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Review

Plant virus expression vectors set the stage as production platforms for biopharmaceutical proteins

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ABSTRACT

Transgenic plants present enormous potential as a cost-effective and safe platform for large-scale production of vaccines and other therapeutic proteins. A number of different technologies are under development for the production of pharmaceutical proteins from plant tissues. One method used to express high levels of protein in plants involves the employment of plant virus expression vectors. Plant virus vectors have been designed to carry vaccine epitopes as well as full therapeutic proteins such as monoclonal antibodies in plant tissue both safely and effectively. Biopharmaceuticals such as these offer enormous potential on many levels, from providing relief to those who have little access to modern medicine, to playing an active role in the battle against cancer. This review describes the current design and status of plant virus expression vectors used as production platforms for biopharmaceutical proteins.

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Introduction

It is a sad fact that a leading cause of infant mortality in developing countries continues to be from infectious diseases which are quite preventable. New technologies which can both deliver vaccines en mass and without the need for syringes, as well as induce a strong mucosal immune response, could revolutionize the accessibility of much needed vaccines in developing countries. Approaches range from the use of inhalation devices which deliver the vaccine antigen in the form of an aerosol spray, to patches containing microneedles which can deliver the desired vaccine antigen across the skin barrier. A third tactic involves the development of plant production platforms as delivery systems for oral vaccines.

Plant-made vaccines have the potential to enhance vaccine coverage in children and infants, particularly in resource-poor regions where infectious diseases remain a problem (Rybicki, 2009, Yusibov et al., 2011). Plants have the advantage of being capable of producing biopharmaceutical proteins that retain the same structural integrity and activity as their mammalian-derived counterparts. Plants expressing vaccine antigens can be produced stably by means of transgenic plant technologies, or transiently by agroinfiltration or infection using recombinant virus expression vectors (Daniell et al., 2009, Penney et al., 2011). Proteins can be produced in plants from constitutive promoters, or alternatively, if the protein proves to be toxic to

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the plant, from inducible promoters. In addition to this, seed-specific promoters can enable recombinant proteins to be stored within plant seed such as corn or rice at ambient temperatures for prolonged periods of time.

Plant virus expression vectors are particularly attractive as they require no lengthy steps of plant transformation, yet they can express substantial levels of the gene of interest on a massive scale rapidly, often within a few days. This production level can be easily scaled up if needed, by merely increasing the number of host plants. Moreover, vaccine proteins produced in plants can be purified using a few simple steps, or in some cases, require only partial purification (Boehm, 2007, Paul and Ma, 2011). These properties make plant virus expression vectors feasible for use to address infectious diseases in the Third World today.

Their rapidity and easy scalability make plant virus expression vectors attractive candidates for other reasons besides providing vaccines and therapeutic proteins to those who reside in developing countries. Production platforms based upon plant viruses are now being used to generate vaccines to combat specific cancers, to offset global pandemics such as H1N1 influenza, and even to produce vaccines against potential biological warfare agents. Objectives such as these together present a powerful driving force towards the further advancement of plant viruses as a technology to produce biopharmaceuticals in plants.

Plant viruses have also been developed as expression platforms for the large-scale production of proteins for industrial, pharmaceutical, veterinary, and agricultural uses (Desai et al., 2010; Rybicki, 2010). This review highlights the current status of vaccine and therapeutic protein production using plant virus expression vectors. More recent developments regarding the construction of vectors based on Tobacco mosaic virus, Potato virus X, Cowpea mosaic virus and two Geminiviruses are described, and examples of vaccines produced using these approaches are discussed. The review concludes with an overlook of the future of plant virus expression vectors for the production of vaccine and therapeutic proteins in plants.

Technology platforms used to produce vaccine proteins in plants

Biopharmaceutical proteins have been expressed in plants using a variety of approaches. One approach involves the stable transformation of plant cells which are regenerated into transgenic plants. Two common methods by which to generate transgenic plants are *Agrobacterium*-mediated transformation and biolistic delivery (Daniell et al., 2009). Plant viruses have emerged as an alternative to stable genetic transformation for the expression of foreign proteins in plants (Yusibov et al., 2011). Neither approach can be considered to be exclusively optimal for protein production, although plant virus expression systems have a number of selective advantages for industrial scale protein production. Transgenic plants have the advantage of heritability for production of recombinant proteins; however, yields can often be low. Transient expression using virus vectors, on the other hand, can provide easy scalability, as well as rapid and high expression of industrial levels of recombinant protein. Furthermore, the more recent versions of virus vectors lack the ability to become encapsidated or move from cell to cell, thus mitigating biocontainment issues often associated with the use of transgenic plants as production platforms. The small genomes of plant viruses are also advantageous as they are easy for researchers to manipulate. Moreover, infecting plants with modified viruses is a rapid process, with predicted yields often achieved within a few days or a couple of weeks, and higher yields obtained than what is generally expected from stably transformed plants (Rybicki, 2010).

