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Biotechnological leap in biopharmaceuticals: Current status and challenges ahead

Tazeen Iftikhar Butt¹, Muhammad Shoaib Amjad^{2,3*}, Tatheer Fatima Butt⁴, Wasi Haider Butt⁵, Kainat Fatima Malik⁶, Sidra Sabir³

¹Department of Biotechnology, Women University of Azad Jammu & Kashmir, Bagh, Pakistan

²Department of Botany, Women University of Azad Jammu & Kashmir, Bagh, Pakistan

³Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

⁴Department of Medicine, Fatima Jinnah Medical University, Lahore, Pakistan

⁵Department of Computer Engineering, National University of Sciences and Technology, Islamabad, Pakistan

⁶Department of Plant Science, Quaid-i-Azam University, Islamabad, Pakistan

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ABSTRACT

Fermentation based vaccine production system is replaced by edible vaccine technology. Plant derived vaccines are produced by using transgenic plants which have immunogenic properties. For combating infectious diseases especially in developing countries there is a practical need of new technology to produce and deliver inexpensive vaccines. We have best option of plant derived edible vaccines. There are many benefits of plant derived vaccines *i.e.* they are cheaper, simple and easy to administer, more stable, extra versatile, with more acceptance power and highly efficient. Some plant derived vaccines are under clinical trials to use commercially and many are under research. This paper reviews the current status and future of this technology.

1. Introduction

The plants in which chosen characters are introduced are labeled as transgenic plants. These plants have given many benefits to mankind such as improvement in the growth of crops. The crops are prevented from many diseases by introducing foreign genes into them. They are better tolerant to stresses of various types, including biotic as well as abiotic type stresses. The most important benefit from artificial plants is the formulation of many different types of drugs from them and many preparations are used for fighting diseases caused by microorganisms as well as other disease causing agents[1-7]. The most common cause of deaths in entire globe is discovered to be ailments caused by various infections[8]. If we divide the death rate of our earth into three parts, a large part of it would be due to diseases caused by various infectious substances and organisms. The term vaccine refers to a material of living origin

which has protective effects on human beings. The vaccine is not only helpful to combat diseases of infectious origin but also the diseases caused by sources other than biological infections[6,9]. How this antitoxin substance vaccine works is a matter of great importance, basically vaccination is something which improves the power of one's own body. It potentiates the immunity which is introduced in a human body by nature. Vaccine gives a power to all the processes in our body which are supposed to be there for helping us to combat any diseased condition. We can get antitoxin from different sources. The most common means for getting a vaccine is the biological sources *i.e.* from living organisms as well as from their dead bodies. Antitoxin can be obtained from their cells or materials outside their cells. Some living organisms have the ability to produce different types of substances such as poisons 'toxins or toxoids'. These natural products can be proved fruitful in preparation of vaccine. Nowadays, there is a new trend of vaccination by making a vaccine from different types of vaccine. It is a very interesting and fruitful approach. Making subunit vaccines is also in progress. While working on these antitoxins one may face a serious problems and one of the the problems is moving vaccine from one place to another, because a vaccine is a very sensitive substance and if one wants to make a storage for use later or use at a different place, one should be strictly bound to follow

*Corresponding author: Muhammad Shoaib Amjad, Department of Botany, Women University of Azad Jammu & Kashmir, Bagh, Pakistan.

Tel: +92 3453812987

E-mail: malikshoaib1165@yahoo.com

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strict rules and take a very great care of the product. Following the rules and maintaining everything can be proved helpful[10]. There arises a different problem in some cases if the rules are not strictly followed. There can be different reactions in the vaccination product which may result in production of a toxic substance[8]. If a person wants to achieve success in the subject of vaccination practically, he should follow some rules which are described in the following sections[11-13]: (a) the required product can be manufactured in a huge amount; (b) it is to be noted that the desired substance has to be placed in the normal room temperature for a considerable period of time; (c) introduce the booster; (d) make sure that as the product is boosted into a body, it should be safe from the action of normal enzyme system of body in such a way that it may not get destroyed or digested before performing its desired actions. In 1990s there was a worldwide program and the purpose of that program was to make the public aware about infectious diseases and their protection by use of vaccination. The governments of different countries made different teams to go by town to town and offer vaccination to children for six most dangerous diseases commonly encountered worldwide. It was targeted to reach maximum of population and decrease the population death rate from various diseases caused by infectious agents. This reduced the number of dying children up to 3 million and it was a big success. There is 20% of population of infants worldwide who are able to get the proper vaccine against the most dangerous and common infectious diseases. The six diseases are polio, measles, diphtheria, pertussis, tetanus and tuberculosis. It is really sad to mention that still in the less developed countries a huge population of children is dying of diseases caused by various infections. This is because in those countries the vaccination is not available and if available it is very expensive that the common people are not able to get their babies vaccinated[14]. We can only get a worthy benefit from a vaccine only if it is injected properly and routinely for a mentioned period of time. Now, the researchers are trying to make into account the use of those vaccines which are easy to induce and cheap. Moreover, it should not be difficult to store the vaccine. Researcher are trying to make use of plants for deriving antitoxin[6,15]. The question which has bothered many people is how to make plants useful in the world of vaccination. The answer to this question is simple; biotechnology is helpful in this regard. Crops can be used as bioreactors and after this they can produce lots of molecules of different combinations[16-19]. We can say that a transgenic plant is used as a vehicle to express characters which are injected into them by carriers in the form of proteins commonly known as antigens. Plants are reviewed with regard to their ability to express and produce vaccines[20,21]. Thus the plants are able to serve as a booster preparation tool[22]. The use of antitoxins of plant origin is very useful in areas where we cannot maintain cold chain system[17-19,23]. For the previous 10 years, the researchers have invented a large amount of vaccinations of plant origin. Antigen-specific proteins have been successfully expressed in various plant tissues and have even been tested in animals and human beings[24]. There are also many successful examples of antigen injection into several types of crops for better results[6,25]. There are many good outcomes of edible antitoxins of plant origin. The risk of harmful results is minimized and they are least destined to develop allergy in people. These vaccines prevent the growth of pathological strains to a great extent. They also prevent risk of allergy and trouble of attenuated strains[26].

