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Production and Challenges of Plant based Vaccines

Sonia, Sharma[1] and Neelam P Negi[2]

[1] PG Student, University institute of Biotechnology, Chandigarh University, Mohali, Punjab, India

[2] Assistant Professor, University institute of Biotechnology, Chandigarh University, Mohali, Punjab, India

Email: [2]neelam.biotech@cumail.in

Abstract

Increasing the world population and exposure of diseases haveattractedthe expansion of efficient new vaccines to fight many diseases. Traditional vaccines are being used throughout the world, but it has many limitations such as high-cost production and time-consuming. Hence, the idea of plant-based vaccine technology has emerged which has shown positive results with powerful and effective protection against many diseases. This technology is based on the insertion of the encoded protein with the desired gene for the specific disease into the plant genome by several methods. The most common method for the production of vaccines by plant is *Agrobacterium* mediated gene transformation which is genetically modified with plant viruses and used to generate plant vaccines. With the enhancement of technology, new methods were developed which include electroporation, sonication, agrofilteration, gene gun, biolistic method, and polyethylene glycol treatment (PEG). This technology of producing edible vaccines comes up with many benefits but, there are still some obstacles in the development of 3rd generation vaccines. Besides all these, progressive steps are taken to produce effective vaccines against human and animal-associated diseases. In this review article, we are focusing on the production and challenges vaccines by plants.

Keywords: Vaccine, Transgenic, Plant transformation, plant based vaccines.

INTRODUCTION

As stated in world health organization report, more than twenty million deaths are occurring in a year due to various diseases such as heart disease, polio, HIV, diphtheria, so vaccination is the most effective strategy to protect our immune system against these diseases(Rappuoli et al., 2002). The successful development of vaccines in the19th century was the greatest achievement and the threat of death-causing diseases was reduced by the administration of vaccines. For the successful development of vaccine an antigenic substance prepared from pathogens that enhance our immunity (Stern et al., 2005).). The production of Conventional vaccines involves the utilization of live or killed microorganisms and delivered in the form of injection or oral administration Effective Traditional vaccines are made by live or killed microorganisms and are being accepted by the world but it has many drawbacks such as production process is time-consuming, costly, need refrigerated storage and safety (Hileman et al.,2002). It is not possible to produce all vaccines by traditional methods (Wang et al., 2004; Lycke et al., 2012). So, these pitfalls lead to the development of plant-based vaccines which are also known as 'edible vaccines' that are produced by introduction of antigenic

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protein into plants via genetic engineering techniques (Holmgren et al., 2005; Penny et al., 2011).

This new approach comes with many advantages to overcome the limitations of conventional vaccines such as the production of vaccines with less time, safe, cost-effective, high stability, eco-friendly, no complex storage required and also it eliminates the requirement of skilled personnel for the delivery (Yusibov et al., 2008). In the year 1990,Arntzen and coworkers developed the concept oftransgenic plants for the development of vaccines. Due to their effort, first successful vaccine was developed by protein antigen of *streptococcus mutants* in the model plant tobacco. Later, they started the production of hepatitis toxin subunitin potato tuber as well aspotato plants. In the developing world, edible vaccines give all possible ways ofdiminishing the burden of infectious diseases (Saxena and Rawat 2014).Edible vaccines was approved by National Institute of Allergy and Infectious Diseases (NIAID)for theirmarvelous effect on the immune system. Today, most of the edible vaccines have been developed and are at the clinical trial phase (Aboul-Ata et al., 2014; Guan et al., 2013). Still, there is no edible vaccine received the approval from the US Food and Drug Administration (USFDA).

Productions of vaccines by plants

The production process involves the incorporation of transgenes into the plant cells in which vectors are integrated with target antigen sequence, after that it is injected into the plant cell and this process is done by transient or stable transformation system. Stable transformation involves nuclear or plastid integration (Alpeter et al., 2005) and causes permanent changes in the recipient cell. Transient transformation involves the production of desired proteins into the host cell genome (Chen and Lai 2005) and this expression is achieved by *Agrobacterium*-mediated transformation and particle bombardment method (Alpeter et al., 2005). Production of vaccines is done by two methods direct or indirect gene delivery method.