Transient expression of vaccine proteins in plants

Plant virus expression vectors have been under further development as robust and efficient systems by which to produce vaccine and therapeutic proteins. Two major plant RNA virus expression vector systems have been engineered for the production of immunogenic peptides and proteins in plants. These include epitope presentation systems (short antigenic peptides fused to the CP that are displayed on the surface of assembled virus particles) and polypeptide expression systems (systems which express the entire recombinant protein) (Yusibov et al., 2011). Plant DNA viruses, such as geminiviruses, have also been under exploration as potential protein expression platforms. DNA virus vectors lack the size restrictions and issues with instability that their RNA virus vector counterparts experience. Geminiviruses in particular, with their broad host range, accumulate to extremely high copy numbers in inoculated cells, resulting in greatly elevated levels of gene expression (Chen et al., 2011).

Plant viruses which have been engineered to produce vaccines and therapeutic proteins include Tobacco mosaic virus, Potato virus X, Cucumber mosaic virus, Cowpea mosaic virus and Alfalfa mosaic virus (Yusibov et al., 2011). Viruses such as these have been used as vectors for the expression of recombinant proteins for over two decades. Several biopharmaceutical proteins have been expressed using vectors based on full-length plant virus genomes (Cañizares et al., 2005a; Koprowski and Yusibov, 2001; Lomonossoff and Hamilton, 1999). Full-length virus vectors can spread systemically within a plant and are readily transmitted to new plants if scale-up is required. However, biocontainment can be an issue for their use in certain circumstances. For example, some plant viruses used as vectors can be transmitted mechanically; others are transmitted by insect vectors. As a result, it is paramount that precautions be taken to prevent the unwanted spread of virus vectors to nontarget organisms. Viruses can be engineered so that they lack the genes necessary to move from cell to cell, be transmitted by insects to other plants or undergo any unexpected recombination events that limit their usefulness as expression vectors. Target plants harboring virus vectors can also be separated from other crops via physical isolation, such as being strictly housed within greenhouses or in cell culture.

The use of plant virus vectors is also influenced by insert size and host range. For example, virus vectors which have been engineered for epitope presentation frequently exhibit restrictions with respect to the actual size of an epitope which can be effectively displayed on the surface of a virus particle without disturbing capsid protein conformation and virus particle assembly. Similarly, viruses which have been developed as polypeptide expression systems are restricted with respect to insert size as a result of difficulties with respect to encapsidation and/or genome stability. To circumvent these problems, deconstructed or deleted versions of plant virus-based expression systems have been under development (Gils et al., 2005; Gleba et al., 2004). Deconstructed versions of the RNA viruses Tobacco mosaic virus (TMV), Potato virus X (PVX), and Cowpea mosaic virus (CPMV) RNA-2 as well as the geminivirus Beet curly top virus (BCTV) have successfully been used been used to produce a variety of proteins in plants (Lim et al., 2010; Cañizares et al., 2005b; Chung et al., 2011).

Human and animal trials for plant-derived vaccines

Tobacco mosaic virus and potato virus X

Tobacco mosaic virus (TMV) and Potato virus X (PVX), are rod shaped viruses with single-stranded RNA genomes, and have been widely used as virus expression vectors for biopharmaceutical

protein production, both as a single genomic molecule or in a deconstructed vector format. Since the genome of TMV, for example, is encapsidated by over 2000 copies of coat protein, it represents an optimal epitope presentation system. However, due to size limitations, only small epitopes can be incorporated on the virus particle surface. Some vaccine epitopes which have been expressed on TMV include antigens from Foot and Mouth Disease Virus, HIV-1, malaria and rotavirus (Wigdorovitz et al., 1999; Yusibov et al., 1999; Turpenet al., 1995). TMV has also been used as a polypeptide presentation system. By incorporating an additional CP subgenomic promoter onto the virus genome, recombinant TMV viruses can express both their own coat protein as well as a protein of interest. TMV vectors utilized in this manner express plasmodium antigen, α- trichosanthin, a tumor-derived ScFv, bovine herpes virus-gD protein, major birch pollen antigen, and human growth hormone (Gils et al., 2005; Péres-Filgueira et al., 2003; Kumangai et al., 1993; Wagner et al., 2004; Webster et al., 2009).