2. Transgenic plants for the production of plant derived edible vaccines

Edible vaccines are cost-effective, easy to be administered, storable and widely acceptable. Oral administration of edible vaccines proves to be promising for reducing the incidence of various diseases like hepatitis and diarrhea, especially in the developing world which face the problem of storing and administering vaccines[27]. The use of transgenic plants has helped to create diverse antigen expression. However there are also some major hurdles that have prevented commercial production of plant-based vaccines[28]. Now almost every line has its own antigenic expression in plants[29]. Talking about the first plant derived vaccine, it was obtained from streptococcus mutants and was expressed in tobacco[30]. The easy way of storing a vaccine of plant origin is to store it in the form of seed or fruit. This form can be easily moved to various places. The storage of vaccine in seed form also protect that vaccine from being degraded. The cultivation of crops to produce various plant derived vaccine is a good procedure and it also needs very few inputs. Other important diseases which can be cured by vaccination produced from plants include autoimmune disorders like Type I diabetes, multiple sclerosis, rheumatoid arthritis, etc. We select plants which exhibit least toxic effects and are excellent in expressing great antigenic properties. Today researchers know a large amount of plants which are gifted with these properties[6]. There is a great development in the field of genetic engineering and this development has helped a lot to extent the expression system from model plants to various other plants which exhibit the property of large amount of protein content. Nowadays, there are many plants under study and they are discussed in details for better results. These plants include leafy crops, pulses plants, seed plants of various types, fruit plants, vegetable plants, as well as plant tissues and their cultures (Table 1). Scheme for making candidate vaccine antigen in plant is given if Figure 1.

Table 1

Transgenic plants for the production of recombinant vaccines against various diseases.

Transgenic Plants	Protection against	Recombinant vaccines
<i>Arabidopsis</i>	Mouth and foot virus	Mouth and foot virus
Tomato	Rabies virus	Rabies glycoprotein
Potato	<i>Vibrio cholera</i>	Cholera toxin B subunit
Tobacco	Human cytomegalovirus	Human cytomegalovirus glycoprotein B
Tobacco	Herpes simplex virus	Herpes virus B surface antigen

There is also research on hydroponic systems, algae and halobios. Co-expression of adjuvant along with antigen has also been done in the same plant[31]. Rice is a staple food of many countries, so researchers studied to introduce transgenes into rice plants and this has now been done successfully[32]. Furtado *et al.* studied the differences between storage protein gene and non-storage promoter[33]. His study of storage protein promoter was done on barley and wheat, and it revealed that expression was leaky; moreover the expression was directed in endosperm other than embryo. The expression was also noted in seed maternal tissues, leaf and root tissues; moreover endosperm specific expression was directed by rice promoters[33]. Alfalfa (*Medicago sativa*) is among those plants which have a good amount of protein contents. This property makes alfalfa a suitable bioreactor for producing recombinant proteins. Alfalfa

also has very little amount of secondary metabolites[34]. The most suitable crops for enhancing antigenic concentration are cereal crops. They are proved to be useful for improving antigenic concentration as well as lowering oral dose. This property of cereal crops is due to a good amount of soluble protein in their endosperm[6]. Some other plants that are reported as candidates to express vaccines include potato, tomato and carrot[35]. There are some antigen genes which can be transformed to tomato. These include hepatitis B surface antigen (HBsAg), HIV-gag and rabies capsid protein[36]. While studying the proplastids of carrot cells cultured in lab a recombinant protein expression with very good quality was observed[37]. Carrot is now used as a means to deliver vaccine which needs to be introduced orally. Another benefit of this is that no cooking is required while using a vegetable like carrot[38]. Lettuce (*Lactuca sativa*), celery cabbage (*Brassica rapa* var. *pekinensis*), cauliflower (*Brassica oleracea* var. *botrytis*) are also taken into considerations. However, there is a problem while using these vegetables because they have a low expression level[39]. Banana (*Musa acuminata*) was the earliest fruit which was used for the transgenic program of plants[40]. The ideal species for vaccine production is papaya (*Carica papaya*) as mentioned by Trivedi and Nath[41]. The scientists are also engaged in producing pharmacotherapeutics from algae in addition to fruit, vegetable and cereal crops[42]. The species which can be used for this purpose include *Chlamydomonas reinhardtii*[43], *Phaeodactylum tricornutum*[44], *Amphidinium carterae*, *Symbiodinium microadriaticum*[45] and *Cylindrotheca fusiformis*[46]. There is a very successful production of two very important groups of medicines, antitoxins and anti-bodies from the study of chloroplasts[47,48]. The plant chloroplasts which are developed while using recombinant techniques exhibit many important properties such as high expression levels. Moreover, the ability to develop edible vaccine is another exciting feature of transgenic chloroplasts. They can be used to produce antimicrobials[49]. The important organelle of plants called plastid is also under study for previous few years and many vaccines are produced while using plastids. The transplastomic plants are ranked second in the field of expression systems. This study revealed that while using plastid for producing vaccines, the gaps in traditional production systems can be fulfilled. Plastids have a silent character and they can combine both prokaryotic and eukaryotic characters in a single expression system. The organisms such as viruses and bacteria can be used for the amplification of these characters[47]. For a successful antigen expression of plants there was use of some other famous organisms such as *Escherichia coli* (*E. coli*), enterotoxin B subunit which is heat labile (LT-B) which resides in tobacco and potato[50], rabies virus G protein being noticed in tomato[51], hepatitis B virus surface antigen in potato and tobacco[52], Norwalk virus capsid protein in potato and tobacco[53], the B subunit of cholera in potato[54]. The suitable method of administering antigen expression related to plants or plant products is parenteral and includes intravenous and intramuscular administration. The vaccination administered by oral route includes vaccination produced from homogenized leaves, fruits or vegetables. The way of transferring a purified antigen which contains plant tissue can be done by packing it in capsule form or making a powder pill out of it. The benefit of using vaccine packed in the form of capsule is that it can be made to dissolve in a particular part of stomach of our body; moreover the vaccine can be made to carry its effect in a particular selected part. The intravenous

