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(54) **Title**: CLOSTEROVIRUS-BASED NUCLEIC ACID MOLECULES AND USES THEREOF

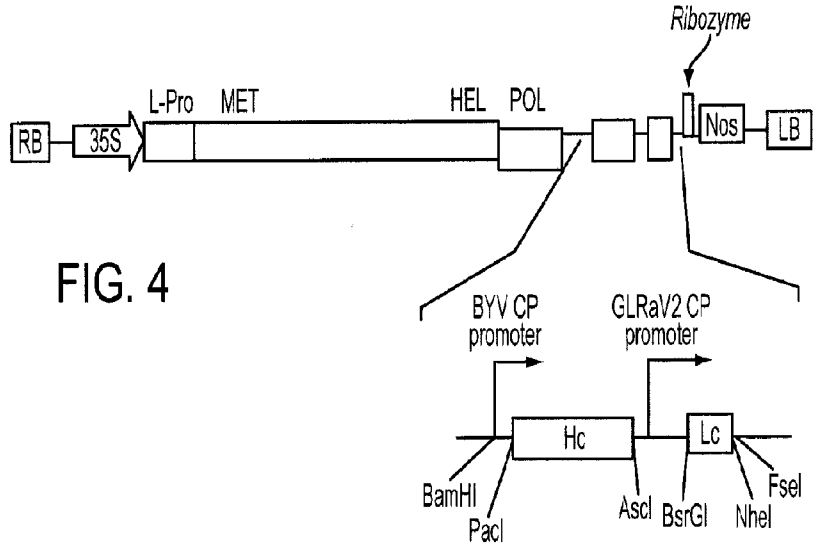

FIG. 4

(57) **Abstract**: The present invention relates to novel nucleic acid molecules for producing target polypeptides in plant cells. More specifically, the novel nucleic acid molecules comprise a minireplicon derived from a Closteroviridae virus and heterologous polynucleotides encoding the target polypeptides. Also provided are compositions comprising the target polypeptides and uses thereof.

WO 2012/048221 A1

CLOSTEROVIRUS-BASED NUCLEIC ACID MOLECULES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/391,333, filed October 8, 2010, the contents of which are incorporated herein by reference in their entireties for all purposes.

FIELD OF THE INVENTION

The present invention relates generally to novel nucleic acid molecules for producing target polypeptides in plant cells. More specifically, the nucleic acid molecules comprise a minireplicon derived from a *Closteroviridae* virus and polynucleotides encoding the target polypeptides.

BACKGROUND OF THE INVENTION

Production of recombinant proteins, including monoclonal antibodies (mAbs), vaccine antigens, enzymes, dual vaccines, fusion molecules and virus-like particles (VLPs), using plant viral vectors is an attractive alternative to traditional mammalian cell-based expression systems. Due to the high speed of virus replication, plant viral vectors have the potential to rapidly produce large quantities of foreign proteins.

A tobacco mosaic virus (TMV)-based vector is the most widely used vector for transient expression of plant-produced subunit vaccines and therapeutic proteins (Streatfield; Yusibov at al.; Gleba at al.; Rybicki). However, expression of high molecular weight proteins or co-expression of multiple polypeptide chains or proteins using TMV vectors is challenging. There remains a need for improved plant viral vectors for producing single large target proteins as well as simultaneously producing multiple target proteins in plants.

SUMMARY OF THE INVENTION

The disclosed subject matter of the present invention relates to novel nucleic acid molecules for producing target polypeptides in plant cells.

According to one aspect of the present invention, an isolated nucleic acid molecule for producing one or more target polypeptides in a plant cell is provided. The nucleic acid comprises a minireplicon derived from a *Closteroviridae* virus and one or more heterologous polynucleotides. The nucleic acid molecule is capable of replicating in the plant cell. The one or more heterologous polynucleotides encode the one or more target polypeptides. The nucleic acid molecule may further comprise a polynucleotide encoding one or more movement proteins derived from the *Closteroviridae* virus. The *Closteroviridae* virus may be *Beet yellows virus*.

The plant cell may be in a plant, a plant part, or a cell culture medium. The plant may be a whole growing plant. The plant part may be selected from the group consisting of leaves, stems, roots, floral tissues, seeds and petioles.

In one embodiment, the one or more target polypeptides comprise one or more subunits of a protein, and are capable of forming the protein in the plant cell. The protein may be an enzyme.

In another embodiment, the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and the first polypeptide is capable of modifying the second polypeptide in the plant cell.

In yet another embodiment, the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and the first polypeptide is capable of affecting expression of the second polypeptide in the plant cell. The first polypeptide may be a silencing suppressor.

In a further embodiment, the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and the first polypeptide is capable of increasing production of the second polypeptide in the plant cell.

In yet a further embodiment, the one or more target polypeptides comprise a heavy chain and a light chain of an antibody, and are capable of forming the antibody in the plant cell.

The one or more target polypeptides may comprise an immunogenic polypeptide. The one or more target polypeptides comprise a polypeptide of at least 100 kD.

For each nucleic acid molecule of the present invention, a vector comprising the nucleic acid molecule is provided.

According to another aspect of the present invention, a method for producing one or more target polypeptides in a plant cell is provided. The method comprises (a) introducing a nucleic acid molecule into the plant cell; and (b) maintaining the plant cell under conditions permitting production of the one or more target polypeptides in the plant cell. The nucleic acid molecule comprises a minireplicon derived from a *Closteroviridae* virus and one or more heterologous polynucleotides. The nucleic acid molecule is capable of replicating in the plant cell. The one or more heterologous polynucleotides encode the one or more target polypeptides. The one or more target polypeptides comprise a polypeptide of at least 100 kD. The method may further comprise purifying at least one of the one or more target polypeptides from the plant cell. The nucleic acid molecule may further comprise a polynucleotide encoding one or more movement proteins derived from the *Closteroviridae* virus. The *Closteroviridae* virus may be *Beet yellows virus*.

In the production method of the present invention, the plant cell may be in a plant, a plant part, or a cell culture medium. The plant may be a whole growing plant. The plant part may be selected from the group consisting of leaves, stems, roots, floral tissues, seeds and petioles.

A composition comprising at least one of the one or more target polypeptides produced by the method of the present invention is provided. A method of treating a subject in need of at least one of the one or more target polypeptides produced thereby is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the at least one of the one or more target polypeptides.

In one embodiment, the one or more target polypeptides comprise one or more subunits of a protein. In the production method, the maintaining conditions further permit production of the protein in the plant cell. A composition comprising the protein produced thereby is provided. A method of treating a subject in need of the protein is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the protein. The protein may be an enzyme.

In another embodiment, the one or more target polypeptides comprise a first polypeptide and a second polypeptide. In the production method, the maintaining conditions further permit modifying, affecting expression, and/or increasing production of the second polypeptide by the first polypeptide in the plant cell. A composition comprising the second polypeptide is provided. A method of treating a subject in need of the second polypeptide is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the second polypeptide. The first polypeptide may be a silencing suppressor.

In yet another embodiment, the one or more target polypeptides comprise a heavy chain and a light chain of an antibody. In the production method, the maintaining conditions further permit production of the antibody in the plant cell. A composition comprising the antibody is provided. A method of treating a subject in need of the antibody is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the antibody.

In a further embodiment, the one or more target polypeptides comprise an immunogenic polypeptide. A composition comprising the immunogenic polypeptide produced thereby is provided. A method for inducing an immune response in a subject is also provided. The induction method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the immunogenic polypeptide. A method for inducing a protective immune response against a pathogen

in a subject is further provided. The protective induction method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the immunogenic polypeptide. The immunogenic polypeptide is derived from the pathogen.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram illustrating the organization of the BYV genome. L-Pro – papain-like leader proteinase; Met, Hel, and Pol – methyltransferase, RNA helicase, and RNA-dependent RNA polymerase domains of the replicase, respectively; p6 – 6 kD protein; Hsp70h – a Hsp70 homolog; p64 – 64 kD protein; CPm and CP – minor and major capsid proteins, respectively; p20 and p21 – 20 and 21 kD proteins, respectively.

Figure 2 is a diagram illustrating a T-DNA region of a BYV-based launch vector encoding Hc and Lc of an anti-PA antibody. 35S – *Cauliflower mosaic virus* 35S promoter; NOS – *Nopaline synthase* terminator; LB and RB – left and right borders of T-DNA, respectively.

Figure 3A shows *N. benthamiana* leaves systematically infected with the BYV-based launch vector encoding Hc and Lc of an anti-PA antibody. Figure 3B shows Western blot analysis of Hc, Lc and total anti-PA IgG expression in the systematically infected *N. benthamiana* leaves at 30, 32, 34, 36 and 39 days post infiltration (dpi). The maximum expression of total anti-PA IgG was observed on day 34, with 53 mg/kg of fresh leaf weight. *Standards, ng of total human IgG.

Figure 4 is a diagram illustrating a T-DNA region of a T-DNA-based BYV minireplicon vector for expression of Hc and Lc of an anti-PA antibody. The Hc and Lc genes are under the control of the BYV CP promoter and the GLRaV2 CP promoter, respectively.

Figure 5 is a diagram illustrating a T-DNA region of a T-DNA-based BYV minireplicon vector for expression of Hc and Lc of the anti-PA antibody. The Hc and Lc genes are under the control of the BYV CP promoter and BYSV CP promoter, respectively.

Figure 6A-B shows Western blot analysis of Hc and Lc expression in *N. benthamiana* leaves systematically infected by a modified miniBYV vector at 5 and 7 dpi, respectively. Figure 6C shows calculated amounts of Lc and Hc expression in the systematically infected *N. benthamiana* leaves at 5, 7 and 9 dpi. Lanes: 100, 50 and 25 (ng) of the human mAb standard. Q3, #1, Q3, #2, Q3, #3, Q4, #1, Q4, #2, Q4, #3 – different clones of the miniBYV replicon carrying Hc and Lc of the anti-PA mAb.

Figure 7 is a diagram illustrating a T-DNA region of a miniBYV replicon vector for expression of the anthrax Protective Antigen 83 (PA83) under the control of the BYV promoter.