Both full length virus vectors and their deconstructed versions can be delivered via agroinfection. Deconstructed vector strategies have been employed using TMV and the results have been enormously successful. The genome of TMV has been 'deconstructed' into two separate modules, as depicted in Fig. 1. One module contains the portions of the genome required for replication, while the other contains cassettes designed for insertion of foreign genes. In this way, biopharmaceutical proteins can be expressed at high levels in a viral replicon system in plants (Gleba et al., 2004). A technique for transfecting plants with these recombinant virus vector modules, known as 'magnifection', was also established by Icon Genetics. Using the magnifection approach, an *Agrobacterium tumefaciens* suspension could be infiltrated into the intercellular space of all mature leaves of a tobacco plant, resulting in virus infection that is more synchronous and faster than systemic infection. Deconstructed versions of TMV have been used to generate HPV E7 protein, as well as Norwalk virus-like particles in plants (Noris et al., 2011; Santi et al., 2008). Another example of the use of the deconstructed virus system can be demonstrated with the L1 protein of canine oral papillomavirus, which was expressed in transgenic tobacco chloroplasts using a Potato virus X vector. The transgenic plants used in this production system expressed a suppressor of gene silencing, which was able to improve protein expression even more (Azhakanandum et al., 2007). Similarly, Mallory et al. (2002), coexpressed PVX-based 'amplicons' along with a viral suppressor of gene silencing in transgenic tobacco lines, resulting in greatly increased levels of transgene expression. Combined transgene-virus systems such as these will not be discussed further in this review.

TMV vectors have been used widely by other researchers. For example, Lindbo (2007a), co-introduced a 35S driven TMV vector as well as a version of a viral suppressor of RNA silencing into plants, resulting in the production of high levels of protein (between 600 and 1200 micrograms of recombinant protein per gram of infiltrated tissue) within a week post infection. Another TMV RNA based overexpression vector (TRBO) constructed by the same author, which lacked the TMV CP gene coding sequence and so was unable to form virus particles, possessed a higher agroinfection efficiency and produced up to 100 times more recombinant protein than the suppressor of silencing-enhanced system (Lindbo, 2007b).

In another example, a vaccine against the endemic disease plague, derived from the causative bacterial agent *Yersinia pestis*, has been developed using the deconstructed tobacco mosaic virus-based system (Molina et al., 2004). Plague antigens that were purified from the Icon genetics TMV-based deconstructed system were used in animal trials and shown to generate systemic immune responses as well as provide protection against an aerosol challenge by virulent *Y. pestis*. (Santi et al., 2006). Similarly, Saejung et al., (2007) expressed the envelope protein of Dengue virus using a tobacco mosaic virus-based transient expression system. Mice which were immunized intramuscularly with envelope protein D2EIII induced an anti-Dengue virus antibody response that exhibited neutralizing activity against Dengue type 2 virus. Production levels as great as 300 mg/kg leaf fresh weight were determined for HBsAg expressed in the MagnICON^{TM} viral vector expression system; furthermore, these could self-assemble into VLPs (Huang et al., 2008). As a final example, Webster et al., (2009) described the rapid expression of the malarial antigen PyMSP4/5 in *Nicotiana benthamiana* leaves using the same deconstructed tobacco mosaic virus-based expression system outlined above. Not only were levels of malarial antigen up to 10% of total soluble protein or 1–2 mg/g of fresh weight produced; the antigen retained its immunogenicity following long-term storage at ambient temperature within freeze-dried leaves. Malaria-specific antibodies were observed in mice fed this plant-derived malaria antigen along with a mucosal adjuvant, demonstrating that large quantities of malaria vaccine can be produced and stored using this TMV-based production system.

Deconstructed versions of TMV expression systems have recently moved to the forefront of the up and coming discipline of personalized medicine, including a potential treatment for various forms of cancer. For example, deconstructed TMV