and intramuscular methods of administration can be used in case of purified component. Systemic as well as mucosal immunization can be induced by using oral vaccine. The administration of an antigen by oral means elicit more mucosal response than by means of intramuscular or intravenous injections. The most important antigens are said to those which have the ability to produce immunoglobulin A (IgA) antibody and which have the power to exhibit maximal mucosal response.

The diarrheal diseases which are caused by different types of microorganisms including rotavirus, Norwalk virus, *Vibrio cholerae* (*V. cholerae*), enterotoxigenic *E. coli* (ETEC) can be best treated with mucosal immunity. The mucosal immunity can also be useful in cases of respiratory diseases such as pneumonia. Multicomponent vaccines are vaccines which are obtained from second generation plants. They help in providing immunity against several pathogens. The ETEC LT-B and the capsid protein of Norwalk virus were able to be expressed in plants and by this induction the immune response against both of them was developed successfully.

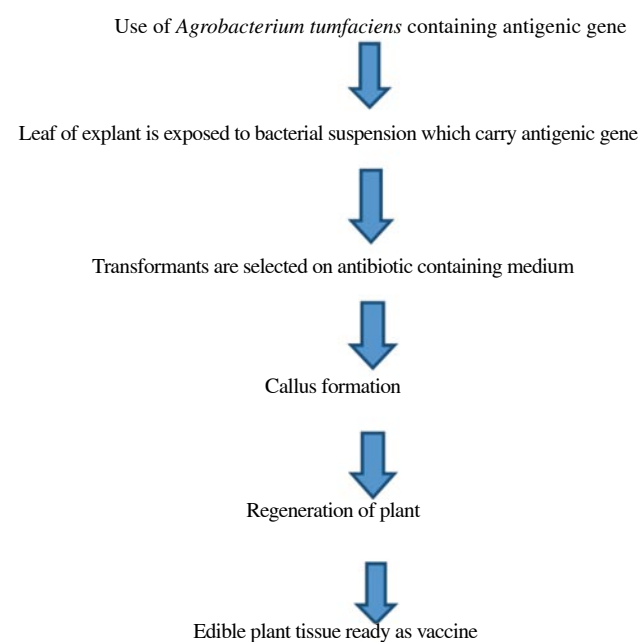


Figure 1. Scheme for making candidate vaccine antigen in plant.

3. Why do we need to use plant vaccines?

The vaccination system related to plants has many important properties; the most important to be mentioned at the top includes their ability by which they never facilitate the growth of human or animal pathogens (such as virions or prions). They never transport pathogens in accompany with target subunit vaccine[55]. The plant derived vaccines are cheap when compared with processes such as fermentation or production of vaccine in bioreactors. They can easily be habituated in a cheap farm or green house. They also sometimes even need not any purifying when induced in the edible part of the plant as for example fruit, grain, or even leaves. There is also stability associated with the maintenance of antigenic protein in edible plant cells. These vaccines have a greater power to be delivered by oral means than by injecting intramuscularly. This provides an easy approach of administration for animals and humans[56]. These vaccines offer immunity to mucosal surfaces (first

line of defense) such as in nose, esophagus and mouth. This prevents attack of pathogens on the mucosal surfaces which are mostly the first part of body being exposed for disease causing organism[15]. The overall price of immunization is generally reduced by using plant derived vaccines. This makes them useful in under developed countries.

4. General consideration of plant vaccines

4.1. History

The currently used vaccines can easily be replaced by plant derived vaccines. By expressing surface protein antigen A of streptococcus mutants, the first vaccine of plants was developed in tobacco[30]. There was also development of another vaccine which followed the first one by expression of hepatitis B surface antigens in plants[57]. Now there are many different types of proteins which are successfully expressed in plants, such as *E. coli* heat-labile enterotoxin antigen[58], granulocyte macrophage colony stimulating factor[59], human serum albumin[60], enkephalins[61] and glucocerebrosidase, surface antigen of Norwalk virus[53], VP1 antigen from foot and mouth disease virus[62,63], B subunit of cholera toxin[54], antigen of rabies virus[51], transmissible gastroenteritis coronavirus's S protein[64,65], G and F proteins of respiratory syncytial virus[66], rotaviral VP6 protein[67], rinderpest[68], and the measles[69] from the major surface antigen of *Plasmodium falciparum* (an epitope)[70] and virus hemagglutinin proteins. Some plant based vaccines under clinical trials are given in Table 2.

Table 2

Some plant based vaccines under clinical trials.

