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Virus Vectors for Production of Pharmaceuticals in Plants

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Abstract

Over the past two decades, plants have been developed as inexpensive, efficacious and safe production platforms for vaccines and other biopharmaceuticals. One of the up and coming ways to generate vaccines quickly and in large amounts in plants is through the use of deconstructed plant virus expression vectors. This review discusses several of the state-of-the-art plant virus expression systems that have been engineered for vaccine production. Recent advances in tobamovirus, cucumovirus, geminivirus, and several other virus expression vector systems are described in this review, and examples of vaccines generated from each are described.

Keywords: Plant-based vaccines; Virus expression vector; Immune response

Introduction

Plant-derived vaccines and other pharmaceutical proteins have made great advances over the past two decades and are now beginning to be found in the marketplace. Plant-derived vaccines are efficacious, safe, and inexpensive and lack cold chain requirements. They can easily be scaled up or down for large scale production, merely by increasing or decreasing the amount of plant biomass utilized. Plant-made vaccines can find their forte in places where conventional vaccines fail to deliver. For example, due to their inexpense, plant-made vaccines offer hope to the impoverished who reside in rural communities within developing countries. Similarly, governments who are interested in stockpiling vaccines against pandemic infectious diseases can generate them from plants simply and rapidly [1,2].

Vaccines were first generated from constructs that were stably integrated in transgenic plants; however, new technologies which focus on transient expression systems have increasingly become popular. Virus expression vectors have been developed to act in a 'deconstructed' form, that is, lacking the open reading frames that encode movement and coat protein functions, so that the virus cannot become encapsidated or infect unintended plants but instead remains bio-contained. Plant viruses which have been engineered to be delivery vehicles for vaccine proteins provide a number of advantages over transgenic plants; these include rapid and high expression levels and the freedom from some of the concerns that are frequently brought up with respect to genetically modified crops, such as transmission of the transgenes to weedy relatives [3].

Original studies using plant viruses constructed as expression vectors involved infecting the host plant, resulting in a low yield of infection due to tissue specific limitations of the virus as well as the lack of synchrony of the virus life cycle. More recently, agroinfiltration of the virus vector into host leaves using a syringe, or into many plants en masse under a vacuum, has become the method of choice [4]. Using this approach, the virus vector can be introduced to every external cell of the host at the same time, resulting in a highly temporally controlled infection which can result in much higher levels of vaccine protein production.

Plant viruses have been developed as vectors for heterologous protein production as well as for plant functional genomics studies, by employing virus induced gene silencing (VIGS) to down-regulate specific host transcripts. Both of these technologies offer great promise for the agricultural and pharmaceutical industries alike. The following review describes recent progress in plant virus expression vector development, and their use for the production of vaccines and other therapeutic proteins.

Positive-sense RNA virus expression vectors

Positive-sense, single-stranded RNA viruses are the largest group of plant viruses that have been engineered into expression vectors for vaccine production. These include the tombusvirus, the potexvirus, the cucumovirus and the comovirus groups.

Tombusviruses: Tobacco mosaic virus (TMV) is one of the first plant viruses that have been engineered for use in biopharmaceutical production. The first generation of virus expression vectors derived from TMV were based on usage of the entire viral genome and were inoculated onto the leaves of the host, where the recombinant virus underwent a natural infection cycle. More recently, a series of second generation 'deconstructed' expression vectors, consisting of only essential elements of the virus genome necessary for replication were included on a series of plasmid constructs. One of the preliminary series of deconstructed TMV vector modules, known as the MagnICON® vector system, was developed and transfected into plants in a process known as 'magnifection.' This involves the vacuum infiltration of a suspension of *Agrobacterium* cells into the leaves of tobacco plants, in such a way that infection takes place in every cell, and in a synchronous fashion [5]. Mixtures of different combinations of modules can be transformed into the *Agrobacterium*, making it easy to produce an assortment of biopharmaceuticals rapidly. This system has been used to produce a number of vaccine proteins, including plasmodium antigen, bovine herpes virus-gD protein, and the envelope protein of Dengue virus [6-8]. The expression system can be further modified by altering transcript splice sites and codon usage patterns to further improve this expression platform. Co-expression of a suppressor of gene silencing P19 in conjunction with the expression modules can also enhance protein expression [9].

Another way to increase gene expression in a TMV-based vector

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is by placing the open reading frame closer to the 3' terminus. The CP open reading frame has been removed in this TRBO (TMV RNA-based overexpression) vector, and is a much more powerful production platform than the earlier system using P19 [10].

