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EDITORIAL

Is it possible to ensure COVID19 vaccine supply by using plants?

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Abstract
Any disease that spreads quickly and crossed the geographical barrier is termed as pandemic. After the initial occurrence of Covid-19 from China, World Health Organization had declared novel corona viral outbreak as pandemic on March, 2020. Since then, COVID-19 continued to devastate people all around the world. Human civilization has witnessed one of its greatest crises by facing 180 million of confirmed cases with 38.9 lakh deaths across the world till end of June 2021. India alone contributes 30 million of positive cases and has lost 3.92 lakh valuable lives (data as on 24th of June 2021 from CSSEGIS and Data (http://github.com/CSSEGISandData/COVID-19); (the number increases in each day). Bio-medical experts from all around the world are working tirelessly to limit the disease and find potential cures for this viral infection. Vaccination is the most effective strategy to prevent the spread of any viral disease. Virologists have developed some effective vaccines, but production or supply lags far behind the present demand across the globe. Plant-derived vaccines (PDVs) based on modified virus like particles (VLPs) can be a feasible alternative in this case. A summarized account about the efficacy of the first plant-derived Covid 19 vaccine, CoVLP is discussed. PDVs and VLPs are also reviewed briefly, along with their benefits and drawbacks.

Keywords Covid 19 · Pandemic · Plant derived vaccine · Therapeutics · Viral like particles

Introduction

Since the last couple of years mankind has continuously been facing its greatest challenge of the century. The global pandemic emanated during December 2019 from Wuhan, Hubei province. Nevertheless SARS CoV-2 is now grasping the whole world by its multiple waves of devastations. Primitive acellular nature along with RNA as genetic material has given the severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) an extra potency to modify against human immune barriers and continuously succumb complex orchestration of human immune functions [8]. Wide host ranges, climatic variations and indefinite applications of non-confirmed trials of medications create a huge pressure on the viral particles to mutate faster than usual. Despite all these mutation events the basic mechanism of interaction of spike (S) protein of viruses with human ACE 2 receptor (angiotensin-converting enzyme 2) is the key regulatory event in this host–pathogen interaction [12]. In this gloomy situation the only ray of hope has cropped up with the discovery of some effective vaccines against these viruses [6]. Rapid vaccination drive to the whole population is the only fastest and cost-effective way to save the mankind in this present situation. The main constrain of this process is the breaking of the supply chain against huge demand. Most of the effective vaccine production is carried out by animal cell culture and ova culture systems which are taking months to produce considerable amount of clinical grade vaccine dose. Another major restraint of this production method is storage, stability as well as frequent contamination or infection of the cell culture systems. In this situation plant derived vaccines (PDVs) have proved to be potent alternative. PDVs are safe, free from any subsequent human pathogenic contamination and predominantly high-quality clinical grade doses can be produced within weeks [17]. The "molecular farming" using plants is not completely new. First, biofarming of human growth hormone was successfully done on 1986; subsequently, human serum albumin was produced in tobacco cell culture [6, 19]. First plant derived vaccines

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were made against Hepatitis B virus in 1992 [13]. Besides vaccines, plants are also the repertoire of innumerable therapeutic metabolites. Several studies have shown potentiality of plant-based biomolecules for treating SARS-CoV-2 either alone or synergistically with other probable drugs [16]. The proper study on these huge resources of medicinal plants and application of cutting-edge technologies to find out actual mode of action of these hidden treasures would provide many future wonder drugs [16, 18].

Virus like particles (VLPs): the possible alternative for conventional vaccines

VLPs are the self-assembling, non-replicative, non-pathogenic supramolecular particles used to target specific host and drug delivery system [7]. Literally VLPs have a very wide sense of application i.e., any acellular body which have similarity with viruses can be grouped under VLPs. Pathogenic epitopes displayed in a non-pathogenic coat or carrier system largely popularized this amazing technology to revolutionize drug delivery system as well as development of a plant derived vaccine though engineered VLPs. They have several positive features in development of vaccines. As the VLPs lack the entire genome except the "epitope" they are unable to produce any disease in host cell, moreover, the coat proteins are in maximum cases engineered from different viruses. So, incompatibility with coat protein is another point for its ineffectiveness. Lipid enveloped coat is found to be very effective carrier for VLPs. The comparatively better stability of lipid system in cellular environment makes them supercilious delivery system. Scientists have also tried the "liposomes" as delivery carriers for VLPs but this system needs further standardization. VLPs are engineered such a way, that epitope proteins are widely displayed along with densely repeated amino acid motifs, which help the humoral immune system to instigate B cell more efficiently [2, 4]. Another important factor is the size, due to very low size, delivery system by VLPs become more efficient as readily taken up by antigen presenting cell (APC), which in turn help in activation of T cell for cytotoxicity and eventually boost up immunity[1].