Product	Plant host	Against disease
Newcastle disease virus	Tobacco cell	Newcastle disease
HN	Suspension potato	
Personalized anti-idiotypic dcFVs	<i>N. benthamiana</i>	Non-Hodgkin's lymphoma
H5N1 influenza HA VLP	<i>N. benthamiana</i>	H5N1 "avian" influenza
H1N1 influenza HAcI	<i>N. benthamiana</i>	H1N1 "swine" influenza

N. benthamiana: *Nicotiana benthamiana*. HA: Hemagglutinin; VLP: Virus-like particles; HAcI: Recombinant influenza haemagglutinin antigen subtype C.

4.2. Advantages of edible vaccine

Plant derived vaccines are very easy to be delivered and have the property of offering protection to mucosal surfaces. These vaccines are cheap. They can be cheaply prepared and stored. The transportation of these vaccines is also not expensive. They are stable at room temperature. They don't need special temperature such as to be stored in cold which was a very expensive method for traditional drugs previously[13]. Moreover, the seeds of transgenic plants could be dried as there is less moisture content in seeds and the plants with oil or their aqueous extracts possess more storage opportunities[71]. For production of edible vaccines, costly equipments and machines are not necessary as they could be easily made on soil rich motherland and the cost for growing plants is also low compared to cell culture grown in fermenters. Plant derived vaccines are available very easily. They are cheap to manufacture because they don't need special processes like sterilization. The traditional drugs need this

process. The vaccine which can be used enterally are safe in many aspects. The risk of contamination is least. They don't need to be induced by injections in many cases. Contamination by environment is also least. The vaccines which are derived from plants can be used to produce more vaccines. The vaccines which are produced latter are known as second generation vaccines. These vaccines have the ability to approach M cells. Using enteral vaccination is safer than others because of least risk of infections. There is least involvement of pathogens. The mass production from edible vaccine is easier than from those of animal systems.

5. Approaches to produce plant derived vaccines

Expression of antigens as vaccines and of antibodies against antigens of pathogens in transgenic plants is a convenient and inexpensive source for various bacterial, viral, helminths, protozoan and autoimmune diseases with lower costs[10]. Some are discussed below.

5.1. Models for vaccine production

Following are some models for the production of vaccine candidates.

5.1.1. Viral

5.1.1.1. Hepatitis B virus

Over 3%–6% of people around world are affected by hepatitis B virus according to one estimation. The amount of carriers of hepatitis B virus is over 300–400 million. There are about 40 million carriers in India, which is a very huge number. While going into different stages of hepatitis, in acute stage, we see various symptoms of inflammation in liver. This includes portal triads. The substance causing inflammation is mainly composed of lymphocytes. There is ballooning of single cells in the parenchyma of liver. There is also formation of acidophilic bodies. The damage to liver spreads from the portal triads to outside, while transformation into chronic stage. The appearance of live in this stage is piecemeal necrosis appearance. The lobules of liver are also engaged in inflammation. The formation of fibrous tissue starts with the progression of disease and it leads to cirrhosis. The division of hepatitis B virus occurs in the hepatocytes. The study of hepatocytes revealed the presence of viral DNA and HBsAg in the nucleus and cytoplasm revealed the presence of HBsAg[72]. The blood is infected with hepatitis B virus and the virus is easily transported through blood to all of the body fluid systems. The virus can be transmitted from one person to the other. The transmission of virus can occur by blood. The transmission through blood occurs when infected syringes are used in injections or blood transfusions. The transmission can also occur by sexual contact and tattooing. The vaccination of hepatitis B is possible. The test for HBsAg was done in mice and it was expressed in transgenic potato plant[73]. The production of many small surface antigens for hepatitis B virus (S-HBsAg) is done by Pniewski *et al.* and they were shown in genetically modified glufosinate-resistant lettuce[74]. The mice was immunized with lyophilized plants by use of oral means. This showed the presence of secretory IgA and serum antibodies. The transformation of HBsAg gene mediated by *Agrobacterium tumefaciens* into tomato was explained by Li *et al.*[75]. The hepatitis B virus antigen was demonstrated in tomato

plant experimentally by Lou[76]. In Brazil there was transgenic lettuce plant which carried recombinant hepatitis B virus antigen HBsAg[77]. The early study on oral transgenic plant derived vaccines against hepatitis B virus was discussed by Tackett[78]. The antigen specific antibodies of serum were derived with A phase I clinical trial by using plant derived hepatitis B vaccine[78].

5.1.1.2. Influenza virus H5N1

The production of hemagglutinin was explained by Shoji *et al.*[79]. He described its production from the A/Indonesia/05/05 strain of H5N1 influenza virus by using the technique of transient expression in plants[79]. The ferrets were immunized by hemagglutinin as indicated by results and were prevented from being infected by homologous virus. The form in which plant derived vaccine can exist is the solution form. In the solution form the production of influenza virus can be quick and in a huge amount. The expression of recombinant hemagglutinin was demonstrated by Kalthoff *et al.*[80]. This study was done by using *N. benthamiana* because *N. benthamiana* optimized expression levels[80]. This can be used for the prevention of chicken from very dangerous and life taking infections of heterologous highly pathogenic influenza viruses H5N1. This expression has 96% homology to recombinant hemagglutinin by plant-expressed hemagglutinin. The expression of HAcI was done by Jul-Larsen *et al.*[81]. This expression was produced from the 2009 pandemic H1N1 virus and was induced in tobacco plants[81]. The recombinant HAcI from tobacco plant is said to be a promising vaccine candidate and both B and T types of cells can recognize this expression. The benefits of influenza vaccination derived from plants were explained by Shoji *et al.*[82]. This vaccination is cheap, takes less time and is less like prone to pathogens[82]. A/California/04/09 (H1N1) and A/Indonesia/05/05 (H5N1) strains were used to make a great amount of recombinant hemagglutination proteins. The plans used in this method were *N. benthamiana*. Their immunology was tested (serum hemagglutination inhibition and virus neutralizing antibodies). Moreover their safety in animal models was also demonstrated. An alternative of transient expression system of plants was produced by Madhun *et al.*[83]. The influenza vaccine can be administered through a intranasal needle free device. For the purpose of strong mucosa and humoral immunity we can use plant derived influenza vaccine composed of antigen H5N1 (A/Anhui/1/05) only or in combination with a formulation of bis-(3',5')-cyclic dimeric guanosine monophosphate. There was search for effective and protective adjuvant for the enhancement of H5N1 intranasal vaccine from the extracts obtained from mushroom mycelia. This was a good approach[84].