Figure 8A shows Western blot analysis of PA83 expression in systematically infected *N. benthamiana* leaves at 5, 7 and 9 dpi. Figure 8B shows the amounts of total protein (TP) and total soluble protein (TSP) expression in the systematically infected *N. benthamiana* leaves at 5, 7 and 9 dpi. Lanes 1-9: 1, PA standard, 50 ng; 2, PA standard, 25 ng; 3, PA standard, 10 ng; 4, pCB-miniBYV-PA83, TP; 5, pCB-miniBYV-PA83, TSP; 6, pGR-D4-PA83, TP*; 7, pGR-D4-PA83, TSP*; 8, pClean 238-PA83, TP; 9, pClean238-PA83, TSP. * Without a silencing suppressor P1HcPro.

Figure 9 is a diagram illustrating a T-DNA region of a miniBYV replicon vector for co-expression of target protein Pfs48 and an enzyme capable of modifying the target protein.

Figure 10 shows co-expression of target protein Pfs48 and an enzyme PNGaseF capable of deglycosylating target protein Pfs48. Detection was made using anti-His antibody (A) and anti-FLAG antibody (B), respectively.

Figure 11 is a diagram illustrating a T-DNA region of a miniBYV replicon vector for expression of three open reading frames (ORFs) for three different targets using a combination of strong and weak closteroviral promoters (i.e., BYV CP promoter, GLRaV2 CP promoter, and BYSV CP promoter).

Figure 12A-H shows the nucleic acid sequence of a T-DNA region of a BYV launch vector (SEQ ID NO: 1) according to some embodiments of the disclosed subject matter. A BYV sequence (upper case) with multiple cloning sites (bold) along with the 35S promoter (lower case) and the NOS terminator (lower case italic) are introduced between the left border (LB) and right border (RB) of the T-DNA sequence (underline). The multiple cloning sites (bold) include PacI (14,254-14,261 bp), AscI (14,262-14,269), BsrGI (14,270-14,275), NheI (14,276-14,281) and FseI (14,285-14,292).

Figure 13A-E shows the nucleic acid sequence of a T-DNA region of a miniBYV launch vector (SEQ ID NO: 2) according to some embodiments of the disclosed subject matter. A miniBYV sequence (upper case) with multiple cloning sites (bold) along with the 35S promoter with a dual enhancer (lower case) and the NOS terminator (lower case italic) are introduced between the left border (LB) and right border (RB) of the T-DNA sequence (underline). The multiple cloning sites (bold) include BamHI (10,278-10,285), PacI (10,286-10,293), AscI (10,294-10,301), BsrGI (10,302-10,307), NheI (10,308-10,313) and FseI (10,317-10,324).

Figure 14 shows the nucleic acid sequences of (A) BYV CP promoter (SEQ ID NO: 3), (B) BYSV CP promoter (SEQ ID NO: 4), and (C) GLRaV2 CP promoter (SEQ ID NO: 5).

Figure 15 shows (A) an amino acid sequence of Lc of an anti-PA antibody with the PR1a signal peptide from *Nicotiana tavacum* on the N-terminus (underline) and (SEQ ID NO: 6), and (B) a corresponding nucleic acid sequence with a TGA stop codon (underline) (SEQ ID NO: 7).

Figure 16 shows (A) an amino acid sequence of Hc of an anti-PA antibody with the PR1a signal peptide from *Nicotiana tavacum* on the N-terminus (underline) (SEQ ID NO: 8) and (B) a corresponding nucleic acid sequence with a TGA stop codon (underline) (SEQ ID NO: 9).

Figure 17 shows (A) an amino acid sequence of PA83 with the PR1a signal peptide from *Nicotiana tavacum* on the N-terminus (underline) and 6His (bold) for purification and KDEL (italic) as an ER retention signal on the C-terminus (SEQ ID NO: 10), and (B) a corresponding nucleic acid sequence with a TGA stop codon (underline) (SEQ ID NO: 11).

Figure 18 shows (A) an amino acid sequence of Pfs48 with the PR1a signal peptide from *Nicotiana tavacum* on the N-terminus (underline) and 6His (bold) for purification and KDEL (italic) as an ER retention signal on the C-terminus (SEQ ID NO: 12) and (B) a corresponding nucleic acid sequence with a TGA stop codon (underline) (SEQ ID NO: 13).

Figure 19 shows (A) an amino acid sequence of PNGaseF with the PR1a signal peptide from *Nicotiana tavacum* on the N-terminus (underline) and a FLAG tag (bold) for detection and KDEL (italic) as an ER retention signal on the C-terminus (SEQ ID NO: 14) and (B) a corresponding nucleic acid sequence with a TGA stop codon (underline) (SEQ ID NO: 15).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that novel nucleic acid molecules comprising a *Beet yellows virus* (BYV) minireplicon can be used to produce a single or multiple heterologous target polypeptides in a plant cell. The BYV minireplicon may be used for production of therapeutically active proteins, subunit vaccines, protein adjuvants, enzymes, monoclonal antibodies (mAbs) and virus-like particles (VLP).

BYV is a member of the alphavirus supergroup of positive-strand RNA viruses belonging to the genus *Closterovirus*, family *Closteroviridae.* The 15.5 kb monopartite genome of BYV encodes 8 open reading frames (ORFs) (Fig. 1). Three groups of proteins are recognized in the BYV genome. The first group of proteins is responsible for

virus replication, and includes methyltransferase (Met), helicase (Hel), and RNA polymerase (Pol) (ORF 1A and 1B). The second group of proteins is responsible for the virus cell-to-cell movement (ORFs 2-6), and includes P6, HSP70h, CP, CPm and p64. The knockout of any one of these proteins results in an arrest of the virus cell-to-cell movement. The third group of proteins includes viral structural components such as Hsp70H, CP, CPm, p64 and p20 (ORFs 3-7). p20 also known as the viral long distance transport factor. p21- the BYV silencing suppressor (ORF 8).

BYV contains a replication gene block which covers more than 50% of the BYV genome and includes genes necessary for BYV replication. The BYV replication gene block is formed by the domain of papain-like leader proteinase (L-Pro), methyltransferase (Met), helicase-like domain region of viral replicase (Hel), and RNA-depended RNA polymerase (Pol). RNA-depended RNA polymerase is expressed from +1 frameshift. A larger replication protein which contains methyltransferase, helicase, and polymerase is produced in smaller quantities compared to the methyltransferase-helicase polyprotein due to the low frequency of frameshifting (Fig. 1). Flexious BYV virions are ~1300 nm in length and ~12 nm in diameter, and contain five structural proteins. The major capsid protein (CP) encapsidates ~95% of the virion body. A short virion tail which is necessary for the BYV cell-to-cell and systemic movement contains minor CP (CPm); Hsp70h, a homolog of cellular heat shock proteins; p64, a 64 kD protein with unknown functions; and p20, a long distance transport factor. Other proteins of BYV are p6, a small transmembrane protein required for BYV cell-to-cell movement and localized in the endoplasmic reticulum of host cell; and p21, a BYV silencing suppressor involved in binding of short interfering RNA.

The term "protein" used herein refers to a biological molecule comprising amino acid residues. A protein may comprise one or more polypeptides. Each polypeptide may be a subunit of protein. For example, the protein may be an antibody consisting of two Hc and two Lc. The protein may be in a native or modified form, and may exhibit a biological function when its polypeptide or polypeptides are properly folded or assembled.

The term "polypeptide" used herein refers to a polymer of amino acid residues with no limitation with respect to the minimum length of the polymer. Preferably, the polypeptide has at least 20 amino acids. A polypeptide may be a full-length protein, or a fragment or variant thereof.

The term "fragment" of a protein as used herein refers to a polypeptide having an amino acid sequence that is the same as a part, but not all, of the amino acid sequence of the protein. Preferably, a fragment of a protein retains the same function as the protein.

The term "variant" of a protein as used herein refers to a polypeptide having an amino acid that is the same as the amino acid sequence of the protein except having at least one modification, for example, glycosylation, phosphorylation, a deletion, an addition or a substitution. The variant may have an amino acid at least about 80%, 90%, 95%, or 99%, preferably at least about 90%, more preferably at least about 95%, identical to the amino acid sequence of the protein. Preferably, a variant of a protein retains the same function as the protein.

The term "derived from" used herein refers to the origin or source, and may include naturally occurring, recombinant, unpurified or purified molecules.

According to one aspect of the present invention, a nucleic acid molecule for producing one or more target polypeptides in a plant cell is provided. The nucleic acid molecule comprises a minireplicon derived from a *Closteroviridae* virus and one or more heterologous polynucleotides, and is capable of replicating in the plant cell. The one or more heterologous polynucleotides encode the one or more target polypeptides.

A *Closteroviridae* virus may be any virus in the family of *Closteroviridae*. For example, the *Closteroviridae* virus may be *Beet Yellows virus*, *Grapevine leafroll-associated virus* 2 (GLRaV2), *Beet yellows stunt virus* (BYSV), *Citrus tristeza virus* (CTV), *Carrot yellow leaf virus* (CYLV), or *Lettuce infectious yellows* virus *(LIYV)*. Preferably, the *Closteroviridae* virus is *Beet yellows virus*.

A plant cell may be a cell in any plants, plant parts (e.g., leaves, stems, roots, floral tissues, seeds and petioles) or cell culture media. The plant may be a whole growing plant. The cell culture media may be any media suitable for growing plant cells, preferably in suspension. The plant cell is preferably susceptible to infection by a *Closteroviridae* virus. More preferably, the plant cell is susceptible to BYV infection. The plant cell is preferably suitable for expression of a target polypeptide. For example, the plant cell may be cells in *N. benthamiana* leaves. Other suitable plants include *Nicotiana clevelandii, Beta vulgaris, Spinacia oleracea*, *Brassica* spp, *Lactuca sativa*, *Pisum sativum*, *Nicotiana tabacum*, *Plantago lanceolata*, *Tetragonia tetragonioides*, *Montia perfoliata*, *Beta vulgari*, *Spinacia oleracea*, *Stellaria media*, *Brassica* spp., *Lactuca sativa*, *Pisum sativum*, *Nicotiana tabacum*, *Plantago lanceolata*, *Montia perfoliata*, *Tetragonia tetragonioides*, *Chenopodium foliosum*, and *Nicotiana benthamiana*.