Plants as vaccine production system with one-of-a-kind delivery mechanism

Plants hold an easy but efficient production powerhouse due to its uniform and proliferous growth patterns. It is very facile to manage cultural parameters in plant cell culture system in vitro. Advent of suspension culture promotes the rapid growth of the cultured cells in a controlled environmental set up. Single cell condition also provides easy culture scale ups by subculturing as well as effortless recovery of the vaccines. Biopharmaceuticals produced by plant cell cultures also shows better stability than animal cell culture system. *Nicotiana tabacum* 1 (NT1) and tobacco bright yellow (BT 2) cell lines are widely used for production of plant derived pharma products [9]. Although suspension culture of *Daucus carota*, *Oryza sativa*, *Lycopersicon esculentum* as well as *Glycine max* also recently been used for the molecular farming of vaccines and other medicinal components of human interest. Tobacco cell suspension culture is popularly used for the production of interferon α (IFNα), human interleukin 2 (IL2), Hepatitis B surface antigen (HBsAg), human growth hormone etc. [3, 11, 19, 20, 24]. Rotavirus VP6 protein was expressed in tomato suspension culture [5]. Hairy root culture of *Nicotiana tabacum* and *N. plumbaginifolia* also popularly utilized for bio-farming. Interestingly, microalga *Chlamydomonas reindardtii*is now popularly maneuvered for vaccine aquaculture and production of different adjuvants [9].

Covid 19 vaccine created from plants: humanity's saviour?

The entire scientific community across the world are devoting their efforts to develop a vaccine that can overcome all the hurdles and available for every living human being on this Earth. The phase I clinical trial report of a plant derived Covid 19 vaccine, CoVLP by MedicagoInc, Canada brings new hope to save the human races in a green way. The original work was published by Ward et al., 2021 in Nature Medicine, explaining futuristic approach to meet the high demand for vaccines in most convenient way [22]. This technology utilizes *Nicotiana benthamiana* as plant system for the vaccine production and disarmed *Agrobacterium tumefaciens* for transfer of episomal DNA bearing SARS CoV-2 spike protein gene. This is the first reported plant derived Covid 19 vaccine which set up a new path for war against Covid 19. The platform used by the Medicago Inc is well characterized and proved to be efficient previously against seasonal influenza and hemagglutinin vaccine against Avian races [14, 15, 23]. The robust and well interpreted experimental design comprising 180 ELISA (against nucleocapsid of SARS CoV-2) negative volunteers from two cities (Montreal and Quebec City) of Canada were tested. The test was performed with intramuscular injection of CoVLP vaccine (alone or adjuvanted with ASO3 or CpG1018). The result outcome was very much promising with no serious adverse effects (AEs). Some side effects, which are mild to moderate, were observed in very negligible percentage of volunteers which were disappeared within days after first as well as booster dose (21 days apart from first dose). This percentage of AE is permissible and will believe not to hamper the

general health of the population. The generation of neutralizing antibody (NAbs) is extremely propitious. CoVLP with adjuvant was found to produce even more NAbs as compared to serum antibodies of Covid 19 survivors. CoVLP was also sufficiently induce interferon γ (IFN γ) and interleukin 4 (IL4), that ensures T cell activation by this vaccine in both adjuvanted and non-adjuvanted forms (Fig. 1). Although, all the experimental data shown here are promising but long-term immunogenic data is required for successful clinical release of this vaccine.

What makes PDVs so appealing?

At present, there are 16 vaccine candidates are in the market which has approved for vaccination across the world, and more than 85 vaccine candidates are in developmental or in different phases of trial events. The approved vaccines were largely based on three popular methods of vaccine developments, (1) inactivated viral particles [CoronaVac by Sinovac; BBIBP by Sinopharm; Covaxin (BBV152) by Bharat Biotech, ICMR, Ocugen, etc.], (2) mRNA based

