectors such as pEAQ in *Nicotiana benthamiana* plants (Huang et al. 2006; Mechtcheriakova et al. 2006; Peyret et al. 2019; Peyret and Lomonossoff 2013). These VLPs are morphologically and immunogenically quite similar to yeast- or *E. coli*-derived HBV VLPs (Huang et al. 2006).

Due to their particulate nature and ability to display epitopes on their surface in a dense repetitive array, HBV VLPs are capable of eliciting strong cellular and humoral immune responses whilst presenting no cytotoxicity in humans (Francis et al. 1990). Indeed in recent years there has been a great deal of interest in utilizing plant expressed HBV VLPs as carriers of immunogenic epitopes or peptides for vaccine development (Roose et al. 2013).

Human papillomavirus (HPV)

Researchers discovered that the expression of main capsid protein of HPV (L1) in enabled the formation of VLPs and consequently two commercial HPV L1-based VLP vaccines, Gardasil® and Cervarix™, were produced in yeast and insect cells, respectively (Biemelt et al. 2003). However, the high cost of producing HPV VLPs in these expression system encouraged researchers to produce these VLPs in plants. At first, VLPs were produced by stable transgenic methods in potato and tobacco plants and were found to be similar in shape and size to commercially produced VLPs, but the yield was low. Transient expression with MagnICON vectors in *N. benthamiana*, however, increased the VLP yield to more acceptable levels (Chen and Lai 2013). HPV VLPs have also been produced in tobacco leaves using pEAQ vector (Matić et al. 2012). Studies have shown that plant-derived HPV VLPs are as effective as commercial vaccines in stimulating the immune system of animal models (Chen and Lai 2013).

Table 1 Some companies using plant-based platform for pharmaceutical production

Company	Host	Technology	Products	Websites
Medicago	*Nicotiana benthamiana*	VLP*Express*™	Influenza VLP vaccine candidate; SARS-CoV-2 VLP vaccine candidate	https://www.medicago.com/en/
Icon Genetics	*Nicotiana benthamiana*	magnICON®-based expression vector	Vaccine candidate non-Hodgkin's lymphoma; ZMapp™ for Ebola virus disease	https://www.icongenetics.com/
Leaf Expression Systems	*Nicotiana benthamiana*	Hypertrans® Protein Expression System	Cowpea Mosaic Virus VLPs; ecombinant SARS-CoV N-protein (Nucleoprotein, His-Tag); SARS-CoV-2 Nucleocapsid (N) Protein	https://www.leafexpressionsystems.com/
PlanetBiotechnology	Tobacco leaves	Transient and Stable Expression	CMG2-Fc; TEM8-Fc; BSG-Fc;AtCry1	https://www.planetbiotechnology.com/
Cape Bio Pharms	*Nicotiana benthamiana*	Transient Expression Vector	PtX™ SARS-CoV-2 Spike Protein (S1, Rabbit FC); PtX™ SARS-CoV-2 Spike Protein (RBD, Rabbit FC); CB_0002.5 PtX™ SARS-CoV-2 Spike Protein (S1-His); Anti-HIV-1 p24 humanized IgG1 antibody conjugated to Horseradish Peroxidase (HRP); tX™ Anti-HIV-1 p24 Human IgG1 antibody	https://www.capebiopharms.com/
Healthgen Biotechnology	*Oryza sativa* (Rice)	Oryz^{HiExp},platform	Recombinant Human Serum Albumin (OsrHSA™); Recombinant Epidermal Growth Factor (OsrEGF)	https://www.oryzogen.net/
Mapp Biopharmaceutical	Tobacco leaves	magnICON®-Based Expression Vector	ZMapp™ for Ebola virus disease	https://mappbio.com/
Kentucky BioProcessing	*Nicotiana benthamiana*	magnICON®-Based Expression Vector	ZMapp™ Ebola virus disease; accine candidate for SARS-CoV-2 (pre-clinical phase)	https://kentuckybioprocessing.com/

VLP Virus-like particle, *SARS-CoV-2* Severe acute respiratory syndrome coronavirus 2, *IgG* Immunoglobulin G

Influenza virus

Influenza virus haemagglutinin protein (HA) when expressed in plant expression systems assembles into VLPs. Medicago has harnessed this technology to produce about ten million doses of VLP-based H1N1 Influenza vaccine in 1 month according to Phase I cGMP regulations (www.darpa.mil/NewsEvents/Releases/2012/07/25.aspx). This company is currently the front runner worldwide in the production of plant-based influenza VLPs. Monovalent VLP-based vaccines were first developed and produced as a response to the H7N9 and H5N1 flu pandemics and were subsequently followed by the development of a plant-expressed quadrivalent HA-based VLP to fight seasonal flu, which has successfully completed the phase 1, 2, and 3 clinical trials (Pillet et al. 2016, 2019). Recently, two phase 3 trials have shown that plant-based HA VLPs provide better protection in adults against influenza viruses compared with commercial flu vaccines that derived from eggs (Ward et al. 2020). In addition, it has been shown that HA-only VLPs produced in plants afforded protection to participants in Phase I/II clinical trials while no negative effects on immune reactions or aggravation of plant-based allergies were observed due to plant-derived glycosylation (Ward et al. 2014). Although, there has been much success achieved with the HA protein, other Influenza virus proteins such as M1 Matrix protein, NA neuraminidase, and the NP nucleoprotein remain to be explored as vaccine candidates due to their more conserved natures.