Potexviruses: Another well characterized single-stranded RNA virus expression vector system has been engineered from the potexviruses. Potato virus X vectors, for example have been used to express vaccine proteins such as the Human papillomavirus-16 L2 minor capsid protein and a conserved influenza epitope [11,12]. These antigens can be expressed from the PVX genome itself in plants or as part of a fusion protein with the PVX CP.

Recently, the potexvirus Narcissus mosaic virus (NMV) was developed as an expression vector to be used in the plant host *N. benthamiana* for secondary metabolite production [13] NMV does not cause visible pathogenic symptoms in *N. benthamiana.* The NMV expression vector was constructed to include the Gateway system to assist in the high throughput cloning of foreign genes. The R2R3 MYB transcription factor AtMYB75 (PAP1), that promotes anthocyanin biosynthesis in *Arabidopsis,* was used to induce visible pigment production and significant changes in metabolite production in infected plants.

Cucumovirus: Cucumber mosaic virus (CMV) has a trimeric genome and a wide host range. Since CMV virions take the form of an icosahedrons, their use as vaccine vectors is limited due to size constraints, however, CMV has also been used successfully as an antigen presentation system. For example, an epitope corresponding to the capsid protein of porcine circovirus type 2 (PCV2) has been placed on the surface of a CMV expression vector; chimeric CMV: PCV2 particles were then injected parentally into mice and pigs. After demonstrating a PCV specific antibody response, pigs challenged with virus demonstrated a partial protection against infection [14]. More recently, mice fed chimeric CMV: PCV2 were shown to elicit a mucosal and serum immune response, and piglets fed chimeric CMV: PCV2 exhibited porcine circovirus-specific antibodies, demonstrating that this plant-derived virus expression system could be used for mucosal vaccine production [15].

Cowpea mosaic virus

The icosahedral Cowpea mosaic virus (CPMV) has also been designed to generate full length proteins or fusion proteins which can later be proteolytically cleaved as well as an epitope presentation system in the form of virus-like particles (VLPs) [16]. One recent example is the use of CPMV VLPs to carry influenza virus antigens as a novel vaccine platform. This technology, designed by Medicago, fully protected against lethal viral challenge in both animal trials as well as in a Phase 1 human clinical trial. More recently, a non-replicating expression system based on CPMV, known as pEAQ, has been engineered and has been greatly successful in producing foreign proteins at high levels in the absence of replication. The gene of interest is inserted between the 5' leader and 3' non-transcribed region of RNA-2 [17]. A highly efficient Cowpea Mosaic Virus hyper-translational "CPMV-HT" expression system, based on the previous system, can generate even higher yields of recombinant protein [18]. This construct contains a modified 5' UTR, lacking upstream AUG codons and an unmodified 3' UTR derived from CPMV RNA-2. The efficiency of this virus expression system has been explored further, through the development of a series of 5' and 3' UTR mutants [19]. The authors found that a Y shaped stem- loop secondary structure found within the 3' UTR played a significant role in enhancing reporter gene expression, and its disruption greatly reduced expression levels. The enhancing role of the Y-shaped structure was confirmed by substitutions with similar structures from the 3' UTRs of other plant RNA viruses, and acted by enhancing the levels of mRNA accumulation. The 5' and 3' UTRs were found to exert their enhancing effects independently of each other, implying that in the future, a spectrum of expression vectors based on CPMV with controlled levels of mRNA transcripts and translation efficiencies could be produced. This system has been employed to generate Bluetongue virus (BTV)-like particles in plants as a vaccine which can protect sheep against live virus infection [20].

Plant DNA viruses

Plant DNA viruses, in particular, geminiviruses; have been engineered as highly effective virus expression vectors. Geminiviruses named for their twinned capsid morphology; are small single stranded DNA viruses which have been shown as expression vectors to replicate to extremely high copy numbers and have expressed vaccine proteins ranging from Hepatitis A VP1 virus to monoclonal antibodies against Ebola virus [21,22]. The circular genome of geminiviruses replicate by rolling circle replication, using the replication initiator protein Rep. Rep is essential for virus replication. Unlike their RNA virus counterparts, DNA viruses can tolerate large inserts and remain stable after many generations of replication. The first geminivirus expression vectors expressed Rep independently from the rest of the virus genome, under constitutive, inducible or developmental promoters. In this way, vector replication could be induced and foreign gene expression could be enhanced enormously.