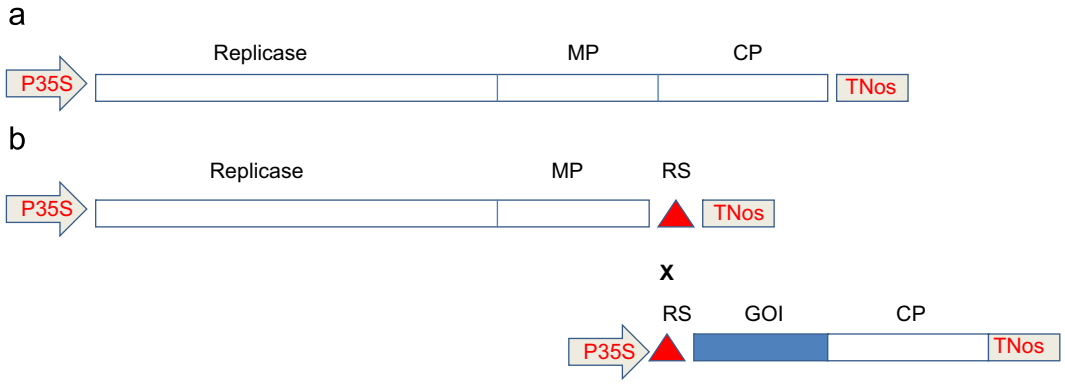

Fig. 1. Schematic representation of deconstructed version of Tobacco mosaic virus (TMV). (a) Full-length TMV expression vector. (b) Deconstructed expression vector for production of active replicons in plants. Virus replicase, MP: movement protein, CP; coat protein RS: recombination site. P35S: CaMV 35S promoter, Tnos: nopaline synthetase terminator. X refers to the site of recombination. The constructs are delivered into plant cells via agroinfection; when a deconstructed virus is used, the two modules recombine at specific sites into the nucleus.

expression vectors are currently being explored as a means by which to potentially combat diseases such as Non-Hodgkins Lymphoma (NHL). The fifth highest cause of death in North America, NHL refers to a tumor disease involving degenerate B-cells which multiply uncontrollably, accumulating in the lymph nodes, bone marrow and other tissues. Degenerate B-cells of each individual NHL patient express a unique idiotype, which can be used as a tumor marker. These idiotypic regions derived from tumor cells which have been isolated from individual patients can be rapidly and inexpensively expressed in plants using a virus expression vector agroinfiltrated into tobacco leaves (Fig. 2). This strategy enables the required amounts of lymphoma vaccine for each patient to be expressed very rapidly and at low cost. Plant-made vaccines against NHL could act as powerful short-term therapy which could be administered immediately after diagnosis to keep tumors in check (McCormick et al., 2008).

Alternatively, Musiychuk et al., 2007 have constructed a novel 'launch vector', composed of TMV-based expression vector sequences that are harbored on the Agrobacterium binary vector pBI121. Upon introduction into plant tissue, multiple ssDNA copies of target sequences are released, a property based on the presence of left and right border sequences of the binary vector. The TMV vector sequences launched in this fashion have been shown to generate 100 mg quantities of protein per kg of plant tissue in less than a week. Vaccines against the oncogenic E7 protein of HPV, the H5N1 influenza virus HA and NA domains, and the F1 and V antigens of *Y. pestis* have been effectively produced using this launch vector system and were shown to be protective using animal models (Massa et al., 2007; Mett et al., 2008; Chichester et al., 2009).

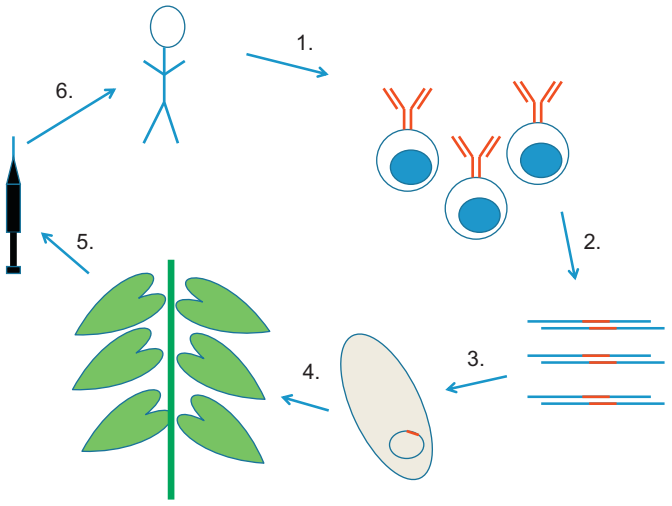

Fig. 2. TMV based plant-derived vaccine for Non-Hodgkins Lymphoma. (1) Proliferating B-cells expressing unique idiotype are isolated from patient with NHL. (2) cDNA containing idiotypic region is cloned into a viral vector. (3) Viral vector containing the idiotype cDNA is transformed into Agrobacterium. (4) Agrobacterium harboring viral vector is infiltrated onto whole plants. (5) Plants are harvested for vaccine protein containing idiotype. (6) Purified vaccine protein is infected into original patient to elicit immune response to cells expressing idiotype.

Cowpea mosaic virus

A number of other plant RNA viruses have been investigated extensively for their potential to produce biopharmaceutical proteins. Cowpea mosaic virus (CPMV) has been developed for the production of vaccine and therapeutic proteins in plants with great success (Canizares et al., 2005a, 2005b). CPMV represents an ideal candidate since it replicates well in host plants, is very stable, and can easily be extracted and purified from plants. Initially, CPMV was developed as an epitope presentation system. CPMV capsid has been well studied; it is comprised of 60 subunits each of both large (L) and small (S) coat protein subunits that are arranged to form an icosahedral virus surrounding a bipartite RNA genome. Exposed loops on the surface of the capsid protein have been identified and several of these loops have been used as the site of insertion of foreign peptides. Recombinant virus particles expressing foreign epitope sequences have been demonstrated to produce yields similar to that of wild type viruses, and many epitopes displayed on the surface of CPMV have been able to successfully elicit strong immune responses (Lomonossoff and Hamilton, 1999).