A minireplicon derived from a *Closteroviridae* virus is a polynucleotide, comprising a nucleic acid sequence encoding only proteins, each of which corresponds to a natural viral replication protein of the *Closteroviridae* virus required for replication of the virus. Each encoded protein exhibits the same function as its corresponding natural viral replication protein, and may have an amino acid sequence at least about 80%, 85%, 90%, 95%, or 99%, preferably at least about 95%, more preferably at least about 99%,

most preferably 100%, identical to that of its corresponding natural viral replication protein. The minireplicon may be generated from the genome of the *Closteroviridae* virus by deleting nucleic acid sequences, including genes, not required for the replication of the virus. For example, a BYV minireplicon may be a replication gene block formed by the L-Pro domain, methyltransferase (Met), helicase (Hel) and RNA-dependent RNA polymerase (Pol) as shown in Fig. 1.

For each nucleic acid molecule of the present invention, a vector comprising the nucleic acid is provided. The vector may include border sequences of a bacterial transfer DNA at either end, and be situated in a bacterial transfer DNA, to allow for delivery of the nucleic acid of the present invention into a plant cell. Specifically, the vector may comprise one or more nucleic acid sequences derived from a Ti plasmid of a binary vector (e.g., right border (RB) and left border (LB) in Fig. 2). Such a vector, including elements of a Ti plasmid and a viral vector, is also called a launch vector. This vector may also be used for co-expression of a target polynucleotide of interest with a protein, such as a silencing suppressor or a modifying enzyme such as PNGaseF, to modify, affect expression and/or increase production the target polypeptide. The protein may facilitate maturation or accumulation of the target polypeptide.

A heterologous polynucleotide is a polynucleotide that is foreign, not native, to the *Closteroviridae* virus and the target cell. It may comprise a nucleic acid sequence encoding a target polypeptide, which may be expressed in a plant cell.

In the nucleic acid molecule of the present invention, each heterologous polynucleotide encodes a target polypeptide, and may be operatively linked to a viral promoter derived from any virus in the family *Closteroviridae*. Examples of suitable viral promoters include BYV promoters, *Grapevine leafroll-associated* virus 2 (GLRaV2) promoters, and/or *Beet Yellows stunt* virus (BYSV) promoters. Preferably, polynucleotides in the same nucleic acid molecule are operably linked to different viral promoters. Exemplary viral promoters include BYV CP promoter (SEQ ID NO: 3; Fig. 14), BYSV CP promoter (SEQ ID NO: 4; Fig. 14), and GLRaV2 CP promoter (SEQ ID NO: 5; Fig. 14).

The nucleic acid molecule of the present invention may further comprise a polynucleotide encoding one or more movement proteins derived from the *Closteroviridae* virus. Examples of the movement proteins include p6, Hsp70h, p64, CPm, CP and p20 of BYV. The movement proteins may enhance the movement of the nucleic acid molecule from one plant cell to another and cause systemic spread of the nucleic acid molecule, thereby increasing plant production level of the target polypeptide encoded by the heterologous polynucleotide by, for example, at least about 1%, 5%,

10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 200%, 500% or 1000%.

The nucleic acid molecule of the present invention may comprise one (e.g., Fig. 7), two (e.g., Figs. 2, 4, 5, and 9), three (e.g., Fig. 11) or more heterologous polynucleotides, each of which encodes a target polypeptide. Preferably, the nucleic acid molecule comprises two or more heterologous polynucleotides encoding two or more target polypeptides, and the target polypeptides are expressed from the same minireplicon of the nucleic acid molecule within a plant cell. The target polypeptide may constitute a subunit of a protein. The target polypeptides may be capable of forming a protein such as an enzyme or antibody. For example, the nucleic acid molecule may comprise two heterologous polynucleotides encoding Hc (Fig. 16) and Lc (Fig. 15) of an antibody. In some embodiments, the nucleic acid may encode two target polypeptides, in which a first target polypeptide is capable of modifying, affecting expression and/or increasing production of a second target polypeptide in a plant cell. The first target polypeptide may facilitate maturation of the second polypeptide, which becomes biologically active. The first target polypeptide may also facilitate accumulation of the second polypeptide.

A target polypeptide may be any polypeptide capable of forming or becoming a functional protein (e.g., an enzyme or antibody) or a vaccine candidate. A target polypeptide may be of any size. It may have at least about 6, 10, 50, 100, 200, 300, 400, 500, 750, or 1000 amino acids, preferably at least about 100 amino acids, more preferably at least about 500 amino acids, most preferably at least 750 amino acids. It may also be at least about 10, 20, 50, 75, 100, 125, 150, or 200 kD, preferably at least about 100 kD, more preferably at least about 125 kD, most preferably at least about 150 kD.

A target polypeptide may be immunogenic. It may comprise one or more epitopes (linear and/or conformational) that are capable of stimulating the immune system of a subject to make a humoral and/or cellular antigen-specific immune response. A humoral immune response refers to an immune response mediated by antibodies produced by B lymphocytes, or B cells, while a cellular immune response refers to an immune response mediated by T lymphocytes, or T cells, and/or other white blood cells. In general, a B-cell epitope contains at least about 5 amino acids but can be 3-4 amino acids, while a T-cell epitope includes at least about 7-9 amino acids and a helper T-cell epitope includes at least 12-20 amino acids. A target polypeptide may be derived from a protein (e.g., a surface protein or toxin subunit) of a pathogenic organism or pathogen.

A "subject" may be an animal. For example, the animal may be an agricultural animal (e.g., horse, cow and chicken) or a pet (e.g., dog and cat). Preferably, the subject is a mammal. Most preferably, the subject is a human. The subject may be a male or female. The subject may also be a newborn, child or adult. The subject may have suffered a disease or medical condition.

For each of the nucleic acid molecules of the present invention, a method for producing one, two, three or more target polypeptides in a plant cell is provided. The method comprises (a) introducing the nucleic acid molecule into a plant cell; and (b) maintaining the plant cell under conditions permitting production of the target polypeptide(s) in the plant cell. The nucleic acid molecule comprises a minireplicon derived from a *Closteroviridae* virus, and is capable of replicating in the plant cell. The nucleic acid molecule further comprises one, two, three or more heterologous polynucleotides, each of which encodes a target polypeptide. The plant cell may be a cell in a plant, a plant part (e.g., leaf, stem, root, floral tissue, seed or petiole) or a cell culture medium. The plant may be a whole growing plant. Preferably, the plant cell is in a plant leaf.

The nucleic acid molecule of the present invention may be introduced into a plant cell using techniques known in the art. For example, the nucleic acid molecule may be delivered into the plant cell via infiltration, bombardment, or manual inoculation. The nucleic acid molecule could be used as a part of an inducible system activated by, for example, chemical, light or heat shock. Preferably, the nucleic acid molecule is introduced into a plant cell via infiltration.

For production of a target polypeptide by a plant or plant cells infected by a vector of the present invention, the infected plant or plant cells are maintained under conditions permitting for the production. Such conditions include suitable temperature, humidity, pressure, timing, and illumination. As described below in Examples 2 and 4-6, nucleic acid molecules of the present invention have been introduced into plant cells, which were maintained under conditions permitting production of one or two polypeptides, and such production was observed. The production method may further comprise purifying at least one of the target polypeptide(s) from the plant. The target polypeptide may be purified from the plant using techniques known in the art. For example, the target polypeptide may be purified from the plant using an antibody or a receptor capable of binding the target polypeptide. The purification process may comprise extraction of the target from *N. benthamiana* using extraction buffer. After low speed centrifugation, supernatant may be clarified by filtration and used for chromatography. The purified product may be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99%, preferably at least about 50%, more

preferably at least about 75%, most preferably at least about 95%, pure. The target polypeptide may be used in a crude plant extract. For example, an industrial enzyme expressed in plant tissues according to the present invention, and a crude plant extract containing the enzyme may be used in an industrial process.

In the production method according to the present invention, the *Closteroviridae* virus may be any virus in the family of *Closteroviridae*. Examples of the *Closteroviridae* virus include BYV, *Grapevine leafroll-associated* virus 2 (GLRaV2), and *Beet Yellows stunt* virus (BYSV). Preferably, the *Closteroviridae* virus is BYV. The vector may further comprise a polynucleotide encoding one or more movement proteins derived from the *Closteroviridae* virus. The movement proteins may enhance movement of the heterologous polynucleotide from one plant cell to another plant cell, and thereby increase plant production level of the target polypeptide encoded by the heterologous polynucleotide by, for example, at least about 1%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 200%, 500% or 1000%.

For each production method of the present invention, a composition comprising the one, two, three or more target polypeptides produced thereby is provided. Also provided is a method of treating a subject in need of the one, two, three or more target polypeptides. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the target polypeptide(s). The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier or diluents. Suitable carriers or diluents are known in the art and include, but are not limited to, saline, buffered saline, mannitol, L-histidine, polysorbate 80, dextrose, water, glycerol, ethanol, and combinations thereof. The pharmaceutical composition may optionally contain an adjuvant. The pharmaceutical composition may have a pH of about 4.0-10.0, preferably 5.6-7.0.

The term "an effective amount" refers to an amount of a pharmaceutical composition comprising the target polypeptide(s) required to achieve a stated goal (e.g., treating a subject in need of the target polypeptide(s), or inducing an immune response in a subject). The effective amount of the pharmaceutical composition comprising the target polypeptide(s) may vary depending upon the stated goal, the physical characteristics of the subject, the nature and severity of the need of the target polypeptide(s), the existence of related or unrelated medical conditions, the nature of the target polypeptide(s), the composition comprising the target polypeptide(s), the means of administering the composition to the subject, and the administration route. A specific dose for a given subject may generally be set by the judgment of a physician. The pharmaceutical composition may be administered to the subject in one or multiple doses.

The target polypeptide(s) may be formulated in a pharmaceutical composition of the present invention. The pharmaceutical composition may be formulated for administration to a subject via various routes, for example, oral, sublingual, intranasal, intraocular, rectal, transdermal, mucosal, topical or parenteral administration.