3.1) Direct Method of Gene Delivery

This method includes the direct insertion of DNA or RNA into the plant cells (Naderi and Fakheri 2015). A biolistic method also called as gene gun is an example of direct gene delivery and act as alternative method for nuclear transformation (Saxena and Rawat 2014; Mishra et al., 2008; Kim and Yang 2010). In this DNA or RNA is coated with micro-carrier such as gold and tungsten to deliver the gene into the nucleus, and pressure is created by helium gas which allows the coated DNA to travel at a high speed within a vacuum and penetrated the host cell to form stable integration. It is used to deliver foreign DNA into the plant genome (Alpeter et al.,2005) but it is costly method and sometimes can harm the plant also (Mason et al., 1992; Gomez 2010).

By using the biolistic method, chloroplast and nuclear transformation, the two different types of antigen expression in plants are found. The main aim of chloroplast transformation is to enhance the production ofrecombinant proteins from a single transformation step. In this method, the integration of specific DNA into the chloroplast genome.In nuclear transformation, the introduction of the desired gene occurred in the nucleus of the plant by

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non-homologous plant cells (Gomez et al., 2009; Streatfield et al., 2005). This transformation showsthe low expression level of antigen and increases the requirement of a large amount of plant materialto produce the right dose and also random insertion of genes causes pleiotropic effect (Monila et al., 2005; Walmsley 2005). Chloroplast transformation has more advantages over the nuclear transformation as it eliminates gene silencing effect, low-cost production, fast, require less technical work and specific insertion of the transgene into the chloroplast genome (Gomez et al., 2009; Rigano and Wamsley 2005; Rosales-Mendoza et al, 2012; Saxena and Rawat 2014). As there is no Glycosylation process occurs in the plant plastid so it is not a good option for the production of heterologous proteins. Between the nuclear and chloroplast transformation, the chloroplast is the most widely used transformation for the production of edible vaccines.Chloroplast transformation overcomes the limitations of nuclear transformation as it maintains the continuous supply of sources and the high yield of antigen in chloroplast transformation reduces the requirement of plant material for vaccinations. Here are some examples of chloroplast derived vaccines which prevent bacterial diseases such as cholera, Lyme disease, and plaque, it also fights against viral disease like rotavirus and canine parvovirus(CPV) (Verma and Daniell 2007). The Tobacco plant has been used as the model plant for the production of most of these vaccines. *Lactuca sativa* and *Zea mays* plants have been produced two edible vaccines which fight against dengue and rabies virus respectively.

3.2) Indirect gene delivery method-

This method shows more success as compared to the direct one. Plant cells are infected with plant-bacteria or viruses which naturally poison the plant and integrate into the plant genome (Esmael et al., 2013). It includes *Agrobacterium* mediated gene transfer and genetically engineered plant virus.

3.2.1)Gene transfer Method by *Agrobacterium*

Agrobacterium is a soil living gram-negative bacteria thatinfect plant cells and carries T-DNA genes to the nucleus.(Gomez et al., 2010; Kimm and Yang 2010).*Agrobacterium* tumefaciens and *Agrobacterium*rhizogenes are the two strains of *agrobacterium* which are used as biological vectors. *A. trumefaciens* is a tumor inducing vector, whereas *A. rhizogenes* is a root inducing vector. *Agrobacterium* starting communicate with the wound present in theplant cell and this wounded plant generate phenolic compounds that depending on species involved. A protein called vir A which is recognizes by the *Agrobacterium* detects the chemical signal which arise from the wounded plant and activates the anotherVir gene which guides to transfer the T-DNA inside the plant cell. (Mishra et al., 2008; Sharma and Sood 2011).The most preferred genetic transformation method is *A.tumefaciens*. The plasmid is used as a carrier for the transfer of genes into plants through the T DNA region. Theproduction of the vaccine will modulate the Ti plasmid region by digesting the plant hormone encoding genes and inserting a heterologous gene to construct a recombinant plasmid vector (Sharma and Sood 2011). This recombinant vector is further transformed into the host with the help of *Agrobacterium.* Many vaccines have been generated by this method such as TB, dengue, Ebola (William et al., 2002).