CPMV has also been engineered to express the 2A protein of Foot and Mouth Disease Virus, which acts as a catalytic peptide to promote cleavage between the viral proteins and GFP. Foreign proteins have been expressed as fusions with the CPMV coat protein or movement protein, with the addition of an integral proteolytic cleavage site to allow the target protein to be released (Gopinath et al., 2000).

Replicating RNA virus-based expression systems offer several drawbacks; they may intrinsically alter the host cell, causing perturbations to the endomembrane system which could result in variations in the quality of recombinant protein or in different post-translational modification patterns. In addition to this, RNA virus- based replication has been associated with a higher rate of accumulated mutations, due to the poor fidelity of RNA-dependent RNA polymerases (RdRp). Recently, a novel nonreplicating expression system based on a disabled version of RNA-2 of CPMV has been developed which involves positioning a gene of interest

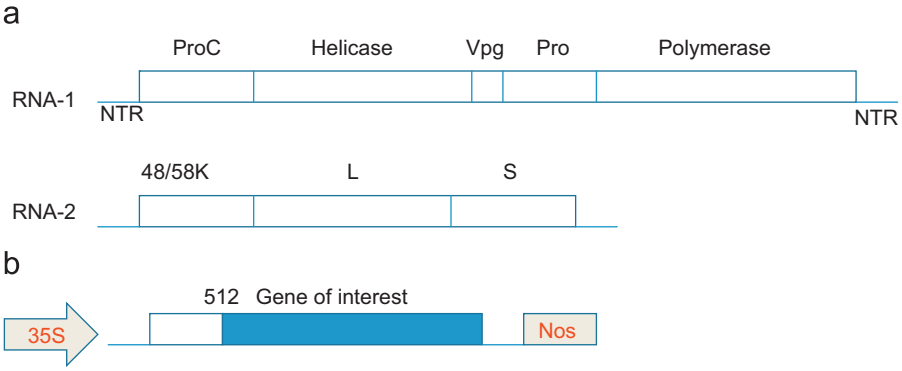

Fig. 3. Transient expression vector based on CPMV RNA-2. (a) Genomic organization of CPMV RNAs-1 and 2. (b) CPMV RNA-2 nonreplicating construct for high expression of vaccine proteins. This is the RNA that is inoculated into plants for transient gene expression. NTR: nontranslated regions, 512: nucleotide position of CPMV RNA-2 first open reading frame, fused to gene of interest.

between the 5′ leader sequence and 3′ untranslated region (UTR) of RNA-2 (Fig. 3). *Agrobacterium*-mediated transient transformation is used to obtain high-level expression of the foreign gene of interest in the absence of virus replication, a feature that makes this system extremely advantageous (Sainsbury and Lomonossoff, 2008). Deletion of an in-frame initiation codon located upstream of the main translation initiation site of RNA-2 resulted in a massive increase in foreign protein accumulation. In fact, proteins ranging in diversity from a full-size IgG to a self-assembling virus-like particle could be expressed to high levels (10% and 20% of total extractable protein, respectively) (Sainsbury et al., 2010a). A CPMV non-replicating system was further shown to maintain high quality of purified anti-HIV-1 antibody produced in plants (Sainsbury et al., 2010b). More recently, a CPMV-derived expression vector was used to transiently express a chimeric Human papillomavirus 16 L1 protein carrying the M2e epitope of influenza A virus in tobacco plants. The chimeric proteins were able to assemble into higher order structures, including virus-like particles, and react with anti-influenza antibodies, thus demonstrating the potential of this system for vaccine production (Matic et al., 2011).

Geminiviruses

Another system under development for the production of plant-made biopharmaceuticals is the use of a small plant DNA virus known as geminivirus. Geminiviruses, named for their twinned capsid morphology, are characterized by a small genome and a broad host range, and can replicate to extremely high copy numbers in infected cells (Timmermans et al., 1994). A number of geminiviruses have been used to express foreign proteins in plants; two geminiviruses have been developed further using the deconstructed vector strategy. Bean yellow dwarf virus (BeYDV), a mastrevirus, has been engineered to express its replication initiator protein (Rep) under independent promoter control (Fig. 4). This approach has been used to produce a vaccine against Staphlococcus Enterotoxin B (SEB), considered to be a potential biowarfare agent, as well as antigens derived from Hepatitis B virus, Norwalk virus, HIV and HPV (Hefferon and Fan, 2004; Huang et al., 2009; Regnard et al., 2010). Monoclonal antibodies against Ebola virus have also been created using this technique (Huang et al., 2010). Beet curly top virus, (BCTV), a curtovirus, has also been developed as a deconstructed geminivirus to express foreign proteins. In this instance, the authors were able to demonstrate that accumulation of the protein of interest could be further enhanced by co-delivering an additional plasmid which expresses a strong viral suppressor of RNA silencing (Kim et al., 2007).