In a production method according to the present invention, the one, two, three or more target polypeptides may be one or more subunits of a protein, and the maintaining conditions may further permit production of the protein in the plant cell. A composition comprising the protein produced thereby is provided. A method of treating a subject in need of the protein is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the protein. The protein may be an enzyme.

In a production method according to the present invention, a first target polypeptide may be capable of modifying, affecting expression, and/or increasing production of a second target polypeptide in the plant cell, and the maintaining conditions may further permit modifying, affecting expression, and/or increasing production of the second target polypeptide by the first target polypeptide in the plant cell. A composition comprising the second target polypeptide produced thereby is provided. A method of treating a subject in need of the modified target polypeptide is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the polypeptide.

In a production method according to the present invention, the target polypeptides may be Hc and Lc of an antibody, and the maintaining conditions may further permit production of the antibody in the plant cell. A composition comprising the antibody produced thereby is provided. A method of treating a subject in need of the antibody is also provided. The treatment method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the antibody.

In a production method according to the present invention, the target polypeptide may be immunogenic. A composition comprising the immunogenic target polypeptide is provided. A method for inducing an immune response in a subject is also provided. The immunogenic method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the immunogenic target polypeptide. Where the immunogenic target polypeptide is derived from a pathogen, the immunogenic method may be used for inducing a protective immune response against the pathogen in a subject by administering to the subject an effective amount of a pharmaceutical composition comprising the immunogenic target polypeptide. The pathogen may be an intracellular or extracellular pathogen.

The term "about" as used herein when referring to a measurable value such as an amount, a percentage, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate.

All documents, books, manuals, papers, patents, published patent applications, guides, abstracts, and other references cited herein are incorporated by reference in their entirety. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Example 1. Construction of BYV-based launch vector for monoclonal antibody expression

A BYV-based launch vector for simultaneous expression of two target polypeptides within the same host cell was constructed (Fig. 2). The BYV launch vector was used as a carrier for expression of two ORFs, Hc and Lc of a mAb against PA of anthrax. For cloning of two foreign genes (Lc and Hc of the anti-PA mAb), a multiple cloning site (MCS) was introduced into the BYV genome between the CPm and CP coding sequences (SEQ ID NO: 1, Fig. 12). The MCS contains 5 restriction sites, PacI/AscI/BsrGI/NheI/FseI, in addition to the native BamHI restriction site. After inserting the MCS, two heterologous closteroviral CP promoters were introduced into the BYV genome: the GLRaV2 CP promoter and the BYSV CP promoter (Fig. 2). As a result, the sequences of Hc (SEQ ID NO: 9; Fig. 16) and Lc (SEQ ID NO: 7; Fig. 15) of the anti-PA mAb were cloned under the control of the BYV CP and the GLRaV2 CP promoters, respectively. Meanwhile, the BYSV CP promoter drives the BYV CP (Fig. 2). The resulting construct pCB-BYV-PA-HcLc was transformed into *Agrobacterium tumefaciens* strain GV3101.

Example 2. Expression of anti-PA mAb in systemically BYV-infected leaves

To confirm the stability of the virus and the expression and assembly of the anti-PA mAb, 5-week-old *N. benthamiana* leaves were manually co-infiltrated by overnight-grown (at 28 °C) cultures of agrobacteria carrying a BYV vector encoding Hc and Lc of the anti-PA mAb and agrobacteria carrying a binary vector encoding a silencing suppressor P1HcPro from *Turnip mosaic virus* (Kasschau et al, 2003), at a ratio of 1.0:0.2 OD_{600}. After 30 dpi, systemic symptoms of the BYV infection were observed (Fig. 3A). In particular, the infected leaves showed clearing veins as the systemic symptoms. Samples were collected from systemically infected leaves at 30, 32, 34, 36 and 39 dpi.

To demonstrate the expression of Lc and Hc of the anti-PA mAb, Western blot analysis was employed (Fig. 3B). To assess the Hc and Lc expression levels, horseradish peroxidase (HRP)-conjugated goat anti-human Hc and Lc antibodies (Bethyl Laboratories Inc.) were used at dilution of 1:5000 and 1:2000, respectively. A purified anti-PA mAb was used to serve as a positive control. The expression levels were calculated using GeneGnome5 gel imaging and analysis systems from Synoptics Ltd. Using the same technique under non-reducing conditions, the expression level of the assembled anti-PA mAb was calculated. The maximum expression level was determined to be 53 mg/kg of fresh leaf weight at 34 dpi.

Example 3. Construction of a T-DNA-based BYV minireplicon

To decrease the production time and increase the antibody expression level, a T-DNA-based BYV minireplicon (miniBYV) was engineered by removing all genes which are not necessary for viral replication from the BYV-based launch vector as described in Example 1 (Figs. 1, 2 and 4). Using the native BamHI restriction site, the same MCS as the one inserted into the whole-length BYV-based vector in Example 1 was introduced into the miniBYV replicon (SEQ ID NO: 2; Fig. 13) (Figs. 2 and 4). This strategy allowed for using heterologous closteroviral subgenomic promoters to express two foreign genes from a single miniBYV replicon. In addition, two closteroviral promoters, the BYV CP promoter and GLRaV2 CP promoter, were introduced to drive Hc (SEQ ID NO: 9; Fig. 16) and Lc (SEQ ID NO: 7; Fig. 15) of the anti-PA mAb, respectively.

To prevent splicing and increase the efficiency of viral invasiveness, the canonical splicing sites were removed from the viral replicase sequence. To increase the transcription level of miniBYV RNA, *Cauliflower mosaic virus* 35S promoter with a dual enhancer was inserted upstream of the 5' end of the miniBYV sequence (Fig. 4).

To increase the amount of the synthesized initial transcript, the 35S promoter with dual enhancers was introduced upstream of the miniBYV sequence to generate a modified miniBYV vector (Fig. 5). The weak GLRaV2 CP promoter was replaced by the strong BYSV CP promoter. The BYV replicase was analyzed for the presence of the canonical splicing sites using the SplicePredictor software from the Center for Bioinformatics and Biological Statistics, Iowa State University. *Arabidopsis* was used as a splicing site model. To avoid potential splicing, the high-scoring canonical acceptor splicing site within the BYV replicase was mutated by substituting the nucleotide 2219 (GenBank Accession No. AF 190581) from A to C, which was confirmed by sequencing. This also allowed for knocking out the donor site in the position 3606. The final size of T-DNA insert carrying miniBYV with Hc and Lc of the mAb was 13,746 bp (Fig. 5).

Example 4. Expression of anti-PA mAb in leaves systemically infected with the modified miniBYV vector (pCB-BYV-Hc-Lc)

To examine the expression level of Hc and Lc of the mAb from the modified miniBYV vector, 5-week-old *N. benthamiana* plants were infiltrated as described in Example 2 using two clones (Q3 and Q4, confirmed by sequencing) and three agro colonies were collected for each clone. To confirm that both Hc and Lc were expressed, the leaf disks were taken at 5, 7 and 9 dpi, analyzed by Western blotting and calculated as described above. The results demonstrate a three-fold increase in the expression level of Hc and Lc of the anti-PA mAb using the modified miniBYV launch vector carrying two strong promoters (Fig. 6) and suggest that the miniBYV vector can be used for antibody production.

Example 5. Expression of a large protein (PA83) using the miniBYV vector

To investigate a possibility of using the miniBYV vector for expressing large proteins, anthrax Protective Antigen 83 (PA83, GenBank accession no. M22589) with a molecular weight of 83 kD from *Bacillus anthracis* was used. The sequence of PA83 with added PR-1a signal peptide from *Nicotiana tabacum*, 6xHistidine affinity purification tag and an endoplasmic reticulum (ER) retention signal (KDEL) (SEQ ID NO: 11; Fig. 17) was plant optimized by GENEART Inc. (Germany) and cloned into the miniBYV vector using PacI/NheI restriction sites (Fig. 7). The final construct was confirmed by sequencing. A binary vector carrying the miniBYV-PA83 vector was transformed into the GV3101 strain of agrobacteria. The expression level of PA83 from the miniBYV was compared to other vectors such as TMV-based launch vector pGR-D4-PA83 and a regular binary vector pClean283-PA83 carrying a dual 35S promoter with a TEV leader. Five-month-old *N. benthamiana* leaves were manually infiltrated as described above.

The total protein (TP) and total soluble protein (TSP) expression levels at 5, 7 and 9 dpi were analyzed (Fig. 8A). The infiltration and analysis were repeated three times for 7 dpi to confirm the calculated numbers. As shown in Fig. 8B, the highest expression level was observed for PA83 using the miniBYV vector at 7 dpi (268 ± 22 mg/kg) with 83% solubility. The expression level was at least two times higher for the miniBYV replicon compared to the regular binary vector (pClean) and more than 60% higher compared to the TMV-based vector (D4) (Fig. 8).

Using an immobilized metal ion adsorption chromatography (IMAC) column, the PA83 protein expressed from the miniBYV replicon was purified. The purification process consisted of an extraction of the target from *N. benthamiana* leaf tissue with extraction buffer (50 mM Na-Phosphate pH 8.0, 500 mM NaCl, 20 mM Imidazole and 1 mM diethylcarbamic acid [DIECA]) at the 3:1 v/w ratio. After clarification, PA83 was captured using Ni-IMAC by employing the Chelating Sepharose Big Beads resin. The target was eluted with buffer contacting 300 mM Imidazole (obtained by mixing 60% of Buffer B [50 mM Na-Phosphate pH 7.5, 500 mM NaCl, 500 mM Imidazol] with 40% of

Buffer A [50 mM Na-Phosphate pH 7.5, 500 mM NaCl]). Western blot analysis showed that final recovery of PA83 expressed from the miniBYV vector after IMAC purification was about 72%.