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Arakawa and coworkers successfully developed vaccines against cholera toxin subunit in potato by genetic transformation using *Agrobacterium tumefaciens* method (Arakawa et al., 1997). In another study by Castan´on and coworkers (1999) generate protein VP60 against hemorrhage disease in potato. This method was also expressed*Helicobacter pylori*TonBprotein in *Arabidopsis thaliana*(Kalbina et al., 2010). The produced antigen wasrecognized by rabbit anti-TonB antiserum and provide protection against helicobacter infections by oral immunization. Another study displayed thatby insertion of hepatitis B surface antigen gene into tomato plants (Li et al., 2011*)*.The Advantages of this method is that it permits the insertion of more than two heterologous genes, simple and cost-effective but has limitations that it required expensive tool, slow process, poor yield and inserted genes are unstable. To overcome these limitations a new technology for the plant viral vector system with *Agrobacterium* mediate transformation has been introduced (Yusibov et al., 2006; Gleba et al., 2007). This technology is used for the production of heterologous proteins and monoclonal antibodies. Agroinfiltration is another method that can be used to overcome problems like an unstable expression of genes and low yield. This method requires syringes for the transformation of*A.tumefaciens*suspension into the plant cell.(Srinivas et al., 2008; Yang et al., 2000). It increases the level of an antigen in the plant (Kim et al., 2015). It involves syringe and vaccum infiltration. infiltration through syringes requires syringes to transformed *A.tumefaciens*into the injected leaf which comes with several advantages like it allows multiple transgene introduction into the different parts of the leaf and a rapid and cost-effective. In the vaccum infiltration method leaves are put into the buffer having transgene carrying *A.tumefaciens*. It is a complicated method than syringe infiltration but recombinant production is much high.

3.2.2) Genetically modified plant virus

In genetically modified plant virus method, epitope of insert is transformed into the plant virus. The plant virus is modified in such a way that it achieves coat protein gene and it accumulate the epitope.It is expressed only by a generation of infected cells (Yusibov et al., 2002) which leads to transient expression (Saxena and Rawat 2014; Renuga et al., 2014). The advantages of using an infection-mediating plant virus are that it can produce a high level of expression of recombinant proteins in less time. It forms various copies of the antigen on the surface of the viral particles and allows large-scale infection in a plant (Landridge et al., 2000; Santi 2009; Guan et al., 2013; Saxena and Rawat 2014). Expression vectors which are used in genetically modified plant viruses are: Cucumber mosaic virus, tobacco mosaic viru, potato virus X, alfalfa mosaic virus, cowpea mosaic virus (Fujiki et al., 2008). TMV is most commonly used vector which has been used to produce antigens in tobacco plants. TMV vector with epitope of murine hepatitis virus (MHV) were transferred in model plant tobacco and purified hybrid viral particles. These purified hybrid viruses were injected in mice, it develops serum IgA and IgG for TMV envelope proteins.

In one study, a positive correlation was found between immunogen delivery and protection against MHV infection (Koo et al., 1999). Hybrid TMV vector containing the epitope of foot and mouth disease virus (FMDV) and guinea pig were expressed in tobacco plants. Pigs were

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used to evaluate the effects of recombinant viruses (Wu et al., 2003). In a study by Staczek and coworkers observed that the chimeric vaccine produce by *pseudomonas aeruginosa* in a mouse providing immunoprotection against chronic lung infection (Staczek et al., 2000). Till date, many RNA viruses (bamboo mosaic virus, papaya mosaic virus, plum pox, tomato shrub stunt virus and potyvirus) have been added as vectors for the production of plant based vaccines (Lebel et al., 2015).