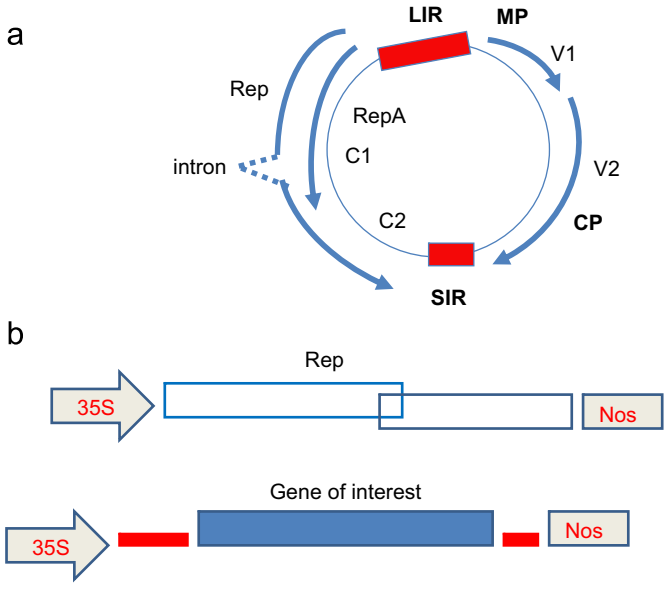

Fig. 4. Geminivirus expression vector constructed for expression of vaccine proteins. (a) Genomic organization of Bean yellow mosaic virus. (b) Deconstructed expression vector. LIR: long intergenic region, SIR: short intergenic region.

Conclusions: onwards and upwards for future commercialization

Plant-derived vaccines can be produced in numerous settings, including cell culture, open fields and under greenhouse conditions. While variable soil and weather conditions can make outdoor production difficult, cell suspension systems and greenhouses offer more precisely controlled environments. Continuous culture of plant cell lines or hairy root systems which are able to secrete the specific protein product into the surrounding media can reduce expensive downstream processing in an effective manner (Penney et al., 2011; Boehm, 2007). Purification from plant tissues is in general more facile and economical than standard purification from their mammalian and bacterial counterparts. The first vaccine to gain regulatory approval and be licensed is a poultry vaccine for Newcastle disease, produced from a cell culture bioreactor system (Sparrow et al., 2007). A number of other plant-derived therapeutic proteins are currently undergoing extensive clinical trials and are approaching market release. Many of these new products incorporate plant virus vectors in the form of deconstructed vector systems, making biopharmaceutical protein production even more rapid and productive than ever. As demonstrated in this review, plant virus expression vectors provide a powerful tool for future biopharmaceutical protein production and will offer alternative solutions ranging from producing novel personalized medicines to combating future pandemics, and of course, to protecting the world's poor against preventable infectious diseases.

References

Azhakanandum, K., Weissinger, S.M., Nicholson, J.S., et al., 2007. Amplicon-plus targeting technology (APTT) for rapid production of a highly unstable vaccine protein in tobacco plants. Plant Moll. Biol. 63, 393–404.
Boehm, R., 2007. Bioproduction of therapeutic proteins in the 21st century and the role of plants and plant cells as production platforms. Ann. N.Y. Acad. Sci. 1102, 121–134.
Canizares, M.C., Nicholson, L., Lomonossoff, G.P., 2005a. Use of viral vectors for vaccine production in plants. Immunol. Cell. Biol. 83 (3), 263–270.
Canizares, M.C., Lomonossoff, G.P., Nicholson, L., 2005b. Development of cowpea mosaic virus-based vectors for the production of vaccines in plants. Expert Rev. Vaccines 4 (5), 687–697.
Chen, Q., He, J., Phoolcharoen, W., et al., 2011. Geminiviral vectors based on bean yellow dwarf virus for production of vaccine antigens and monoclonal antibodies in plants. Hum. Vaccin. 7 (3), 331–338.
Chichester, J.A., Musiychuk, K., Farrance, C.E., et al., 2009. A single component two-valent LcrV-F1 vaccine protects non-human primates against pneumonic plague. Vaccine 27, 3471–3474.
Chung, H.Y., Lee, H.H., Kim, K.I., et al., 2011. 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. 30 (8), 1513–1521.
Daniell, H., Singh, N.D., Mason, H., et al., 2009. Plant-made vaccine antigens and biopharmaceuticals. Trends Plant Sci. 14 (12), 669–679.
Desai, P.N., Shrivastava, N., Padh, H., 2010. Production of heterologous proteins in plants: strategies for optimal expression. Biotechnol. Adv. 28 (4), 427–435.
Gils, M., Kandzia, R., Marillonnet, S., et al., 2005. High-yield production of authentic human growth hormone using a plant virus-based expression system. Plant Biotechnol. J. 3 (6), 613–620.
Gleba, Y., Marillonnet, S., Klimyuk, V., 2004. Engineering viral expression vectors for plants: the 'full virus' and the 'deconstructed virus' strategies. Curr. Opin. Plant Biol. 7 (2), 182–188.
Gopinath, K., Wellink, J., Porta, C., et al., 2000. Engineering cowpea mosaic virus RNA-2 into a vector to express heterologous proteins in plants. Virology 267 (2), 159–173.