Example 6. Co-expression of a target protein and an enzyme using the miniBYV vector

To explore a possibility of using the miniBYV vector for co-expression of a target protein and a modifying enzyme, malaria vaccine candidate protein Pfs48 (from protozoa parasite *Plasmodium falciparum,* accession # AAL74351) and endoglycosidase F from *Elizabethkingia meningoseptica* (PNGaseF, accession no AAA24932) were used. Again, a plant GENEART-optimized sequence of Pfs48 with the PR-1a peptide at the N-terminus and the 6xHis-tag and KDEL on the C-terminus of the protein (SEQ ID NO: 13; Fig. 18) was used. For PNGaseF, the PR-1a peptide on N-terminus of the protein and FLAG-tag and KDEL ER retention signal on the C-terminus (SEQ ID NO: 15; Fig. 19) were used. Both sequences were cloned into the miniBYV vector as shown in Fig. 9. A weak GLRaV2 CP promoter was used to control PNGaseF because the enzyme toxic to the plant tissue when expressed at a higher level.

The expression level of Pfs48 was assessed using a mouse anti-histidine mAb at a 1:2000 dilution (anti-4xHis, Qiagen Inc.). Fig. 10A shows that the electrophoretic mobility of the glycosylated Pfs48 (control, which was expressed w/o PNGaseF) was different (less mobility) compared to the non-glycosylated form of Pfs48 that was co-expressed with PNGaseF. The expression of PNGaseF was confirmed by Western blotting using a rabbit anti-FLAG primary mAb (Sigma) and a goat anti-rabbit secondary Ab (Biorad) (Fig. 10B). These results verify identical compartmentalization of both the target (Pfs48) and the enzyme (PNGaseF), which is probably inside of the virus replication complex. The results also verify that the PNGaseF acted on the Pfs48.

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What is Claimed:

1. An isolated nucleic acid molecule for producing one or more target polypeptides in a plant cell, comprising a minireplicon derived from a *Closteroviridae* virus and one or more heterologous polynucleotides, wherein the nucleic acid molecule is capable of replicating in the plant cell, and wherein the one or more heterologous polynucleotides encode the one or more target polypeptides.

2. The nucleic acid molecule of claim 1, wherein the *Closteroviridae* virus is *Beet yellows virus*.

3. The nucleic acid molecule of claim 1 or 2, wherein the plant cell is in a plant, a plant part, or a cell culture medium.

4. The nucleic acid molecule of any of claims 1-3, wherein the plant part is selected from the group consisting of leaves, stems, roots, floral tissues, seeds and petioles.

5. The nucleic acid molecule of any of claims 1-4, further comprising a polynucleotide encoding one or more movement proteins derived from the *Closteroviridae* virus.

6. The nucleic acid molecule of any of claims 1-5, wherein the one or more target polypeptides comprise one or more subunits of a protein, and wherein the one or more target polypeptides are capable of forming the protein in the plant cell.

7. The nucleic acid molecule of claim 6, wherein the protein is an enzyme.

8. The nucleic acid molecule of any of claims 1-5, wherein the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and wherein the first polypeptide is capable of modifying the second polypeptide in the plant cell.

9. The nucleic acid molecule of any of claims 1-5, wherein the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and wherein the first polypeptide is capable of affecting expression of the second polypeptide in the plant cell.

10. The nucleic acid molecule of claim 9, wherein the first polypeptide is a silencing suppressor.

11. The nucleic acid molecule of any of claims 1-5, wherein the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and wherein the first polypeptide is capable of increasing production of the second polypeptide in the plant cell.

12. The nucleic acid molecule of any of claims 1-5, wherein the one or more target polypeptides comprise a heavy chain and a light chain of an antibody, and wherein the one or more target polypeptides are capable of forming the antibody in the plant cell.

13. The nucleic acid molecule of any of claims 1-12, wherein the one or more target polypeptides comprise an immunogenic polypeptide.

14. The nucleic acid molecule of any of claims 1-13, wherein the one or more target polypeptides comprise a polypeptide of at least 100 kD.

15. A vector comprising the nucleic acid molecule of any of claims 1-14.

16. A method for producing one or more target polypeptides in a plant cell, comprising

(a) introducing a nucleic acid molecule into the plant cell, wherein the nucleic acid molecule comprises a minireplicon derived from a *Closteroviridae* virus and one or more heterologous polynucleotides, wherein the nucleic acid molecule is capable of replicating in the plant cell, and wherein the one or more heterologous polynucleotides encode the one or more target polypeptides; and

(b) maintaining the plant cell under conditions permitting production of the one or more target polypeptides in the plant cell.

17. The method of claim 16, further comprising purifying at least one of the one or more target polypeptides from the plant cell.

18. The method of claim 16 or 17, wherein the *Closteroviridae* virus is *Beet yellows virus*.

19. The method of any of claims 16-18, wherein the plant cell is in a plant, a plant part, or a cell culture medium.

20. The method of claim 19, wherein the plant part is selected from the group consisting of leaves, stems, roots, floral tissues, seeds and petioles.

21. The method of any of claims 16-20, wherein the nucleic acid molecule further comprises a polynucleotide encoding one or more movement proteins derived from the *Closteroviridae* virus.

22. A composition comprising at least one of the one or more target polypeptides produced by the method of any of claims 16-21.

23. A method of treating a subject in need of at least one of the one or more target polypeptides produced by the method of any of claims 16-21, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the at least one of the one or more target polypeptides.

24. The method of any of claims 16-21, wherein the one or more target polypeptides comprise one or more subunits of a protein, and wherein the maintaining conditions further permit production of the protein in the plant cell.

25. The method of claim 24, wherein the protein is an enzyme.

26. A composition comprising the protein produced by the method of claim 24.

27. The composition of claim 26, wherein the protein is an enzyme.

28. A method of treating a subject in need of the protein produced by the method of claim 24, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the protein.

29. The method of any of claims 16-21, wherein the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and wherein the maintaining conditions further permit modifying the second polypeptide by the first polypeptide in the plant cell.

30. The method of any of claims 16-21, wherein the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and wherein the maintaining conditions further permit affecting expression of the second polypeptide by the first polypeptide in the plant cell.

31. The method of claim 30, wherein the first polypeptide is a silencing suppressor.

32. The method of any of claims 16-21, wherein the one or more target polypeptides comprise a first polypeptide and a second polypeptide, and wherein the maintaining conditions further permit increasing production of the second polypeptide by the first polypeptide in the plant cell.

33. A composition comprising the second polypeptide produced by the method of any of claims 29-32.

34. A method of treating a subject in need of the second polypeptide produced by the method of any of claims 29-32, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the second polypeptide.

35. The method of any of claims 16-21, wherein the one or more target polypeptides comprise a heavy chain and a light chain of an antibody, and wherein the maintaining conditions further permit production of the antibody in the plant cell.

36. A composition comprising the antibody produced by the method of claim 35.

37. A method of treating a subject in need of the antibody produced by the method of claim 35, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the antibody.

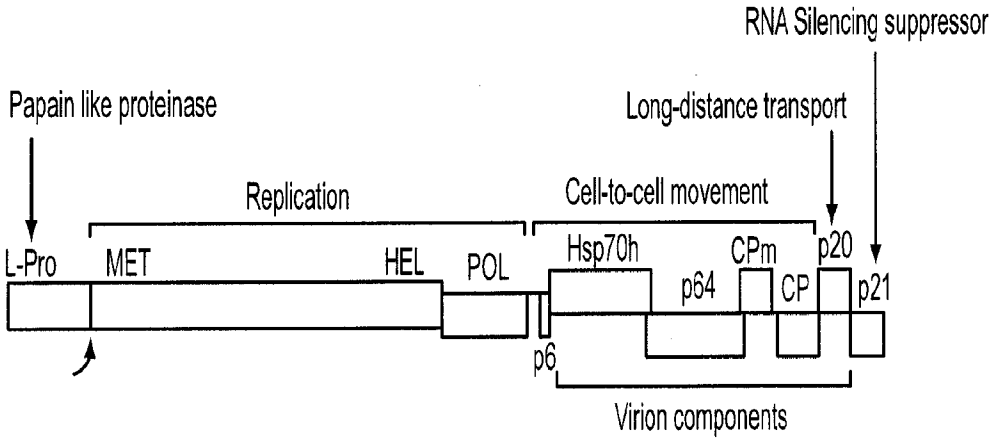
38. The method of any of claims 16-21, wherein the one or more target polypeptides comprise an immunogenic polypeptide.

39. A composition comprising the immunogenic polypeptide produced by the method of claim 38.

40. A method for inducing an immune response in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the immunogenic polypeptide produced by the method of claim 38.

41. A method for inducing a protective immune response against a pathogen in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the immunogenic polypeptide produced by the method of claim 38, wherein the immunogenic polypeptide is derived from the pathogen.

42. The method any of claims 16-21, wherein the one or more target polypeptides comprise a polypeptide of at least 100 kD.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Virion components

FIG. 1

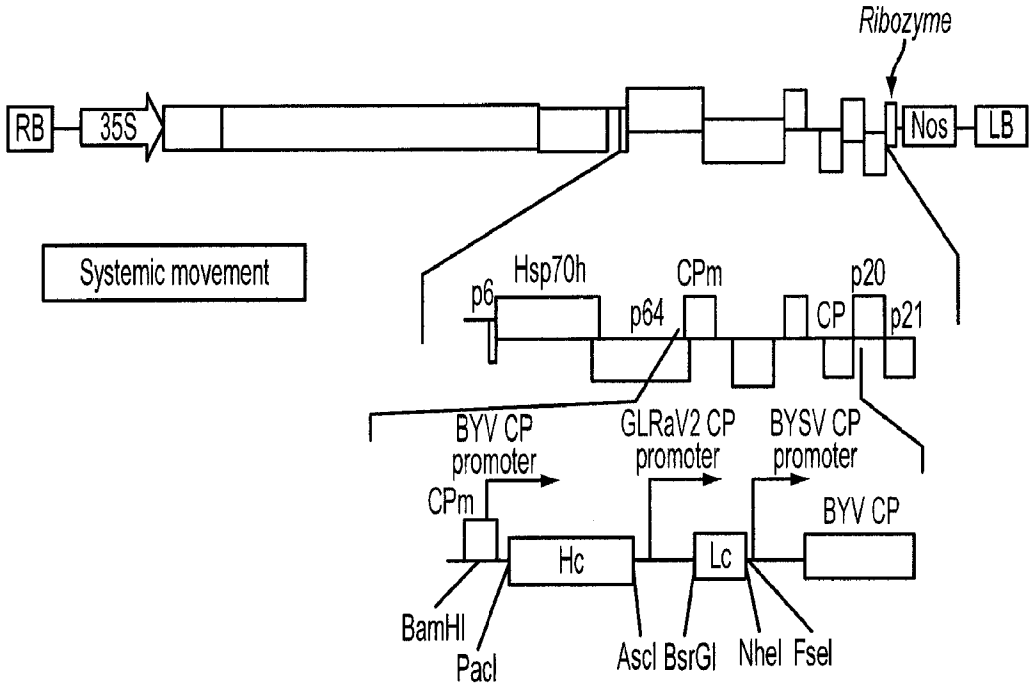
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FIG. 2