Recently, Dugdale et al., [23], engineered the mastrevirus Tobacco yellow dwarf virus (TYDV) as a double-expression cassette system [23,24]. One expression cassette encodes Rep/Rep A under the control of the ethanol inducible AlcA: AlcR promoter, while the second expression cassette contains the open reading fame encoding the foreign protein, also under the control of the ethanol inducible promoter. Thus, by applying a simple ethanol spray, the construct can become activated to undergo rolling circle replication and express the gene of interest. Furthermore, the gene of interest resides in the INPACT (In Plant Activation) cassette, in the form of two halves, which are separated from each other by a synthetic intron. As a result, the foreign gene of interest can only be expressed from replicons which have been activated by the ethanol spray and have been processed to splice out the intron. Having the TYDV sequences responsive to an ethanol spray ensures that expression of the desired protein can be controlled temporally, spatially and in a dose-dependent manner [23].

Conclusion

Plant virus expression vectors have been engineered to function as rapid, inexpensive and robust platforms for vaccine production. The implications of this and other technologies related to molecular pharming in plants are substantial; not only can the world's rural poor benefit from the low cost and improved accessibility of plant made vaccines and other therapeutic proteins, but the capability to upscale and stockpile these vaccines in a time effective manner in

order to combat global pandemics, for example, provides a select advantage over conventional vaccine production. A number of technical challenges concerning plant-based biopharmaceuticals must be overcome in order to bring this technology to fruition. These include limitations with respect to plant virus expression vector host range, poor expression levels of the therapeutic protein in plants, innate differences with respect to the post translational modification profiles of mammalian proteins generated in plants, and potential allergenicity issues related to plant-based products. Public perception of plant biotechnology also remains an issue that must be better addressed. Further research and development, as well as a better informed public should help to resolve these problems.

As research and development progresses, even more sophisticated plant virus expression systems will become available. For example, Cowpea chlorotic mottle virus (CCMV) capsid proteins can package RNA from Sindbis virus, an animal virus, to produce hybrid, cellular nuclease- resistant VLPs which can be delivered to mammalian cells. Moieties involved in subcellular targeting can be conjugated to this hybrid CCMV: Sindbis VLPs so that they can release their RNA contents at appropriate sites within the cell [25]. It is innovations such as these which will broaden the number of applications of plant virus expression vectors in the field of medicine for many years to come.