SARS-CoV-2

Following the success of its production of influenza virus VLPs in plants, Medicago has, through its platform and acquired knowledge, developed a SARS-CoV-2 VLP-based vaccine in plants, and is currently at the forefront of SARS-CoV-2 vaccine development. The SARS-CoV-2 spike gene sequence was introduced into *N. benthamiana* plants with agrobacterium-mediated transformation. Following expression of the desired protein SARS-CoV-2 VLPs assembled which was quite similar in size and shape to the native SARS-CoV-2 virions (Fig. 2) (Ward et al. 2021). This VLP-based SARS-CoV-2 vaccine candidate has shown to be successful in phase I human clinical trials and is currently undergoing phase 2 and 3 clinical trials and is estimated to be produced at a rate of 10 million doses per month (Rosales-Mendoza 2020; Phillip, International 2020).

Chimeric plant-produced VLPs as vaccines

VLPs may also act as a presentation system for the display of heterologous epitopes to the immune system. Foreign epitopes are coupled to the capsid proteins on the surface of VLPs either by genetic fusion or chemical conjugation and presented in high density repetitive arrays (Chen and Lai 2013). These chimeric VLPs (cVLPs) protect the presented antigen and enhance immune cell uptake and stimulation due to their particulate nature and high density epitope presentation (Acosta-Ramírez et al. 2008; Lacasse et al. 2008; Manayani et al. 2007; Maurer et al. 2005; Plummer and Manchester 2011; Work et al. 2008). Although a more stable bond is generated between VLP and antigen via genetic fusion, it cannot be predicted with certainty whether chimeric VLPs will assemble properly, the assembly being dependent on factors such as peptide insert length and charge (Bendahmane et al. 1999). However a number of foreign epitopes have been successfully been inserted into domains of HBcAg, HBsAg and HIV proteins that are dispensable for VLP assembly or fused to the *N*- or *C*-termini of VLP capsid proteins (Chen and Lai 2013). In order to overcome the limitations of insert size and charge on the correct assembly of chimeric VLP, chemical conjugation of the target antigen to the native VLP is performed. Antigens are linked to the VLPs either through covalent bonds i.e. cysteine-lysine linkages (Lechner et al. 2002) or noncovalent bonds i.e. streptavidin–biotin interaction (Chackerian et al. 2006). The specific interaction between Staphylococcus aureus protein A and the antibody constant fragment can also be exploited (Werner et al. 2006).

In the last two decades, HBcAg VLPs have been one of the most widely used platforms for the presentation of foreign antigens. The antigens of a number of pathogens such as foot-and-mouth-disease virus (FMDV), hepatitis C virus (HCV), hepatitis B