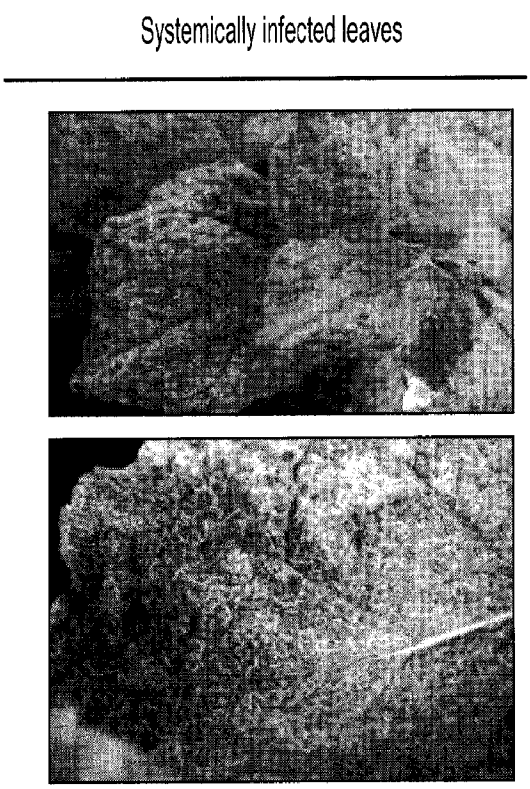
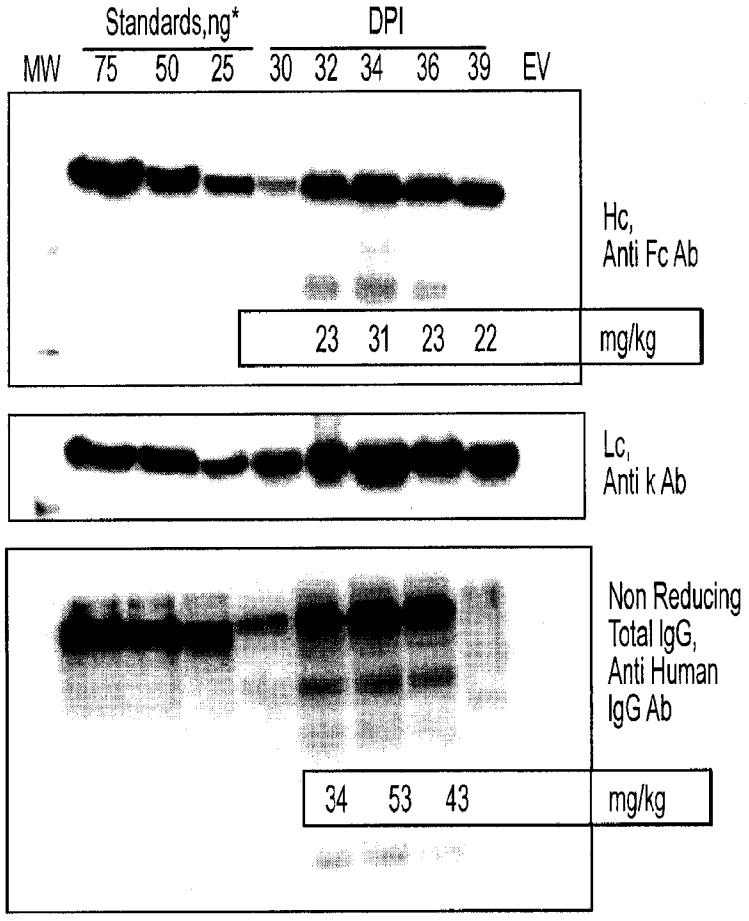

Systemically infected leaves

FIG. 3A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mg/kg

FIG. 3B

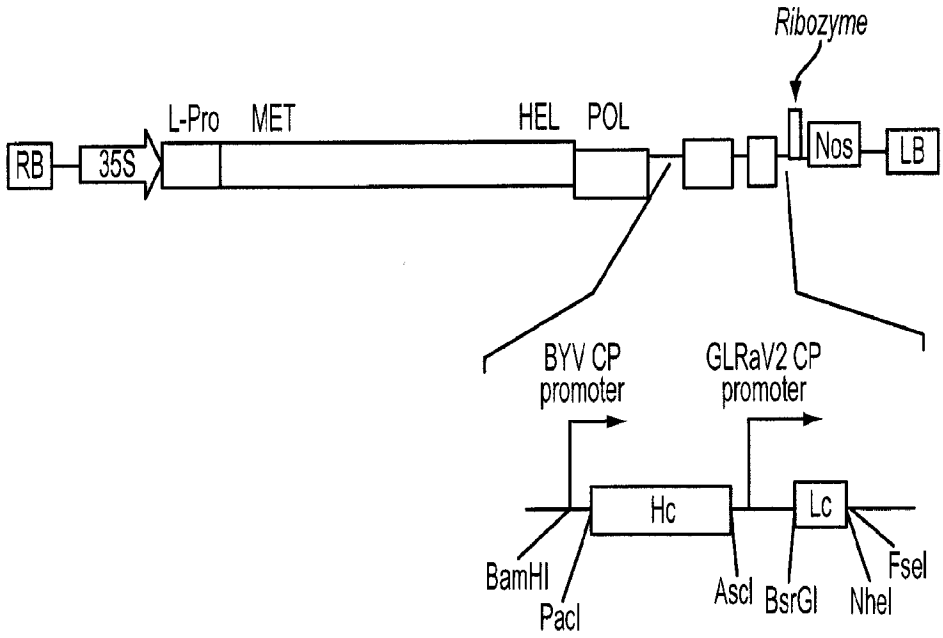
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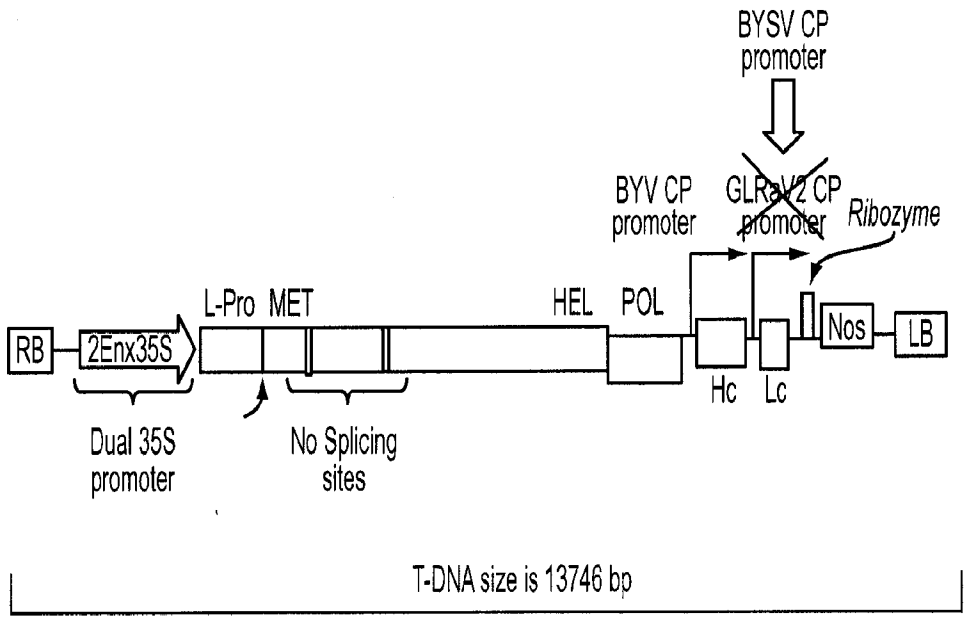
FIG. 4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GLRaV2 CP promoter
Ribozyme
L-Pro
MET
HEL
POL
RB
2Enx35S
Nos
LB
Hc
Lc
Dual 35S promoter
No Splicing sites
T-DNA size is 13746 bp

FIG. 5

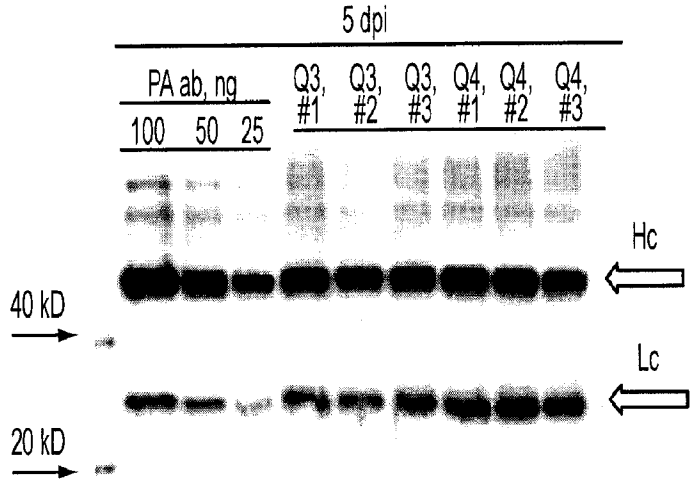

FIG. 6A

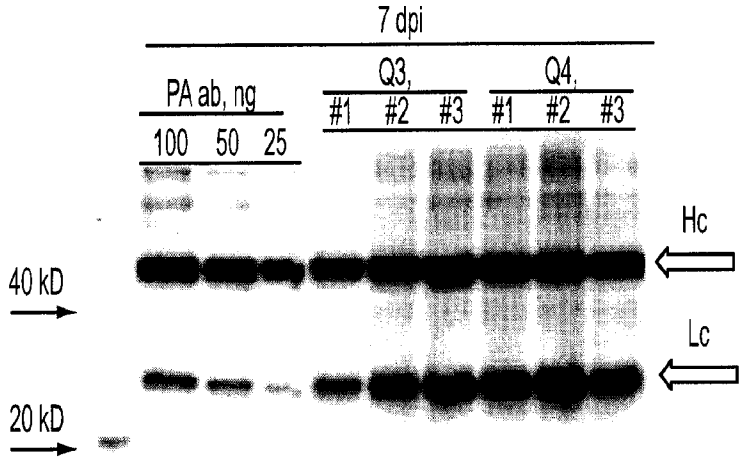

FIG. 6B

dpi	Q3, #1 Lc/Hc	Q3, #2 Lc/Hc	Q3, #3 Lc/Hc	Q4, #1 Lc/Hc	Q4, #2 Lc/Hc	Q4, #3 Lc/Hc
5 dpi	213/102	176/81	245/97	303/113	337/110	290/74
7 dpi	212/51	482/128	595/167	547/140	640/149	437/133
9 dpi	209/68	321/89	488/125	387/122	413/123	541/145