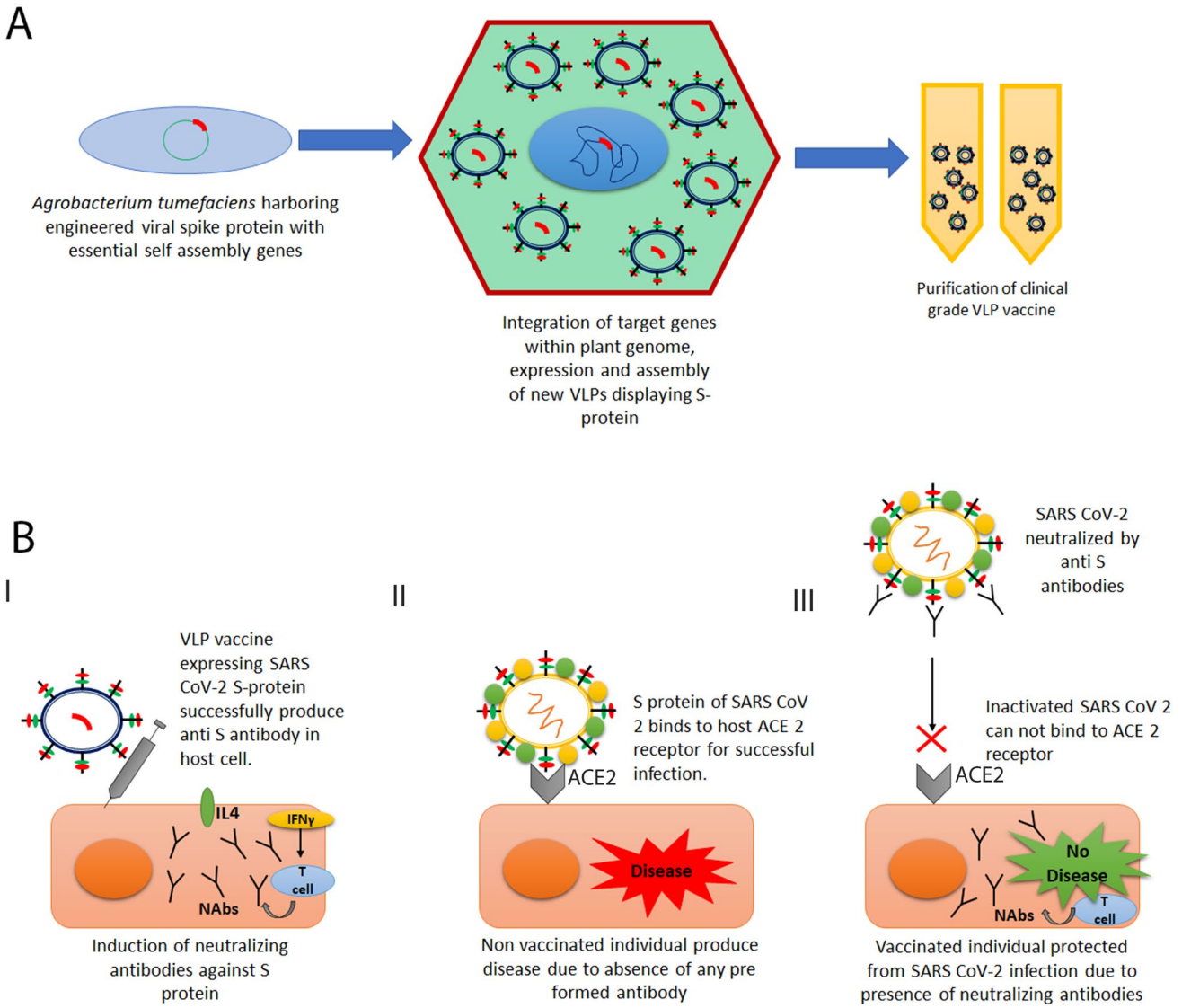

Fig. 1 Plant derived vaccines (PDVs), production and mode of action, **A.** Schematic diagram showing *Agrobacterium* based plant derived vaccine generation platform. **B.** Illustration showing different interaction of CoVLP and SARS CoV-2 with host cells, (I) CoVLP expressing spike (S) protein when administered by intramuscular injection produced sufficient amount of neutralizing antibodies (NAbs). Subsequently induction of interferon γ (IFNγ) and interleukin 4 (IL4) regulates T cell activity; (II) in non-vaccinated individuals SARS CoV-2 spike protein interacts with ACE2 receptor and due to absence of preformed antibodies successfully produce disease; (III) in vaccinated individuals NAbs inactivates SARS CoV-2 and as a result of that virus cannot successfully establish disease. T cells also help in developing antibodies by antibody recall