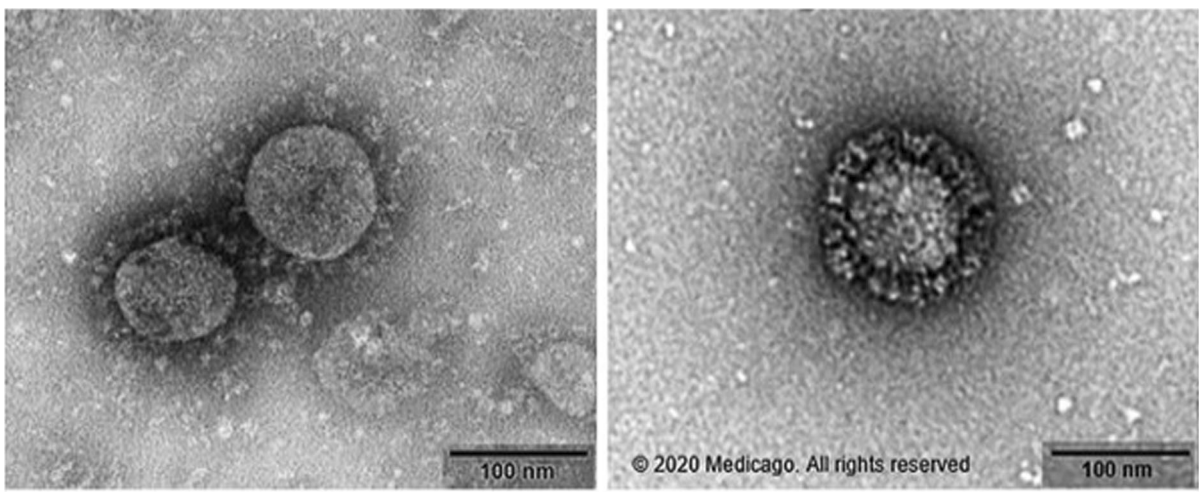

Fig. 2 Comparison of SARS-CoV-2 VLP made by Medicago with wild-type SARS-CoV-2 (Ward et al., 2021) https://www.medicago.com/en/). *SARS-CoV-2* Severe acute respiratory syndrome coronavirus 2, *VLP* Virus-like particle; (Left) Wild-type SARS-CoV-2; (Right) Medicago's Plant-Derived VLP of SARS-CoV-2

virus (HBV), and HIV have been displayed using chimeric HBcAg VLPs (Chen and Li 2006; Clarke et al. 1987; Malik et al. 2012; Stahl and Murray 1989; Ulrich et al. 1992; Yang et al. 2005; Zhang et al. 2007; Zheng et al. 2016). Chimeric HBcAg VLPs presenting antigens from Dengue virus, Hepatitis E virus (HEV), Human papilloma virus (HPV), Influenza virus, malaria parasite, West Nile virus (WNV) and Zika virus have been expressed in plants (Buonaguro et al. 2011; Chen et al. 2011; Diamos et al. 2019, 2020; Pang et al. 2019; Ponndorf et al. 2021; Ravin et al. 2012; Santi et al. 2006; Yang et al. 2017; Zahmanova et al. 2021). Also produced in plants are chimeric hepatitis B surface antigen (HBsAg) VLPs presenting antigens from HCV, HIV as well as the entire GFP protein (Greco et al. 2007; Huang and Mason 2004; Mohammadzadeh et al. 2020).

Another well researched epitope presentation platform are chimeric HPV VLPs (Lee et al. 2012; Schellenbacher et al. 2009; Slupetzky et al. 2001; Varsani et al. 2003). Chimeric HPV CLPs produced in plants have been used to display Influenza epitopes and HPV epitopes, amongst others (Chabeda et al. 2019; Rosa et al. 2009; Matić et al. 2011).

Plant virus particles (PVPs) offer similar advantages to VLPs in enhancing safety, immunogenicity, yield and stability of presented antigens (McCormick and Palmer 2008; Plummer and Manchester 2011; Sainsbury and Lomonossoff 2008). PVPs from Cowpea mosaic virus (CPMV), tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), alfalfa mosaic virus, potato virus X (PVX), and papaya mosaic virus (PapMV) have been studied extensively (Brennan et al. 1999; Chichester et al. 2018; Attar et al. 2004; Jiang et al. 2006; Lacasse et al. 2008; Liu et al. 2005; Pulcini et al. 2013; Wang et al. 2007).

Plant-expressed chimeric Cucumber mosaic virus (CMV) PVPs have been used to present Newcastle disease virus epitopes as well as the Zika virus envelope protein (Cabral-Miranda et al. 2019; Natilla and Nemchinov 2008) while Papaya mosaic virus (PapMV) PVPs have displayed HCV and Influenza epitopes (Babin et al. 2013; Carignan et al. 2015; Denis et al. 2007; Laliberté-Gagné et al. 2019; Thérien et al. 2017). Plant expressed chimeric Cowpea mosaic virus (CPMV) PVPs have been used for the display of HIV epitopes (McLain et al., 1996) as well as more recently SARS-CoV-2 epitopes (Chung et al. 2020). Formulating epitope-based vaccines is an option to reduce the risk of disease enhancement in SARS-CoV-2 infection (Venkataraman et al. 2021).

Conclusions and future perspective

Virus-like particles (VLPs) have been shown to have a number of advantages over traditional and recombinant subunit vaccines including enhanced safety when compared to attenuated and inactivated vaccines and improved immunogenicity and stability when compared to subunit vaccines. When expressed in plant transient expression systems the additional advantages of VLPs include proper eukaryotic modification and assembly, high scalability for high yields, decreased cost for improved product accessibility to developing countries, increased safety due to the lack of contaminating human pathogens, as well as the production speed required to control the outbreak of pandemics, such as SARS-CoV-2.

Several pharmaceutical companies have specialized in producing plant pharmaceuticals and vaccines and efficient, scalable, cost-effective and cGMP-compliant processes have been developed to recover VLPs from plants (Chen and Lai 2013). Although the regulatory hurdle of FDA approval for plant-expressed VLPs still need to be overcome, the great strides accomplished by the plant-produced influenza and SARS-CoV-2 VLP-based vaccine candidates currently in Phase I, II and III clinical trials suggests that the registration of these products, and consequently acceptance of other plant-produced therapeutics and vaccines, is within reach.

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Conflict of interest The authors have declared no conflict of interests.

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