FIG. 6C

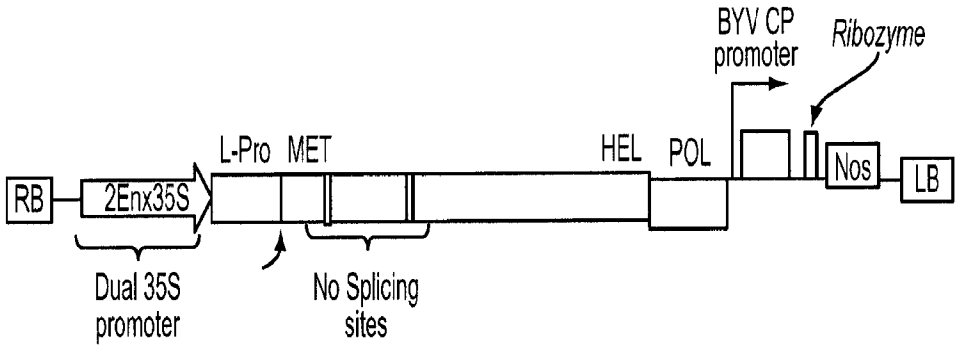

FIG. 7

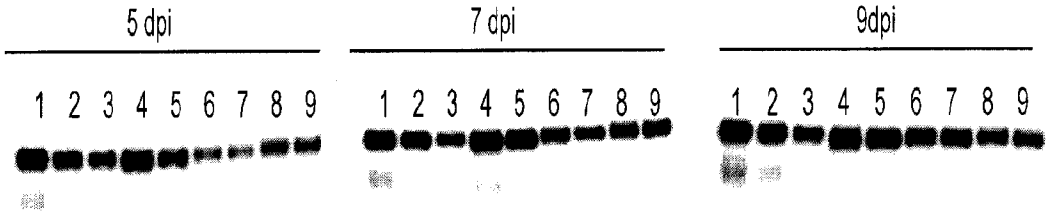

FIG. 8A

Expression, leaves, mg/kg	miniBYV-PA83, #3		pGR-D4-PA83*		pClean-PA83	
	Total Protein	Total Soluble Protein	Total Protein	Total Soluble Protein	Total Protein	Total Soluble Protein
5dpi	200	116	57	47	84	77
7dpi	268±22	222±31	147±30	129±28	85±45	83±67
9dpi	183	178	121	133	92	90

*W/O P1HcPro

FIG. 8B

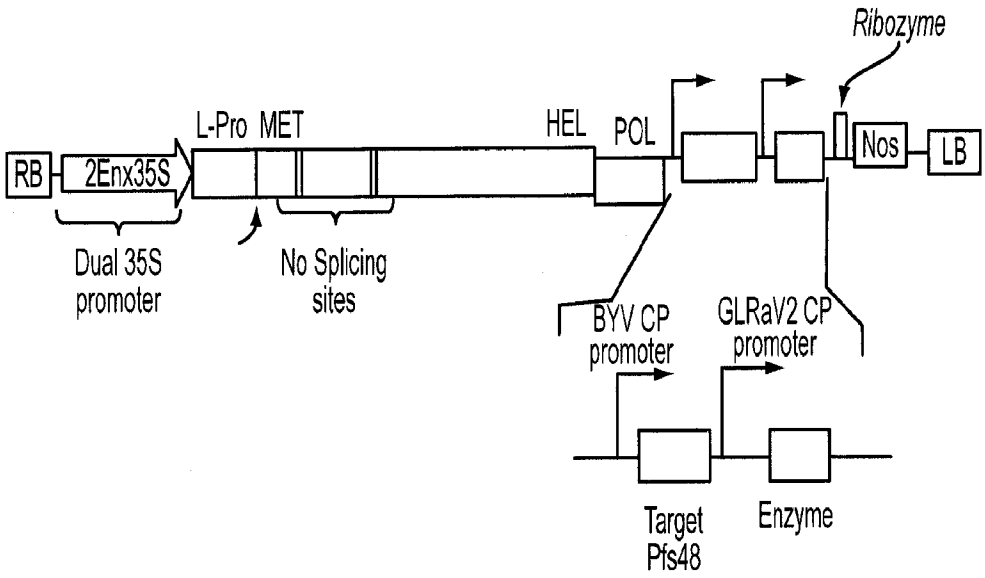

FIG. 9

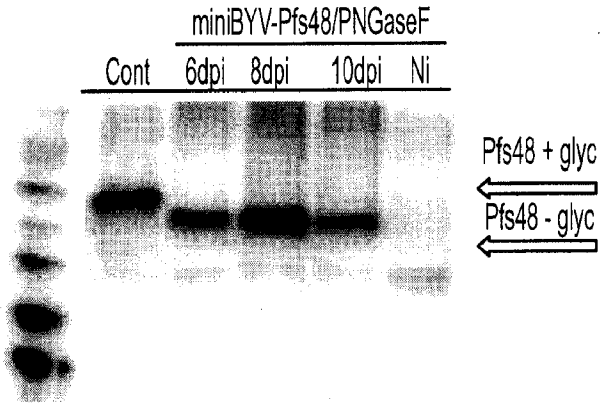

FIG. 10A

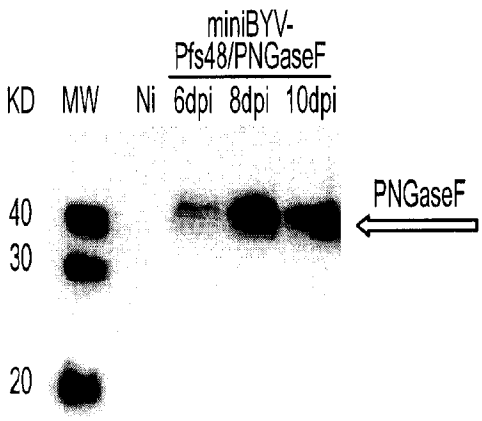

FIG. 10B

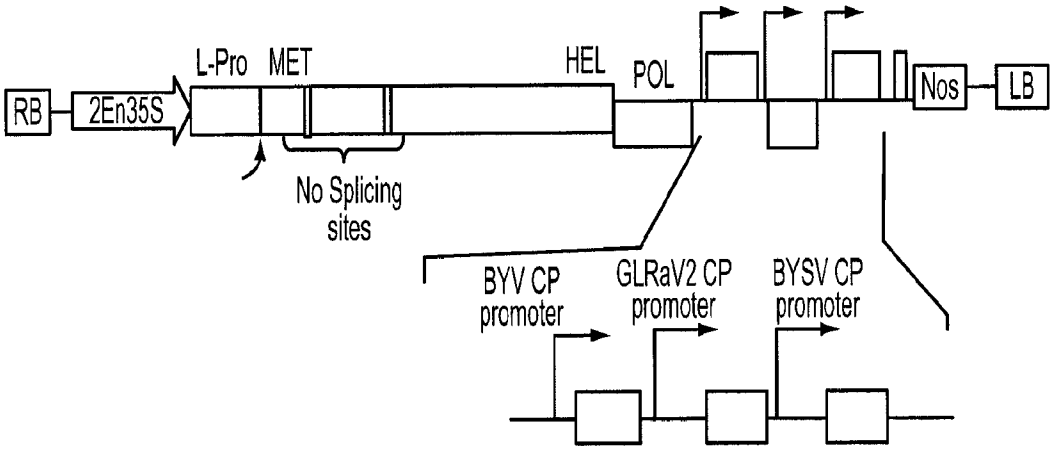
RB
2En35S
L-Pro
MET
No Splicing sites
HEL
POL
Nos
LB
BYV CP promoter
GLRaV2 CP promoter
BYSV CP promoter

