Plant-based recombinant vaccines

Maziar Habibi Pirkoohi¹, Saeid Zibaee²

1. Ferdowsi university of Mashhad
2. Razi vaccine and serum research institute

Corresponding author email: maziar.habibi@stu-mail.um.ac.ir

ABSTRACT: As an alternative for traditional methods, the use of plant-based systems for production of oral vaccines has attracted great attention in recent years. Plants offer many advantages over other methods that make them excellent platforms for producing recombinant vaccines. Here, we review various aspects of this promising technology including the principles of recombinant vaccine production, plant host selection, transformation systems, strategies toward enhancing gene expression level and mechanisms involved in mucosal immunity.

Keywords: antigen; genetic engineering; plant; recombinant vaccine; transformation

INTRODUCTION

The use of green plants for production of therapeutic products is a promising field of biotechnology with high economic potential. Regarding modern tools of genetic engineering and tissue culture methods, it is now possible to develop a wide range of transgenic plants including both monocots and dicots and even micro-algae that can express various recombinant pharmaceutical compounds, including viral and bacterial antigens, antibodies, and many other therapeutic proteins (Buetow and Korban, 2000). Recombinant proteins expression is achieved either directly by stable transformation of plants with Agrobacterium rhizogene and biolistic approach or via transient expression using plant viruses (De Muynck et al., 2010).

For a long time, Recombinant vaccines were exclusively produced in expensive expression platform such as yeast or mammalian cells. Production of recombinant vaccine in bacterial systems, though simple and cost-effective, was not successful owing to improper folding of eukaryotic peptides and occurrence of inclusion bodies in bacterial hosts. (Franklin and Mayfield, 2005). Genetic engineering of higher plants was a milestone in recombinant vaccine production. The goal is to produce transgenic plants that upon oral or parenteral administration induce an immune response in the body. The first report of expressing a vaccine antigen within plants was published in 1990 when Curtiss and Cardineau expressed the Streptococcus mutans surface protein antigen A (SpaA) in tobacco (Curtiss and Cardineau, 1990). This pioneer examination was followed by plant expression of the hepatitis B surface antigen (HbsAg) (kapusta et al., 1999), the E. coli heat-labile enterotoxin responsible for diarrhoea (Haq et al., 1995), and the rabies virus glycoprotein (McGarvey et al., 1995). Proteins produced in these plants induced synthesis of antigen specific mucosal IgA and serum IgG when delivered orally to mice and humans.

Compared to other recombinant protein expression systems, Plants offer several advantages including the possession of eukaryotic posttranslational modification machinery, suitable folding of foreign protein, low cost scale up, target protein stability and safety of use of plant-derived products due to the lack of any mammalian pathogens. Here, we first describe the principles of plant-based recombinant vaccine production. A handful examples demonstrating successful expression of antigen in plants are also cited. Moreover, strategies toward enhancing expression level will be noted, and finally, biosafety issues and future perspectives are discussed.

Expressing vaccine antigen in transgenic plants

Plant choice

Plant-based vaccines are subunit vaccines in which the antigen of interest is expressed in plant tissues. The antigen, or antigens, must be expressed at a sufficiently high level in the chosen plant to allow for the practical oral delivery of a sufficient antigen dose to induce immune response. Several issues should be considered when selecting a plant species as an antigen expression host. The first issue is the form of vaccine delivery. Foreign proteins can be expressed in fresh tissue, such as mature plant leaves and germinating seedlings or in dry tissue, such as the seeds of cereals (Streatfield et al., 2001). Hydroponic culture is another ideal platform because the
system makes it possible to secrete the expressed protein into the surrounding medium (Borisjuk et al., 1999). The plant species selected as expression system should have optimum antigen expression, allows for cost-effective production, and can be manufactured into a practical form for oral delivery. So far, several plant species, including potato, tobacco, tomato, Arabidopsis, soybean, alfalfa, lettuce, lupine, rice, wheat, apple, cowpea, and corn, have been used for production of recombinant vaccine (Lamphear et al., 2004). The most common plant for antigen expression in experimental works is tobacco because of ease of transformation and successful expression of foreign antigens. However, in industry more focus has been placed on plant species, such as cereals, that:

- offer long-term stability of the expressed protein
- are cost effective
- produce large volumes of the desired product
- can be easily processed into a deliverable form for oral vaccination (Streatfield et al., 2001).

**Plant transformation strategies**

Recombinant vaccine expression in plant can be achieved either by stable or transient transformation. In stable transformation, the gene of interest is integrated in nuclear or plastid genome using biolistic or Agrobacterium mediated transformation methods. In Agrobacterium mediated gene transfer, the gene of interest is inserted into the T-region of a disarmed Ti plasmid of Agrobacterium. The recombinant DNA is placed into Agrobacterium; a plant pathogen which is co-cultured with the plant cells or tissues to be transformed. The main disadvantage of this method is that it gives low yield and the process is slow. This method works especially well for dicotelydenous plants like potato, tomato and tobacco. In this manner, the foreign antigen is stably inherited through successive generations (Lal et al., 2007).

In transient transformation technique, the epitope of interest is engineered into a plant virus, usually within the coat protein gene. Infection of target plant by this viral vector results in intracellular production and accumulation of the epitope. The epitope sequence, as well as the viral genome, never become integrated into the plant genome and hence are only expressed by the generation of infected cells (Yusibov et al., 2002). This approach has been successfully applied to tobacco, black-eyed beans and spinach (Dalsgaard et al., 1997).

Transient transformation can also be achieved by Agrobacterium. This method, called “agroinfiltration”, involves the injection or vacuum infiltration of plants parts with a suspension of bacteria harboring the antigen of interest. This approach has a wide spectrum of applications and has been used for the study of molecular processes and production of interesting molecules of monoclonal antibodies (Orzaez et al., 2006) and antigens of human pathogens (Mett et al., 2008).