Hefferon, K.L., Fan, Y., 2004. Expression of a vaccine protein in a plant cell line using a geminivirus-based replicon system. Vaccine 23 (3), 404–410.
Huang, Z., LePore, K., Elkin, G., et al., 2008. High-yield rapid production of hepatitis B surface antigen in plant leaf by a viral expression system. Plant Biotechnol. J. 6 (2), 202–209.
Huang, Z., Chen, Q., Hjelm, B., et al., 2009. A DNA replicon system for rapid high-level production of virus-like particles in plants. Biotechnol. Bioeng. 103 (4), 706–714.
Huang, Z., Phoolcharoen, W., Lai, H., et al., 2010. High-level rapid production of full-size monoclonal antibodies in plants by a single-vector DNA replicon system. Biotechnol. Bioeng. 106 (1), 9–17.
Kim, K.I., Sunter, G., Bisaro, D.M., et al., 2007. Improved expression of recombinant GFP using a replicating vector based on Beet curly top virus in leaf-disks and infiltrated Nicotiana benthamiana leaves. Plant Mol. Biol. 64 (1–2), 103–112.
Koprowski, H., Yusibov, V., 2001. The green revolution: plants as heterologous expression vectors. Vaccine 19, 2735–2741.
Kumangai, M.H., Turpen, T.H., Weizenttl, N., et al., 1993. Rapid, high-level expression of biologically active alpha-trichosanthin in transfected plants by an RNA viral vector. Proc. Natl. Acad. Sci. U.S.A 90 (2), 427–430.
Lim, H.S., Vaira, A.M., Domier, L.L., et al., 2010. Efficiency of VIGS and gene expression in a novel bipartite potexvirus vector delivery system as a function of strength of TGB1 silencing suppression. Virology 402 (1), 149–163.
Lindbo, J.A., 2007a. High-efficiency protein expression in plants from agroinfection-compatible Tobacco mosaic virus expression vectors. BMC Biotechnol. 7, 52.
Lindbo, J.A., 2007b. TRBO: a high-efficiency tobacco mosaic virus RNA-based overexpression vector. Plant Physiol. 145 (4), 1232–1240.
Lomonossoff, G.P., Hamilton, W.D., 1999. Cowpea mosaic virus-based vaccines. Curr. Top. Microbiol. Immunol. 240, 177–189.
Mallory, A.C., Parks, G., Endres, M.W., et al., 2002. The amplicon-plus system for high-level expression of transgenes in plants. Nat. Biotechnol. 20 (6), 622–625.
Matic, S., Rinaldi, R., Masenga, V., et al., 2011. Efficient production of chimeric Human papillomavirus 16 L1 protein bearing the M2e influenza epitope in Nicotiana benthamiana plants. BMC Biotechnol. 11 (1), 106.
Massa, S., Franconi, R., Brandi, R., et al., 2007. Anti-cancer activity of plant-produced HPV16 E7 vaccine. Vaccine 25 (16), 3018–3021.
McCormick, A.A., Reddy, S., Reinl, S.J., et al., 2008. Plant-produced idiotype vaccines for the treatment of non-Hodgkin's lymphoma: safety and immunogenicity in a phase I clinical study. Proc. Natl. Acad. Sci. U.S.A. 105 (29), 10131–10136.
Mett, V., Musiychuk, K., Bi, H., et al., 2008. A plant-produced influenza subunit vaccine protects ferrets against virus challenge. Influenza Other Respir. Viruses 2 (1), 33–40.
Molina, A., Hervás-Stubbs, S., Daniell, H., et al., 2004. High-yield expression of a viral peptide animal vaccine in transgenic tobacco chloroplasts. Plant Biotechnol. J. 2, 141–153.
Musiychuk, K., Stephenson, N., Bi, H., et al., 2007. A launch vector for the production of vaccine antigens in plants. Influenza Other Respir. Viruses 1 (1), 19–25.
Noris, E., Poli, A., Cojoca, R., et al., 2011. A human papillomavirus 8 E7 protein produced in plants is able to trigger the mouse immune system and delay the development of skin lesions. Arch. Virol. 156 (4), 587–595.