5.1.1.3. Japanese encephalitis

The family of viruses flaviviridae contains the Japanese encephalitis virus. It is a single stranded RNA virus with positive polarity. The method of spread of this virus is by a life cycle residing in water birds, mosquitoes and pigs. The disease which is caused by Japanese encephalitis virus can be found in all parts of the earth. The places where this disease commonly occurs are Southeastern Asia and Eastern Asia. The organs most frequently affected by Japanese encephalitis are corpus striatum, thalamus, spinal cord and brainstem. This disease is best managed with the help of preventions. It lacks specific antiviral therapy. The treatment approach is to give a supportive therapy and to manage the symptoms[85]. There

was a production of transgenic rice. The transgenic rice expressed the envelope protein of Japanese encephalitis virus with help of a dual cauliflower mosaic virus. The transgenic rice was used for the immunization of mice which were effected with the antigen of Japanese encephalitis virus. After the administration of this vaccination there was production of specific neutralizing antibodies. The mice were given vaccine via enteral route intraperitoneally[86]. There was also successful work of Appaiahgari *et al.*[87]. He showed Japanese encephalitis virus protein that was enveloped in tobacco plant. The mammals can be protected by this expression system[87].

5.1.1.4. Norwalk virus

The main cause of vomiting and diarrhea by contamination of water and food are caliciviruses. They affect people in all age groups. A capsid protein of Norwalk virus in its capsid form was expressed in transgenic potato and tobacco plants. Mice were inoculated with those potato tubers with expressed antigen of Norwalk virus and there was development of IgG antibody[53]. Some volunteers have also eaten transgenic potato induced by Norwalk virus. Seroconversion was shown as demonstrated by Tackett *et al.*[88].

5.1.1.5. Rabies

The lyssa virus belongs to the family of Rhabdoviridae. The virus is cylindrical in shape, with single RNA, and of negative polarity. In man the virus is frequently spread by contamination of virus infected saliva of scratch wounds or by bite of animal infected with rabies. The mucus membranes of the anus, conjunctiva, mouth and genitalia can also be affected by this virus. There is also infection by aerosol transmission as being demonstrated in animals under experimental study and this has been involved in infection of human beings in many laboratory accidents as well as rabies-infected bat caverns. The virus can also be transmitted from one person to the other. If the blood or semen, *etc.* of an infected person is transplanted into another person then this virus spreads. This infection can be live taking because it caused infection to the central nervous system (CNS). The vesicular stomatitis virus which causes infection in cattle is similar to this virus. This virus mostly occurs in connective tissues that act as point of administration. The replication can also be in the striated tissues. The spread to peripheral nervous system occurs at the neuromuscular junction. Then from the peripheral nervous system it is transmitted to the CNS. The spread occurs in the endometrium layer of the Schwann cells. At last stages the CNS is widely infected. The involvement of a small number of neurons is sufficient to cause structural abnormalities. The disorder is yet not clearly understood. In tomato plant there was demonstration of stable expression of surface protein infected with rabies but there was not any demonstration of immunoprotective ability[51]. There was the identification of a synthetic gene which was coded for the rabies surface glycoprotein (G-protein). It was reported as the most important protein to give protective immunity. This protein was designed strategically for the achievement of huge expression system in recombinant plants[89]. The vaccination for rabies requires glycosylation of the G-protein. The original signal peptide is replaced with *Nicotiana tabacum* PR-S protein which is related to pathogenesis. At the C-end of G-protein retention signal of endoplasmic reticulum was added[60]. Nuclear transformation was the technique use for genetic engineering of tobacco plants. There were some selected transgenic lines. These lines were expressing

the chimeric G-protein at a percentage of 0.38% among the whole soluble leaf protein. The microsomal fraction of tobacco leaf was used for immunization of mouse and the immunization was done by intraperitoneal means. This was more beneficial as compared with the commercial vaccine against virus. The G-protein which is derived from plants for the purpose of immunity is very effective in case of intracerebral lethal challenge occurred by live rabies virus. This led to the conclusion that the most effective and safe production system to express immunoprotective surface protein related to rabies virus is the immunization through plants[90].

