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Exploitation of Plant Viruses as a Vehicle for the Production of Useful Proteins by using Plants as Bioreactor

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SUMMARY

Virus-based transient gene expression has got a lot of attention in recent years and has several advantages over the transgenic approach. Apart from their low cost and simplicity, the transient virus-based gene expression technique can express the protein of interest at a high level in a very short period, within one or two weeks. Among different methods, plant virus-based protein expression systems could be considered as the state of the art technology to develop cost-efficient and effective biopharmaceuticals and immunotherapy products to combat global pandemics and other important infectious diseases in developing countries.

INTRODUCTION

Plants are home to several viruses that use their host machinery to replicate itself and produce their associated proteins which lead to disease conditions in the plant. We can engineer these viruses so that they produce proteins of our interest without affecting the host. Such an approach is highly efficient and successful than the animal system due to its low cost which makes mass production easy, less time consuming, readily available of host plants, higher infection efficiency, and high levels of protein expression. Experiments on the use of plant viruses as a vector for expressing heterologous proteins in plants started after the discovery of the cauliflower mosaic virus in 1960 (Shepherd 1989). The major criteria for utilizing plant viruses as an effective expression vector depends on its ability to infect host plants efficiently. Since most of the plant viral diseases are caused by RNA viruses; gene expression systems based on such viruses are quite efficient and easily transmissible to plants by mechanical inoculation which makes the system highly efficient for rapid inoculation of large acreages of plants in a commercial manner. Foreign proteins are also being expressed efficiently in plants by using phloem limited DNA virus vectors through the agro-infiltration technique by using *Agrobacterium* T-DNA mediated uptake (Gleba et al. 2004; Hefferon 2017). Since the expression is transient, issues related to cross-contamination with plants and permanent change in the viral genome could be relieved. Other important criteria for successful expression of foreign genes in plant systems are sensitive and straightforward monitoring of gene expression in the plants and the absence of substantial interference with virus functions from the plant. There are two different strategies for designing a plant virus-based gene expression vector. In the first strategy or full virus strategy, the entire viral genome is used and the foreign gene of interest is expressed as a fusion product with the coat protein of the parent virus so that the foreign protein epitope is attached and present to the outer surface of the virus. But this approach faced many drawbacks such as the limitation of the size of foreign genes that can be expressed, lower expression level, and other unintended effects of the virus in the host plant. The second strategy which is also known as ‘deconstructed vectors’ helps in overcoming the above limitations where the maximum sequence of the vector is from the foreign gene and only a limited sequence of the virus is retained which are required for replication. It has various advantages over the first strategy such as the increase in size limitation of the foreign gene, enhanced protein expression, and overcoming the tissue or host specificity (Gleba et al. 2004; 2007). In this article, different plant virus-based expression systems which are being reported and successfully used for the production of different biopharmaceuticals and immunotherapy are described.

RNA plant viruses

Tobamovirus based vectors

The full virus strategy was used in the *Tobacco mosaic virus* (TMV) to express the chloramphenicol acyltransferase (CAT) gene by using the subgenomic TMV coat protein mRNA promoter (Dawson *et al.* 1989). But, this strategy has certain limitations such as small insert size and low protein yield. Also, homologous recombination events led to the loss of foreign gene inserts. TMV is also the first plant virus to be used for generating a deconstructed vector system called ‘Magnifection’ by a German firm, Icon Genetics which used the advantages of a high expression RNA virus vector combine with the *Agrobacterium* method of efficient systemic

delivery of DNA molecules (Gleba et al. 2005). Roy et al. (2005) reported the use of a defective RNA based TMV vector to express multiple proteins, human growth hormone, and a lethal factor protein of *Bacillus anthracis* in plants. Also, the expression of such foreign proteins was reported to be enhanced when a silencing suppressor protein such as P19 of the Tomato bushy stunt virus was coexpressed (Voinnet et al. 2003). *Cucumber green mottle mosaic virus* (CGMMV) is another tobamovirus reported to be used as an expression vector. Jailani et al. (2017) compare the efficiency of the expression level of green fluorescent protein (GFP) in CGMMV based vectors in full virus and deconstructed virus strategy. It was observed that there is a 234 fold increase in the expression of GFP when the deconstructed virus was used as compared to 23 fold increase of full virus strategy.

Potexvirus based vectors

Potato virus X (PVX) based vectors are used to express full-length proteins such as N and M protein of Sudden Acquired Respiratory Syndrome Coronavirus (SARS-CoV) antigens to tackle the outbreak of infectious disease (Demurtas et al. 2016) or as a fusion protein in case of Influenza virus vaccine where virus matrix protein 2 (M2e) domain was fused with bacterial flagellin protein to enhances the immunogenicity of the antigen (Mardanova et al.2015).

Potyvirus based vector

The expression strategy of potyviruses involves *cis*-regulated proteolytic polyprotein processing steps due to which the foreign genes cannot be simply inserted between other viral genes. However, this limitation can be overcome by either fusion of foreign genes with the virus genes or the addition of a proteolytic cleavage site adjacent to the foreign gene. These precise engineering requirements have been used to insert the GUS gene between the N-terminal 35-kDa proteinase and the helper component proteinase (HC-Pro) of TEV (Dolja et al. 1992). Inoculation of this construct onto tobacco plants results in high levels of systemic expression of the GUS-HC-Pro fusion product throughout the plant. Although deletion events do eventually occur, the TEV vector is sufficiently stable to maintain GUS gene expression for several mechanical passages through plants.

Comovirus based vector

Cowpea mosaic virus (CPMV) is bipartite (consists of two RNA molecules) and RNA-2 is the target where most of the foreign genes are inserted by deleting its original sequences. Medicago, Inc., developed CPMV based vectors for the expression of virus-like particles that carry Influenza virus HA antigens (D'Aoust et al. 2008). High levels of protein expression were achieved through the use of CPMV vectors called pEAQ series, which employed a Cowpea mosaic virus hypertranslational (CPMV-HT) expression system without virus replication (Peyret and Lomonossoff 2013).

DNA plant viruses

Geminivirus based vectors

Geminiviruses as the name suggests, consist of twined capsid proteins with single-stranded DNA as its genetic material and the virus has wide host ranges. Bean yellow dwarf virus (BeYDV) and Beet curly top virus (BCTV) from the genus *Mastrevirus* and *Curtovirus* respectively have been exploited for the production of monoclonal antibodies to the Ebola, Zika, West Nile viruses and a vaccine against hepatitis A (Huang et al. 2009; Chung et al. 2011).

CONCLUSION

The use of plant virus-based expression vectors is a successful example of exploiting what we thought to be our foe (viruses) to become an ally. Different plant viruses are being exploited to use as a vector for expressing several heterologous proteins inside the plant hosts by either using a full virus or only part of the virus. Deconstructed virus-based vectors are already taking the lead in the field due to their ability to express larger proteins, higher levels of expression, and stability inside the hosts. The system has been already showing success in expressing many important vaccines and antigens against many important infectious diseases such as Influenza, SARS, Hepatitis, Anthrax, etc. around the globe. The utilization of plant viruses as an efficient expression system continues to be one of the promising areas for mass production of cost-effective vaccines and other antibodies to combat global pandemics that we are facing at present. This will address the major limitation faced by developing

countries like India where the accessibility of vaccines and other pharmaceutical products to the poor are in question.

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