4.Efficiency to increase the gene delivery

4.1) Uptake of DNA by chemical stimulation

Several methods have been introduced to increase the efficiency of gene delivery. One such is to introduce the transgene into the plant by chemical stimulant i.e PEG (polyethylene glycol), and the insertion is site specific by the process of dual homologous recombination by flanking sequences (Tiwari et al., 2009). PEG-Mediated transformation is used to extract the cell wall which blocks the entry of DNA. As soon as the protoplast is isolated, it is mixed with DNA in the presence of PEG. Factors such as concentration of PEG and inoculation time must be considered to increase the efficiency of DNA uptake by protoplast (Locatelli et al., 2003; Jeon et al., 2007; Hassanein et al., 2009). Advantage of this method is that it does not harm he protoplast and it is cost-effective protoplast (Jeon et al., 2007). This method has limitations too, as it requires expert for transformation and type of genome also varies (Craig et al., 2005). Due to all these limitations, it is not widely used for the production of plant-based vaccines. By PEG transformation method, Hassan and coworkers succeeded in transforming theM*mmpI* gene and *Lyphotoxin beta* to chloroplast (Hassan et al., 2013). Several other plantssuch as *N. plumbaginifolia* (O'Nell et al., 1993), *Solanumlycopersicum* (Nugent et al., 2005), *Brassica oleracea* var. botrytis (Nugent et al., 2006) use the PEG intermediate transformation.

Sonication

Sonication is the method which uses sound waves to excite the particles in a solution. It induces the formation of small wounds on the tissue and increases the transfer of DNA (naked) into the protoplast (Santarem et al.,1998).It has been observed that when sonication is performed alone in soybean, it harms plant cells due to poor transformation (Santarem et al., 1998) Similarly, in kidney beans, it also caused negative effects (Liu et al., 2005). Studies have shown that sonication with *Agrobacterium* mediated transformation (SAAT) induced wound on plants by ultrasonic waves (Rybicki et al., 2014) and wound allows *Agrobacterium* to enter into the plant cell. It has also been used in the transformation of *Beta vulgaris* and *Chenopodiumrubrum* L (Liu et al., 2005). A heat-sensitive LT recombinant vaccine in *Nicotianatabaccum* was generated by this method.Another approach was developed to improve transformation efficiency (*Agrobacterium*-mediated) by combining sonication with vacuum infiltration (Liu et al., 2005). This approach was successful in transferring the hypocotyls of *Fraxinuspennsylvanica* into pq35GR binary vector which contains *β-glucuronidase genes* and *neomycin phosphate transferase*(Du et al., 2009).

5) The Plant Based Vaccines Challenges

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According to the WHO, nearly 780,000 people have lost their lives each year. Recently, COVID-19 has become a threat to the population and the demand for vaccines is increasing. It is therefore the authorities' challenge to develop a vaccine that should be approved by the population. However, to date not a single herbal vaccine has been approved for human consumption, only a few candidate vaccines are in clinical trials. The vaccines available in today's environment are not completely reliable. There are various limitations with the manufacture of traditional vaccines and these limitations are:

a) The vaccines which developed in eggs, tissue culture may contain the unwanted particles which make them infected.
b) Manufacturing process may lead to the development of a large number of infectious agents which in turn causes the harmful effect on the environment and also hazard the personnel who involved in the manufacturing process.
c) Inactivated vaccines show a weak mode of presentation towards the immune response.
These limitations can be overcome by plant- based vaccines. It is the alternative technology of vaccination to fight against several diseases. It is the technology in which plant species are used as a bioreactor which are environmentally friendly and can produce a large number of recombinant proteins. There is therefore no risk of contamination of these proteins by human and animal pathogens.
Recombinant therapeutic proteins are exogenous proteins which are used for the treatment of diseases in an animals and humans. In the year 1982, the first recombinant protein human insulin was produced in *E.Coli* and it was the major pharmaceutical innovation till date. After that many recombinant proteins have entered in the market and more than hundreds are still in developing with the promise of curing diseases (Shadid et al., 2016; Margolin et al.,2018). Monoclonal antibodies which are copied from immunoglobulin are the most beneficial therapeutic recombinant proteins which targets epitopes with more specificity (Dijk et al.,2001)

In today's world, pharmaceuticals are showing their interest in plants rather than in bacterial, fungal and mammalian cell cultures. But there are few things to keep in mind when producing edible vaccines. The challenges encountered in the production of edible vaccines are its acceptance by population, hence need to generate more awareness among the society for its uses and benefits.Compared to other traditional vaccines, edible vaccines are best alternative for disease prevention, cost effective, efficient and safe.