5.1.1.6. Foot and mouth disease

The foot and mouth disease is caused by a virus known as foot-and-mouth disease virus. This virus belongs to picornavirus. It is a prototypical member of the genus aphthovirus. A, O, C, SAT-3, SAT-2, SAT-1, and Asia-1 are the main serotypes of this virus. There is formation of blisters in the feet and mouth of bovids and some other cloven-hoofed animals. This disease can cause major plague among farm animals because it is greatly infectious[91]. The virus is icosahedral in shape with size of 25–30 nm. It consists of a protein capsid, nonenveloped and it contains a single strand of ribonucleic acid (RNA). The RNA contains a genome with positive encoding. The mode of transmission of this virus is by infected animals via aerosols. This happens by contact with unclean vehicles, farming equipments, contaminated food or cloths, wild predators or by domestic animals. This was the first ever viral disease infecting animals as per mentioned in 1898. There are three basic events of foot and mouth disease pathogenesis and they are characterized as: (i) before-viraemia; this stage is known by the replication at primary sight of replication and infection; (ii) There is sustained viral infection with vesiculation at secondary infection sites and (iii) post-viral infection/convalescence and it includes resolution of disease in clinical terms and it may end in chronic persistent infection. There can be successful expression of foot and mouth disease virus structural protein viral protein (VP1), the protein in plants which is carrying the critical epitopes causing induction of immunologic neutralizing antibodies. The plants in which they can be expressed are potato and *Arabidopsis thaliana* alfalfa which can be used as experimental immunogen, for demonstration of specific protective antibody response of virus[62]. A method was invented to develop a fusion protein formed from a very notable reporter gene, glucuronidase (gus A). This gene is fused to an epitope, and the protein determinants are composed of amino acid residues 135–160 of the anatomical protein VP1 of foot and mouth disease virus (VP135–160). The results showed that a great amount of persons can easily be screened with help of their own enzyme called β -glucuronidase. This enzyme correlates with the VP135–160 expression and its level. There was the development of a protective response in mice against foot and mouth disease virus immunized by plant vaccine[34,62].

5.1.1.7. Newcastle disease

Since the plant-made poultry vaccine against Newcastle disease virus made a breakthrough and went all the way to obtain regulatory approval, research to use plants for expression and delivery of vaccine proteins for animals was intensified[92]. This disease mainly affects domestic poultry and many other bird species. This disease is a problem in almost all countries of the world. This disease

presents initially as a respiratory disease. There is latter development of different symptoms such as diarrhea, depression and nervous manifestations. Death from this disease depends on situation. The virus causing this disease is an RNA virus. There is similarity between new castle virus and paramyxovirus-1 which is a member of genus paramyxovirus-1. The classification of the isolates obtained as virulent factors is as 1 to 3. The levels of these three virulent groups are velogenic, moderately virulent or mesogenic and least virulent or lentogenic.

The least virulent category are being used frequently for the production of live attenuated vaccinations of chickens. The disease can be asymptomatic. It can lead to various levels of mortality and morbidity. The virulent strains of this disease are common in countries from Africa, Asia and some countries of South, Central and North America. Countries like Canada and USA don't have these strains. The imported sources of virulent Newcastle disease virus are pigeons, cormorants and imported psittacine species. Low virulent Newcastle disease virus is prevalent in wild birds e.g. waterfowl as well as in poultry. Birds which are infected pass the virus via exhalation in air, respiratory discharge, and they also pass the virus in faeces. This virus can also be spread via eggs which are laid at the stage of clinical disease and in every part of the carcass when the acute virulent infections are taking place. The aerosols can readily infect chickens. The chickens can also be infected by ingesting infected food or water. The primary source of virus are diseased chickens. The wild and domestic birds may be sources of Newcastle disease virus. The virus can be spread by contaminated faeces, by the shifting of people and affected equipments among poultry flocks.

The recombinant plant in most of the cases showed a very poor amount of protein which was expressed. The technique of western blotting was not appropriate for demonstrating foreign protein particles in recombinant plant. There was low amount of expressed protein for producing a prominent immunity. There are so many benefits of using recombinant plants for producing transgenic proteins, even though there are also disadvantages associated with this process and the disadvantage is that the level of concentration of foreign protein particles in the tissues of plants is very low. This disadvantage became particularly important in places where the extracts of plants are needed to be used without any further processing. The issue of concentration of foreign protein residing in the recombinant plants becomes serious. The best possible way of solving this problem seems to be the identification of recombinant plants which will be able to express a very great level of recombinant protein.

Recombinant plants have become a very useful bioreactor for the production of good quality peptides and medical peptides in biomedical research. There are two expression systems, pGNDVF and pUNDVF which contain the gene for fusion of the protein of Newcastle disease virus in the present days. Newcastle disease virus fusion protein (NDV-F) works by the help of rice glutelin (Gt1) or maize ubiquitin (Ubi) promoter. These genes were produced, and introduced into rice (*Oryza sativa* L.) with the help of transformation mediated by *Agrobacterium*. A total of 12 independent transgenic rice were produced. The experimental study via PCR analysis showed that the NDV-F chimeric gene is in the T-DNA region and it had been implanted into the genome of recombinant rice plants. Western-blot and ELISA analyses elicited that the protein containing NDV-F could be accumulated and expressed in both seed and leaf tissue of many

different rice plants produced by recombinant DNA. The mice were also used to check the immunogenicity of expressed proteins, and the results demonstrated the specific antibodies in mice which were vaccinated intraperitoneally. It was inoculated with raw proteins from recombinant rice plants. The recombinant expression systems based on rice plants can be used as a supplementary bioreactor in engineering of Newcastle disease virus subunit vaccine[93].

5.1.2. Bacterial

5.1.2.1. *V. cholerae*

The cholera is caused by the bacteria named *V. cholerae* and this pathogen is transmitted by contaminated water or food which initiates an acute infection of intestine[94]. The toxin of cholera (CT) is an enterotoxin which was showed in tobacco plant[54]. The oral immunization was shown by Nochi *et al.*[13] with the help of transgenic rice. The transgenic rice was encoding the toxin B subunit of cholera (CTB) which lead to the stimulation of secretory IgA. It is tolerant to gastrointestinal digestion[13]. The gene which encodes for artificial gene CTB was introduced by Karaman *et al.*[95]. This gene is under the control of a promoter located in maize seeds known as γ -zein. The levels CTB were tested with the help of ELISA depending on gangliosides. There was demonstration of anti-CTB IgG and anti-CTB IgA in the fecal samples and sera mice which was immunized by oral mean and was offered protection against holotoxin challenge with CT[95].