vaccines (Comirnaty (BNT162b2) by Pfizer, BioNTech; Fosun Pharma; Moderna COVID-19 Vaccine (mRNA-1273) by Moderna, BARDA, NIAID), (3) Adenoviral or recombinant Adenoviral vaccine (COVID-19 Vaccine AstraZeneca (AZD1222); also known as Vaxzevria and Covishield by Oxford University; Sputnik V and Sputnik light by Gamaleya Research Institute, Acellena Contract Drug Research, and Development) (https://www.raps.org). Adenoviral vector-based vaccines are very much popular because of their easily modifiable small genome as well as greater tendency to activate immune response than other vector-based vaccines. As adenovirus has a wide host range, adenoviral vectors are generally designed using molecular clones of the adenovirus genome from close members of human beings i.e. chimpanzees to eliminate potential cross-contamination from other unknown viruses. The vector containing the epitope was transfected into complementary mammalian cell culture after the reporter gene was successfully removed. Then the vaccine is purified from these cells after the development of plaques [21]. For example, the AstraZeneca vaccine was produced in a genetically modified human embryonic kidney (HEK) cell line (https://www.seruminstitute.com). All the vaccine production systems follow a basic set of protocols. Usually, inactivated vaccines are generated by multiplying viral cells in egg cell cultures. Viral cells are purified from allantoic fluid and subsequently inactivated by several techniques. Live attenuated vaccines also utilized a mammalian cell culture system [10]. These mammalian cell culture or egg cell systems are always at potential risks of contamination from other human pathogenic microbes. Plant-derived vaccines are free from all these potential risks. Hence, PDVs like CoVLP may supersede the conventional vaccine production platforms in the future. Moreover, these cell culture systems have very slow multiplication rates than plant systems. Animal cell culture systems including egg cell and Vero cell culture take about 4–6 months to get the high-quality vaccines but PDVs are produced within months. The transient expression system in plants dramatically increases the production and the ready release of produced vaccines into the culture system will further help in the quick recovery of the product [9]. Besides, these PDVs possess better stability in serum as expressed by greater half-life [24]. Many post-translational modifications e.g., glycosylation, phosphorylation, etc. have taken place more efficiently in plant systems than mammalian cell culture. Nevertheless, genetically modified organism (GMO) enforcement laws of several countries restrict licensing of many animal cell culture-derived vaccines. In most cases, non-crop fast reproducing plants (e.g., tobacco) are utilized as cell culture systems to produce PDVs. Hence, GMO restrictions are also minimum in the case of PDVs. Present pandemic situation necessitates optimal allocation of therapeutics and constant supply of good quality vaccine to mitigate any future outbreaks. Such situation demands a perpetual system to produce huge number of vaccines for the mass. Plant based vaccine production market although segmented at present but has a huge possibility to increase market share in recent future. This will also attract investors and pharma industries to focus more in this technology due to its enormous competence. All these characteristic features have made the PDVs a potential alternative even for future vaccine production tool to cope up with emerging variant of high consequences. SARSCoV-2 variations that have a high rate of transmissibility, increased illness severity, and lower marketed-vaccine-induced protection can be effectively managed by a plant-based vaccine production system.

Conclusion and future perspective

Massive increase in cases of Covid 19 pandemic completely destabilizes health as well as socio economic condition of the world. Huge population density and vulnerable economic condition of the major population makes the situation more complicated in countries like India. Mass vaccination and strict implication of social protocols is the only way to save the human races in this critical situation. The colossal production of the vaccine to reach the entire population on this Earth is the biggest challenge to the scientific society. In this current condition, multifaceted approach of vaccine production is needed to meet the need. For this PDVs can serve as pivotal role to curtail down the huge supply gap of the vaccine. The discovery of CoVLP in this condition brings a new hope to increase the production in future. May many other plants derived vaccines will grab the market in future but low potential yield of protein than mammalian cell culture is a big question to be addressed. Although, standardized 35S promoter, rice α amylase (RAmy3D) and different chemical induced promoters are continuously been croped up to solve this yield problem. Again, allergic reactions against different plant derived products are rare but not completely absent. So, this will also be pertinent with universal application of CoVLP (or other PDVs) in future. Finally, rapid mutation in SARS CoV-2 genome generates several novel variants those have the abilities to evade NAbs effect and capable of masking immune response. Detailed work has to be carried out to fix all these queries in future. In such situations only time will tell us the sustainable efficacy of CoVLP and all the vaccines against Covid 19 in future.

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Declaration

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