References

1. Hamorsky KT, Kouokam JC, Bennett LJ, Baldauf KJ, Kajiura H, Fujiyama K, et al. Rapid and scalable plant-based production of a cholera toxin B subunit variant to aid in mass vaccination against cholera outbreaks. PLoS Negl Trop Dis. 2013; 7: e2046.
2. McCormick AA, Reddy S, Reinl SJ, Cameron TI, Czerwinkski DK, Vojdani F, et al. Plant-produced idiotype vaccines for the treatment of non-Hodgkin's lymphoma: safety and immunogenicity in a phase I clinical study. PNAS, U S A. 2008; 105: 10131-6.
3. Platis D, Drossard J, Fischer R, Ma JK, Labrou NE. New downstream processing strategy for the purification of monoclonal antibodies from transgenic tobacco plants. J Chromatogr A. 2008; 1211: 80-89.
4. Leuzinger K, Dent M, Hurtado J, Stahnke J, Lai H, Zhou X. Efficient agroinfiltration of plants for high-level transient expression of recombinant proteins. J Vis Exp. 2013.
5. Klimyuk V, Pogue G, Herz S, Butler J, Haydon H. Production of Recombinant Antigens and Antibodies in Nicotiana benthamiana Using 'Magnifection' Technology: GMP-Compliant Facilities for Small- and Large-Scale Manufacturing. Curr. Top. Microbiol. Immunol. 2014; 375: 127-154.
6. Webster DE, Wang L, Mulcair M, Ma C, Santi L, Mason HS, et al. Production and characterization of an orally immunogenic Plasmodium antigen in plants using a virus-based expression system. Plant Biotechnol J. 2009; 7: 846-855.
7. Péres-Filgueira DM, Zamorano PI, Dominguez MG, Taboga O, Del Médico Zajac MP, Puntel M, et al. Bovine herpes virus gD protein produced in plants using a recombinant tobacco mosaic virus (TMV) vector possesses authentic antigenicity. Vaccine. 2003; 21: 4201-4204.
8. Saejung W, Fujiyama K, Takasaki T, Ito M, Hori K, Malasit P, et al. Production of dengue 2 envelope domain III in plant using TMV-based vector system. Vaccine. 2007; 25: 6646-6654.
9. Lindbo JA. High-efficiency protein expression in plants from agroinfection-compatible Tobacco mosaic virus expression vectors. BMC Biotechnol. 2007; 7: 52.
10. Lindbo JA. TRBO: a high-efficiency tobacco mosaic virus RNA-based overexpression vector. Plant Physiol. 2007; 145: 1232-1240.
11. Petukhova NV, Gasanova TV, Ivanov PA, Atabekov JG. High-level systemic expression of conserved influenza epitope in plants on the surface of rod-shaped chimeric particles. Viruses. 2014; 6: 1789-1800.
12. Cerovska N, Hoffmeisterova H, Moravec T, Plchova H, Folwarczna J, Synkova H, et al. Transient expression of Human papillomavirus type 16 L2 epitope fused to N- and C-terminus of coat protein of Potato virus X in plants. J Biosci. 2012; 37: 125-133.
13. Zhang H, Wang L, Hunter D, Voogd C, Joyce N, Davies K. A Narcissus mosaic viral vector system for protein expression and flavonoid production. Plant Methods. 2013; 9: 28.
14. Gellért A, Salánki K, Tombácz K, Tuboly T, Balázs E. A cucumber mosaic virus based expression system for the production of porcine circovirus specific vaccines. PLoS One. 2012; 7: e52688.
15. Tombácz K, Gellért A, Salánki K, Balázs E, Tuboly T. Oral immunogenicity of a plant virus vector based porcine circovirus antigen - short communication. Acta Vet Hung. 2013; 61: 547-552.
16. D'Aoust M-A, Lavoie PO, Couture MM, Trépanier S, Guay JM, Dargis M, et al. Influenza virus-like particles produced by transient expression in Nicotiana benthamiana induce a protective immune response against a lethal viral challenge in mice. Plant Biotechnol. J. 2008; 6: 930-940.
17. Peyret H, Lomonossoff GP. The pEAQ vector series: the easy and quick way to produce recombinant proteins in plants. Plant Mol Biol. 2013; 83: 51-58.
18. Sainsbury F, Sack M, Stadlmann J, Quendler H, Fischer R, Lomonossoff GP, et al. Rapid transient production in plants by replicating and non-replicating vectors yields high quality functional anti-HIV antibody. PLoS One. 2010; 5: e13976.
19. Thuenemann EC, Meyers AE, Verwey J, Rybicki EP, Lomonossoff GP. A method for rapid production of heteromultimeric protein complexes in plants: assembly of protective bluetongue virus-like particles. Plant Biotechnol. J. 2013; 11: 839-846.
20. Meshcheriakova YA, Saxena P, Lomonossoff GP. Fine-tuning levels of heterologous gene expression in plants by orthogonal variation of the untranslated regions of a nonreplicating transient expression system. Plant Biotechnol. J. 2014; 12: 1–10.
21. Chung HY, Lee HH, Kim KI, Chung HY, Hwang-BoJ, Park JH, et al. Expression of a recombinant chimeric protein of hepatitis A virus VP1-Fc using a replicating vector based on Beet curly top virus in tobacco leaves and its immunogenicity in mice. Plant Cell Rep. 2011; 30: 1513-1521.
22. Chen Q, He J, Phoolcharoen W, Mason HS. Geminiviral vectors based on bean yellow dwarf virus for production of vaccine antigens and monoclonal antibodies in plants. Hum Vaccin. 2011; 7: 331-338.
23. Dugdale B, Mortimer CL, Kato M, James TA, Harding RM, Dale JL, et al. In plant activation: an inducible, hyperexpression platform for recombinant protein production in plants. Plant Cell. 2013; 25: 2429-2443.
24. Dugdale B, Mortimer CL, Kato M, James TA, Harding RM, Dale JL, et al. Design and construction of an in-plant activation cassette for transgene expression and recombinant protein production in plants. Nat Protoc. 2014; 9: 1010-1027.
25. Azizgolshani O, Garmann RF, Cadena-Nava R, Knobler CM, Gelbart WM. Reconstituted plant viral capsids can release genes to mammalian cells. Virology. 2013; 441: 12-17.

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