A newly developed transformation approach called Magnifection is being used to overcome the limitations possessed by early platforms. It combines the two technologies namely agroinfiltration method and Tobacco Mosaic Virus (TMV)- based viral vectors system. This new approach allows the scalable production of a desired protein with high expression level and yield, low up- and downstream costs, reduced time, and most of all, reduced biosafety concerns (Gleba et al., 2005).

**Enhancing antigen expression level in plants**

The central part in any project of recombinant vaccine production is to express the chosen antigen at a sufficiently high level in the selected plant host. To achieve this, several approaches have been proposed such as codon optimization, the use of strong plant promoters and untranslated leader sequences (Chikwamba et al., 2002). In some cases, signal peptides such as SEKDEL sequence have been used to conduct the antigen in to endoplasmic reticulum, where necessary enzymes and cellular machinery for proper folding are present (Xu et al., 2011). Chloroplast transformation is an effective way to improve foreign antigen accumulation in plant tissues. Since each cell can contain up to 10,000 plastid genomes, transformed chloroplasts will produce a high number of copies of the transgene without inducing the gene silencing phenomena often associated with a high copy number of transgenic plants.

**Oral delivery and mucosal immunity**

The majority of infectious agents enter the body through mucosal membranes. Induction of mucosal immunity is best achieved by direct vaccine delivery to mucosal surfaces (Carter and Langridge., 2002). Plant-derived vaccines have demonstrated the ability to induce both systemic and mucosal immune responses (Kong et al., 2001). The major obstacle to oral vaccination is the digestion of the antigenic protein in the stomach. Vaccines derived from plant cells have been shown to overcome this problem through the protective effect of the plant cell
wall. Like liposomes and microcapsules, the plant cell wall allows gradual release of the antigen onto the vast surface area of the lower digestive tract.

**Diseases targeted for recombinant plant based vaccines**

**Rabies**

Rabies is a viral disease of mammals and is most commonly transmitted through the bite of a rabid animal. The rabies virus glycoprotein (G protein) and a nucleoprotein (N protein) are the major viral proteins responsible for the induction of a protective immune response.

The first recombinant vaccine against rabies was developed in 1997 by Yusibov et al, who expressed G and N proteins of rabies in tobacco. Purified virus particles from the infected tobacco plant tissue were used to immunize mice. Examination in mice receiving intraperitoneally 7 doses (10 μg/dose) of the recombinant vaccine showed that rabies antigen produced by the recombinant viral approach was able to elicit a protective response against rabies (Yusibov et al., 1997).

This pioneer work was followed by another work, in which the immunogenic protein of the virus was transferred into spinach. It was demonstrated that mice intraperitoneally or orally inoculated with virus-infected spinach leaves mounted a local and systemic immune response (Modelska et al., 1998).

**HIV**

Investigations conducted on plant-based HIV vaccines have mainly focused on regulatory protein (Tat), p24 core protein, nef protein or envelope glycoproteins (gp120 and gp41). In one of these studies, immunological properties of plant derived HIV antigens have been proved in mice (Karasev et al., 2005). In another interesting work, Obregon and coworkers showed an improved stability of p24 core protein by fusing it to the constant domains (α2 and α3) of human IgA heavy chain. The fusion protein p24/α2-α3 rendered more protein than p24 alone(Obregon et al., 2006). This result showed that epitope fusion to a secondary protein can be an effective tool for enhancing antigen expression.

**Hepatitis B VLP vaccine**

Infection by HBV is the main cause cirrhosis and liver insufficiency. By means of genetic engineering techniques, a few subunit vaccines have been developed based on the purified Hepatitis B virus surface antigen (HBsAg).

HBsAg is the main envelope protein of hepatitis B virus (HBV), and is an integral membrane protein of the endoplasmic reticulum (ER). HBsAg produced in transgenic plants was similar to virus like particles (VLPs) produced in transgenic yeast, which is the material used in the currently used injectable hepatitis B vaccine. The tobacco HBsAg was immunogenic when injected into mice (Mason et al., 2002). In another work Optimization of HBsAg expression in transgenic potato yielded 16 μg g−1 potato, and showed priming and boosting of serum anti-HBsAg IgG responses on oral delivery in mice (Kong et al., 2001). Electron microscopy showed that HBsAg VLPs accumulate in transgenic plants, thus providing encapsulation and possibly protection from stomach digestion (Kong et al., 2001).

**Gastrointestinal disorders**

According to WHO, cholera vaccine can provide cross protection against enterotoxic E. coli heat labile enterotoxin (LT-B). LT-B was expressed successfully in potato lines. Transgenic potatoes expressing LT-B were fed to mice and it was found that it can induce immune response (Mason et al., 1998). Further investigations showed that cooking raw potatoes does not inactivate the antigen present in edible vaccine. Thus, the spectrum of plants for producing edible vaccine may be expanded beyond raw food plants such as fruits (Richter et al., 1996). LT-B expression and its immunological capabilities in mice were also studied using other plant species such as tomato (Walmsley et al., 2003), tobacco (Kang et al., 2003), and soybean (Moravec et al., 2007).

**Concluding remarks**

The use of green plants for production of vaccine antigens offers many advantages making this method a practical way for propagation of mucosal vaccines on a global scale. Since the pioneer work of Curtiss and Cardineau, many vaccine antigens have been expressed in different plant species to demonstrate the feasibility of oral plant-based vaccines. Despite the promising future and several successes achieved in this field, different issues will have to be established and well defined such as high expression levels, product quality, downstream process costs, regulatory framework, efficacy and safety.
REFERENCES