Paul, M., Ma, J.K., 2011. Plant-made pharmaceuticals: leading products and production platforms. Biotechnol. Appl. Biochem. 58 (1), 58–67.
Penney, C.A., Thomas, D.R., Deen, S.S., et al., 2011. Plant-made vaccines in support of the Millennium Development Goals. Plant Cell Rep. 30 (5), 789–798.
Péres-Filgueira, D.M., Zamorano, P.I., Dominguez, M.G., et al., 2003. Bovine herpes virus gD protein produced in plants using a recombinant tobacco mosaic virus (TMV) vector possesses authentic antigenicity. Vaccine 21, 4201–4204.
Regnard, G.L., Halley-Stott, R.P., Tanzer, F.L., et al., 2010. High level protein expression in plants through the use of a novel autonomously replicating geminivirus shuttle vector. Plant Biotechnol. J. 8 (1), 38–46.
Rybicki, E.P., 2009. Plant-produced vaccines: promise and reality. Drug Discov. Today 14 (1–2), 16–24.
Rybicki, E.P., 2010. Plant-made vaccines for humans and animals. Plant Biotechnol. J. 8 (5), 620–637.
Saejung, W., Fujiyama, K., Takasaki, T., et al., 2007. Production of dengue 2 envelope domain III in plant using TMV-based vector system. Vaccine 25 (36), 6646–6654.
Sainsbury, F., Lomonossoff, G.P., 2008. Extremely high-level and rapid transient protein production in plants without the use of viral replication. Plant Physiol. 148 (3), 1212–1218.
Sainsbury, F., Sack, M., Stadlmann, J., et al., 2010a. Rapid transient production in plants by replicating and non-replicating vectors yields high quality functional anti-HIV antibody. PLoS One 5 (11), e13976.
Sainsbury, F., Cañizares, M.C., Lomonossoff, G.P., 2010b. Cowpea mosaic virus: the plant virus-based biotechnology workhorse. Annu. Rev. Phytopathol. 48, 437–455.
Santi, L., Giritch, A., Roy, C.J., et al., 2006. Protection conferred by recombinant Yersinia pestis antigens produced by a rapid and highly scalable plant expression system. Proc. Natl Acad. Sci. U.S.A 103 (4), 861–866.
Santi, L., Batchelor, L., Huang, Z., et al., 2008. An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles. Vaccine 26 (15), 1846–1854.
Sparrow, P.A., Irwin, J.A., Dale, P.J., et al., 2007. Pharma-Planta: road testing the developing regulatory guidelines for plant-made pharmaceuticals. Transgenic Res. 16 (2), 147–161.
Timmermans, M.C.P., Das, O.P., Messing, J., 1994. Geminiviruses and Their Uses as Extrachromosomal Replicons. Annu. Rev. Plant Phys. Plant Mol. Bio. 45, 79–112.
Turpen, T.H., Reinl, S.J., Charoenvit, Y., et al., 1995. Malarial epitopes expressed on the surface of recombinant tobacco mosaic virus. Biotechnology (N.Y.) 13 (1), 53–57.
Wagner, B., Fuchs, H., Adhami, F., et al., 2004. Plant virus expression systems for transient production of recombinant allergens in Nicotiana benthamiana. Methods 32, 227–234.
Webster, D.E., Wang, L., Mulcair, M., et al., 2009. Production and characterization of an orally immunogenic Plasmodium antigen in plants using a virus-based expression system. Plant Biotechnol. J. 7 (9), 846–855.
Wigdorovitz, A., Carrillo, C., DusSantos, M.J., et al., 1999. Induction of A protective antibody response to foot and mouth disease virus in mice following oral or parenteral immunization with alfalfa transgenic plants expressing the viral structural protein VP1. Virology 255 (2), 347–353.
Yusibov, V., Shivprasad, S., Turpen, T.H., et al., 1999. Plant viral vectors based on tobamoviruses. Curr. Top. Microbiol. Immunol. 240, 81–94.
Yusibov, V., Streatfield, S.J., Kushnir, N., 2011. Clinical development of plant-produced recombinant pharmaceuticals: vaccines, antibodies and beyond. Hum. Vaccin. 7 (3), 313–321.