5.1.2.2. Anthrax

This disease is caused by a bacteria called *Bacillus anthracis* (*B. anthracis*). The disease is transmitted via skin of infected animals, the inhalation of their spores and products in wool fibres or dust particles. There is a complex of toxic components which serve as virulence factor. It is made up of three proteins. The macrophage surface offers attachment of the protective antigen (PA). The lethal factor and edema factor are released after proteolysis and then they undergo endocytosis, and this lead to the blockage of the adenylyl cyclase pathway residing in the cell. The increase of permeability of vessels is the main effect of this toxin complex. This increase in the permeability of vessels can initiate the condition of shock. The immunized antigen was added in transgenic tobacco chloroplasts with help of the pag A gene insertion into the genome of the chloroplast. The measurements of cytotoxicity which causes macrophage lysis assays demonstrated that protective antigen produced in *B. anthracis* was equal to the chloroplast-derived protective antigen. The protective antigen which was derived from chloroplast provides a safer and cleaner plant derived vaccine for anthrax and the price for this is also very low[96]. The first protective antigen expression from stable nuclear recombinant tobacco was published by Koya *et al.*[96]. The researcher named Aziz also worked on the same topic and noted the expression of protective antigen of stable transgenic nucleus in the leaves of tomato[97]. Protective antigen which was expressed in tomato or tobacco was developed by combining with a second *B. anthracis* lethal factor which was protein in nature, and which showed cytoplasmic breaking activity after it was applied to cell lines similar to macrophages. When tomato leaf was inoculated into mice, antisera with neutralizing activity against anthrax lethal toxin can be recovered. The antisera was a combination of lethal factor and protective antigen[97].

5.1.2.3. ETEC

ETEC strains are a main pathogenic group which cause enteric diseases in human beings and live stock. ETEC is able to recognize specific receptors and get attached on them. The receptors are located on the lining of enterocytes present lumen of intestine. The toxin of ETEC is a heat-stable enterotoxin (ST). This enterotoxin is composed of one A and five B subunits. The binding of B subunit is with the sugar particles of ganglioside Gm1 located on the cells which are covering the crypts and villi of the small intestine. The formation of hydrophilic channel into the membrane occurs by attachment of the B subunit to the host cell membrane. The subunit toxin A moves through that transmembrane channel[98]. The study of raw transgenic potato expressing LT-B was done by feeding 11 volunteers by it, there were 10 (91%) people who formed neutralizing antibodies and mucosal response was elicited in 6 (55%) of the individuals[99]. There are many reports based on artificial heat-labile enterotoxin (LT-B) gene and how they are expressed in plants such as tomato, banana, potato and tobacco. These were all tested in mice[100]. The vaccination against these diseases can be achieved by using the expression of fimbrial subunit protein of *E. coli* in recombinant plants. The adhesion of F4 fimbriae and the production of Fae G protein can be done by using transgenic plants as demonstrated by Joensuu[101]. The *E. coli* fimbrial subunits can also be produced by edible soyabean prepared artificially and this was reported by Oakes[102]. The discussion of edible recombinant plant based vaccines for *E. coli* were demonstrated by Tackett[103]. The recombinant gene encoding LT-B: ST which was residing in tobacco displayed protein determinants for both ST and LT-B by means of *Agrobacterium* dependent transformation. The confirmation of plant derived vaccination LT-B: ST was done by demonstration of systemic and mucosal antibody mediated responses developed in mice when they were inoculated enterally with recombinant tobacco plants[104].

5.2. Other potential domains

5.2.1. Cancer therapy

The cancer can also be treated by use of monoclonal antibodies produced by plants in copious amounts. The antibodies are produced from genetically engineered soyabean. This plant produces monoclonal antibody (BR-96) which is used in the treatment of colon cancer, breast overgrowth, ovarian and lung tumors[105,106]. There is also work going on on Non-Hodgkins lymphoma.

5.2.2. Birth control

Tobacco mosaic virus was tested in zona pellucida of mouse (ZB3 protein)[105,106]. This vaccine when induced in body, produced antibodies that were able to block fertilization of eggs in mice.

5.2.3. Chloroplast transformation

The genome of chloroplast is maternally inherited; that's why following transformation of chloroplast, the pollen will be devoid of the protein, thereby decreasing the danger of spread of the recombinant gene to other weed or crops species by means of inter-pollination[37]. There can be accumulation of prominently a huge amount of recombinant protein. There can be the formation of immunological tolerance if the auto-antigens are delivered orally because they are supposed to open the immune system's suppresser cells. This leads to production of immunological tolerance. For

example, the patients suffering from arthritis experienced comfort by intake of collagen. This process is commonly showed in test animals. This process is dose and time dependent. The dose for inducing tolerance is needed in a copious amount and is also needed to be induced again and again. There can also be accidentally development of tolerance due to misidentification and misadministration of plants/fruits. Therefore, it is very necessary to clearly demonstrate recombinant plants and to study effective dosing, feeding schedules and safe methods of antigen induction, and there is a need to study antigens properly to know whether the antigen under study will lead to stimulation or depression of the immune system. The diseases which can be prevented include multiple sclerosis, diabetes type I, transplant rejection and rheumatoid arthritis, *etc.*[107,108]. There was inoculation of mouse with the potatoes which expressed a protein called glutamic acid decarboxylase, linked to CTB subunit and insulin. The mouse immune system was suppressed by using this method. There was a delay in initiation of high blood sugar level[54]. There are also some researches going on for the production of another protein related to diabetes. There is also a study on lupus erythematosus, rheumatoid arthritis, multiple sclerosis and transplant rejection. The need for great quantity of plant self-antigens is seen for autoimmune diseases.