The first licensed vaccine was produced against Newcastle disease virus infection in poultry manufactured in tobacco suspension. Few human herbal vaccines have reached clinical trials, including for *E. coli*, hepatitis B, cholera, and influenza (Tacket et al. 1998,2007; Yusibov et al., 2011). Till today, many plants have been used for the production of vaccines for various diseases (Table 1)

Disease	**Plant**	**References**
Hepatitis B	Potato, lettuce	Sobrino et al., 2001; Brown et al 1991

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Influeza	*Nicotinabenthamania*	Valenzuela et al.,1982; McAleer et al., 1984; Lavanchy 2004; Yano and Maeda 2004
Chlorea	*Rice*	Suzuki and Boyer 1992; Sun et al., 2003
Rabies	Spinach	De et al., 2009

Table1: Plant based vaccines for different diseases in clinical trials

In the United States, the Food & Drug Administration's rules for the development of vaccines ensure their safety, accuracy, strength, and efficacy.A vaccine is approved before the results of FDA studies, safety and public use, the vaccine's effectiveness is evaluated by highly qualified FDA scientists and physicians. The FDA also inspects vaccine manufacturing sites to ensure that they comply with current good manufacturing practice rules (CS et al., 2011).
The fundamental challenge of plant based vaccines is to choose the right antigen and the right host (Rigano et al., 2005; Sharma and Sood2008). Good host and antigen selection not only produces safe vaccines, but also useful in the production of thermostable vaccines. Another challenge is the consistency of dosage as well as the manufacture and production of edible vaccines.

6) Future prospects of Plant-based vaccine

In today's environment, microalgae are established as key source of active molecules such as chlorophyll, fatty acids, enzymes and carotenoids. Itcan be used for the production of recombinant proteins, pharmaceutical growth factors, hormones and anticancer agent. Taxol will be used as edible vaccine due to several advantages like rapid growth and stable expression and it can be taken as tablet form to fight against many diseases (Specht et al., 2014; Yan et al., 2016).

In addition to development methods to improve immunogenicity, studies have been conducted to address the issue of assay consistency. The findings of Rigano and his colleagues have shown that certain food processing technologies can improve the antigenicity.

Inspite of several advantages they cannot compete with microbial and mammalian system as they are well established in terms of good manufacturing practices. Studies from centuries has depicted the unprogressively commercial manufacturing practices. Studies from centuries has depicted the unprogressively commercial manufacturing of therapeutic proteins. Concerning the development of commercial prospective and economical sustainability, technological advancement is necessary to enhance the veterinary vaccines, cosmetics and industrial enzymes due to the drawback of these pharmaceutical products with deficient in regulation unlike therapeutic proteins (Schillberg et al.,2019; Tschofen et al.,2016)

In recent years, several studies have demonstrated the prevention of various breathtaking diseases such as Zika, Nipah, SARS-CoV, MERS-CoV and newest SARS-CoV-2. Diverse

attempts have been employed to commercially produce a vaccine candidate to fight against these diseases but till now there is no vaccine available. If an effective vaccine ever made against such diseases than it would surely impact the developing and underdeveloped countries due to high cost and safety concern. Thus, we can trust on plant based vaccines and it would be a great approach to fulfil the demand of recombinant proteins on the basis of safety concern and low-cost production. However howgood this approach works will be evident in the upcoming years.

CONCLUSION

Plant-based vaccines are acknowledged as evolving vaccines that possess excessive therapeutic value for curing various human and animal diseases. The objective of producing edible vaccine has fascinated scientist over the globe and gained popularity over the decade. Various studies have proven that stable vaccine can be produced in plant cell. Therefore, various efforts have been put to ensure the reliability, steadiness and biological features of antigen expressed in plant cells in addition to purification processes. However more efforts and studies needs to be carried out in this 21st century to remove various harmful factors that are associated with living organisms, processing, production, manufacturing and also public awareness are need to obtain the approval for the utilization of an edible vaccine. By this technology vaccination can be done to fight against various diseases.

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