5.2.4. Recombinant drugs/proteins

The plants can also be renewed by recombinant virus, in addition to be used in production of antibodies and vaccines. The virus which is produced by means of genetic engineering can lead to the production of enzymes, serum protease, drugs such as albumin and interferon. The production was costly and not easy previously. For example, for the treatment of Gaucher's disease the tobacco plant is used to produce glucocerebrosidase. The development of this method lowered its price up to thousand-folds[106]. The treatment of Crohn's disease can be done by using transgenic tobacco. There is also development of industrial processes for the huge production of transgenic therapeutic proteins from plants. Some different novel compounds are HIV inhibiting protein, angiotensin-I (antihypertensive drug) and trichosanthin[106]. There is also production of hirudin (an anticoagulant) from transgenic plants on commercial scales.

6. The future of edible vaccines

The edible vaccines can be affected in future because of development of resistance against genetically modified foods. This can be explained by the example of Zambia government which refused to get aid of maize from US government because of the risk of resistance, even Zambia was at danger of famine[109]. The contamination of transgenic products cannot be avoided. The mode of transmission of transgenes can be by insects sucking them, and the spread to soil microbes if accidentally the plant gets wounds or breakdown in the roots/rootlets and this may pollute the ground and surface water[109].

In recent times a genetically modified corn is approved for consumption by animals. Some measures for isolation were implemented to prevent the transmission of modified genes. But it leads to penalties, monetary loss and criminal charges for alleged violation of the permit to grow gene-altered crops[109]. At elementary level, this event displayed failure. The total loss of money in USA

because of contamination of transgenic products is \$12 billion. Now, the World Health Organization has taken the responsibility of checking the vaccine before used by human being. The World Health Organization checks the efficiency, quality as well as the impact of environment on the mentioned vaccines. There is the testing of greenhouse effects on the genetically modified foods before use. The buffer crops can be used for their protection. The increase in amounts of products is not shown to be done by genetically modified crops, moreover they are not able to show any decrease in the use of pesticides or insecticides. There can also be failure to major crops because of not checking properly the transgenic lines. By mistake sometimes the harmful or lethal gene products or potent allergens may also be induced while producing transgenic vaccines. Other adverse effects include central nervous system toxicity and cytokines induced sickness; α -interferon causes neurotoxicity, dementia, mood/cognitive changes, *etc.* There is a need to use highly toxic herbicides (atrazine, glufosinate ammonium and glyphosate) because of highly tolerant pests and emerging multiple herbicide-resistant volunteers and weeds[109]. The genetically modified foods are studied for their safety. The rats were used to test genetically modified foods and displayed growth factor-like effects in the small intestine and stomach. There is also a discussion on cauliflower mosaic virus-35S promoter. The artificially grown genetically modified crops containing this promoter is especially unstable and can be affected by recombination or mutations by random insertion and horizontal gene transfer. The insertion of random genes can disturb the genomes of its animals and plant hosts, and the effects can be transmitted to the closely located ecosystem. The technique of genetic engineering can play a role in emergence and re-emergence of drug resistance, infectious diseases, reactivation of inactive viruses cancers and danger of autoimmune diseases by aiding in horizontal transference of genes. Bacteria contain transgenic DNA in edibles in human gastrointestinal tract. The spread of antibiotic resistance labeled genes occurs from artificially grown food to disease causing bacteria. This results in difficulty in treatment of infections. If there are little genetic changes in pathogens, there can be huge changes in disease-causing potentials and host spectrums, and afterwards plants unfortunately may become their reservoirs. There is also the danger of developing strains of new disease causing agents, *e.g.* super viruses. By means of shifting of DNA, the persons studying genetics can make many recombinant viruses in laboratory in very little time. The making of bio-weapons can be misused. The use of genetic engineering can proved to be dangerous because it involves the creation of carriers/vectors which are specifically made to cross wide barriers of species, like between animals and plant kingdoms, and transmitting genes by decreasing their protective mechanisms which are functionally useful in treating such genetic assaults[110]. The birth of a Frankenstein by achieved planning would lead to unmitigated disaster. The most dangerous vaccines are perhaps bare DNA vaccines. These vaccines are easily used by all species and can be inserted into genome material of cells. The small pieces of DNA can replicate and multiply indefinitely unlike chemical pollutants. The eating of genetically modified products like maize by animals may be dangerous, including animals as well as human beings who are feeding on the animal products. The environmental and ecological dangers related to edible vaccines must be considered. There is an increase in the world land used for planting transgenic crops from 1.7 to 44.2 million hectares in the year 1996–2000 and the number

of countries growing them is also increased to a great extent. The countries include developing as well as industrial countries[109]. In Canada and USA, at least 350 genetically engineered medicinal products currently are under study. There might be major technical and economical benefits in the events of bioterrorism linked to edible vaccines. This is because of the reason that, their production can be readily scaled up for numbers of doses in a very short period of time (smallpox, anthrax, plague, etc.)[109].

7. Conclusions

The future of edible plant-derived vaccine seems to be more brighter than today. This may prove to be an effective method of immunization. The difficulties faced by the use of conventional vaccines related to distribution, delivery and production can be overcome by use of edible vaccine. The edible vaccines have passed the main problems of developing vaccine technology. The edible vaccines will prove to be a very necessary method in disease preventive arsenal. They will help in fighting complex diseases like malaria, HIV, etc. in a safe and cost-effective delivery system.

Conflict of interest statement

We declare that we have no conflict of interest.

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