FIG. 11

Figure 12A

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1)

ggtttacccgccaatatatcctgtcaagctcacaaaccaaggcaagtaatagagattggagtctctaaaaaggtagttcccactgaatca
aaggccatggagtcaaagattcaaatagaggacctaacagaactcgccgtaaagactggcgaacagttcatacagagtctcttacgac
tcaatgacaagaagaaaatcttcgtcaacatggtggagcacgacacacttgtctactccaaaaatatcaaagatacagtctcagaagac
caaagggcaattgagacttttcaacaaagggtaatatccggaaacctcctcggattccattgcccagctatctgtcactttattgtgaagat
agtggaaaaggaaggtggctcctacaaatgccatcattgcgataaaggaaaggccatcgttgaagatgcctctgccgacagtggtcc
caaagatggacccccacccacgaggagcatcgtggaaaaagaagacgttccaaccacgtcttcaaagcaagtggattgatgtgatat
ctccactgacgtaagggatgacgcacaatcccactatccttcgcaagacccttcctctatataaggaagttcatttcatttggagagGT
TTTTAACCATCCTTCTACTAGACGTACACTCGTACATCCTCTAGTATTTCCTTTATT
TATCTTCCCATAAGCCCCTGCCTTTAACGTTTAACTCTCGCTATCGACATGGCATT
CTTGAACGTTTCTGCTGTCCCCAGCTGTGCTTTCGCACCTGCTTTCGCACCCCACG
CCGGTGCAAGCCCCATTGTGCCCGACAGTTTCCCTTGCGTACCCCGTTATTCGGA
CGACATATCCCATTTCCGTTTAACGTTGTCGCTTGACTTCTCTGTCCCGCGCCCGC
TCTTTTTGAACGCGCGTGTGCACTTGAGAGCTTCTACCGACAACCCCTTACCATC
GCTTCCCCTTGGTTTCCACGCTGAAACCTTTGTTTTGGAATTGAACGGCAGTTCAG
CTCCCTTCTCTATACCGTCCCGCCACATTGACTTCGTTGTGAACCGGCCTTTCTCC
GTGTTCCCTACTGAAGTCCTCTCCGTCAGTTCCCTTCGAACACCTTCTAGGTTGTT
CGCTCTTTTATGCGACTTTTTCCTTTACTGTTCCAAACCAGGTCCTTGCGTTGAAA
TTGCTTCTTTTTCAACTCCACCACCCTGTTTAGTTTCGAACTGCGTTGCACAAATT
CCCACGCACGCCGAAATGGAAAGTATTCGTTTTCCAACGAAGACTCTTCCAGCTG
GTCGGCTCTTACAGTTCCACAAACGAAAATACACGAAACGACCTGAAACTCTAA
TCATACACGAGAGCGGCTTGGCTCTCAAAACCAGCGCGTTAGGTGTTACATCGAA
ACCTAATTCGCGCCCTATCACCGTGAAATCTGCTTCCGGTGAAAAATATGAAGCT
TACGAGATCTCTCGTAAAGACTTCGAACGCTCCCGCAGGAGGCAACAAACTCCTC
GCGTTCGTTCTCACAAGCCCCGGAAGATAAATAAAGCTGTCGAACCTTTCTTCTT
TCCAGAAGAACCAAAGAAAGACAAACGGAAGAGAGCTTCTCTTCCGACTAAAGA
TGAAGGTTTCATTACTTTTGGGACCTTGCGCTTTCCGCTTTCGGAAACCCCTAAGG
AAGAACCTCGTCTTCCTAAATTTCGGGAAGTTGAAATTCCTGTAGTCAAGAAACA
TGCTGTACCAGCAGTAGTTTCTAAACCAGTTCGAACGTTTAGACCGGTCGCTACC
ACTGGCGCTGAGTATGTCAACGCTCGGACCCAGTGTTCGCGCCGTCCAAGAAATC
ATCCGATTTTACGCAGTGCTTCTTATACTTTCGGTTTTAAGAAAATGCCGCTCCAG
CGTTTCATGAAAGAAAAGAAAGATTACTACGTGAAGCGCTCTAAGGTCGTGAGT
AGTTGTAGTGTAACTAAAAGTCCTTTGGAAGCTTTGACCAGCATACTAAAGAACT
TACCACGGTATTCCTACAACTCAGAACGGTTAAAATTTTACGATCATTTTATCGG
CGACGACTTTGAAATTGAAGTGCATCCGCTGCGAGGCGGCAAATTAAGTGTGCTT
CTTATTTTGCCTAAGGGCGAAGCTTACTGCGTAGTCACTGCGGCCACGCCGCAGT
ACCACGCTGCTTTAACTATTGCGCGTGGCGATCGTCCTCGCGTTGGTGAACTTCT
GCAGTACCGACCCGGCGAAGGGTTATGTTACCTCGCCCACGCTGCTCTTTGTTGC
GCTCTTCAGAAGCGCACCTTTCGCGAAGAAGACTTCTTCGTTGGTATGTACCCGA
CGAAGTTTGTCTTCGCTAAACGACTCACTGAAAAGTTGGGTCCTAGTGCTCTCAA
ACATCCTGTGCGCGGAAGACAAGTCTCTCGTTCACTCTTCCACTGTGATGTGGCT
TCTGCTTTTTCG

Figure 12B

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

AGCCCCTTTTACAGTTTGCCGCGTTTCATCGGCGGCGTGGAAGAAGAAGCTCCTG
AGATCACGTCGTCTCTTAAGCACAAGGCGATCGAATCGGTCTACGAACGTGTCAG
CATTCACAAAGACAACTTGTTGGCTCGTAGCGTGGAAAAGGATTTGATCGACTTT
AAAGACGAAATCAAGTCGCTGTCGAAAGAAAAACGTTCCGTTACCGTCCCCTTCT
ATATGGGGGAGGCCGTACAGAGTGGTTTGACGCGCGCGTACCCTCAGTTTAATTT
GAGTTTCACTCACAGTGTCTATTCGGACCACCCTGCGGCGGCCGGTTCCCGTCTG
TTGGAAAACGAAACTTTAGCCTCGATGGCTAAATCTTCCTTTTCTGATATCGGGG
GCTGCCCACTTTTTCACATAAAGAGGGGGAGCACAGATTACCACGTGTGCAGAC
CGATTTACGACATGAAGGACGCTCAACGAAGAGTTTCGAGGGAACTCCAAGCGA
GAGGGCTGGTGGAAAACCTTTCCCGCGAACAACTTGTTGAAGCTCAGGCGCGCG
TTTCGGTGTGCCCTCACACTCTCGGCAATTGTAACGTGAAAAGCGACGTGCTAAT
CATGGTGCAGGTTTACGACGCTTCTTTGAACGAAATTGCGTCGGCGATGGTCCTA
AAGGAATCGAAAGTCGCTTACCTCACCATGGTGACTCCGGGTGAACTTCTAGATG
AACGTGAAGCCTTCGCTATCGACGCTTTGGGGTGCGACGTCGTCGTGGACACCCG
CCGAGACATGGTGCAGTACAAATTCGGCTCATCTTGTTACTGCCACAAACTGTCG
AATATAAAGAATATAATGTTGACTCCCGCATTCACTTTCAGCGGGAATCTTTTTTC
GGTCGAGATGTACGAAAACCGAATGGGCGTCAACTACTACAAAATCACTCGTTC
AGCGTATTCTCCTGAAATACGCGGCGTTAAGACGCTGCGGTACCGCCGAGCCTGC
ACCGAGGTCGTGCAAGTTAAACTGCCACGCTTCGATAAAACTCTAAAGACGTTTC
TCTCCGGGTATGATTACATATATTTGGATGCGAAATTCGTCTCTCGAGTCTTCGAT
TACGTGGTTAGCAACTGTAGCGTTGTTAACAGTAAAACCTTCGAGTGGGTGTGGA
GTTACATAAAATCAAGTAAATCGCGCGTTGTCATAAGTGGGAAAGTGATACATC
GAGATGTGCATATCGACCTCAAACACTCTGAATGTTTTGCGGCTGTGATGCTCGC
GGTTGGGGTGCGTTCTCGCACAACTACTGAATTTTTAGCGAAGAACCTCAATTAT
TACACTGGCGATGCTTCGTGCTTTGAGACTATCCGTTTCCTCTTTCGGGAGTGGAG
CCGGAGGGCTTACGCGGAAATAAATCGCAGTTTTCGAAAGCTTATGAAGAGCAT
TCTTTCCGCTGGGTTAGATTACGAATTTCTCGATCTTGACAACTCGCTCCAACACT
TGCTTGAATACTCAGAGGTTGAAGTGCGCGTTTCAATCGCTCAGAATGGTGAGGT
GGATTGCAACGAAGAGAACCGTGTTTTGACGGAGATAATAGCCGAAGCAGCGGA
CAGGAAATCCATCGCTCAAGGTCTCAGCGGAGCGTTGAGCTCCGTTCCAACGCA
ACCGAGAGGCGGTCTGAGAGGGGGTAGTCGCCGAAGTGGAGTTTTTTTCCTTTAT
AACTTGGTAGAGGAAGTCGGAAATCTTTTCTTTTCCGTCGGTGATGCCGTGCGGT
TTCTTGTTAAGTTATTTAAAACTTTTTCCGACTCTCCCATCTTTCGGGTCGTTCGG
ATGTTCCTGGACTTGGCGGAAGCAGCTTCACCGTTCGTTTCGGTTGTTTCTTTGTG
CGCGTGGTTGCGCGAAGCTGTAAGCGCTTTCTCAGGCTGGGTTGCCGACAGAACG
GTGTCGGAAAGTGTAAAAACCTTTGTAAACCGTACGGTAAAAAGGTTTCTAAACT
TTATGTCTGCAAAAACCCTGACGAAAAAATTTTTTAGGTTTTTCTTATCCGCTTCC
GCCTTAGCTAAAACCGTTGTGAGGAAGGCGAAGGTGATTTTGGAAGCTTACTGG
GAAGTGTGGTTCGAATCGATTCTTTCGGATAGTGGAGAGTATAGTGCTGTTGAAT
TTTGTAGCAGCGTTGTGATCACACTGTTAACGAATTCCGGTCGGCTGCTACCTGG
GTTTAGTCC

Figure 12C

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

ATCCGCTGTCATTACTGAAGTACTGCTTGACCTAGCCACGAAAATATCCATTGAA
GTTCTTCTAAAACAAATTTCTCCTGTCGACTCGACAGCTTCTTCGGCCTTATATCG
CCGCGTCCTAAGCGAGATTCTGTCGAATTTTCGCACAATGGGCGAACACGGGATC
TTCACTAAAGTCTTTTTACTCTGCGGGTTTCTACCCGTGTTTGTTAGGAAGTGCGT
CGCTCTGTGCGTTCCTGGTGACATGGCGACGTACGCCCGGTTTCTCGAATACGGG
GTCGACGATCTCTTCTTTTTGGGAAGGTCCGTGAACTCGATTAAGAACTACCTAT
GTGTGGTTGCTGCCGGGTTGGTGGACTCCATTGTCGATTCCGTTGTTTTGAAACTT
TCCGGTGTCGCCAAAGAGCGAGTGCTTGGTTTTAAATCAAAAATCATAAAAAATT
TTTTAAACGTCTTTAGGAAGGCGAAAGTCGTTACACGTACTTCTTCCAGCACGGA
CTTGTCGGAAGACGAATATTTTTCGTGCGACGAAAGCAAACCCGGCTTGAGAGG
CGGTTCCTCTAGGTTCACGCTTTCGCGATTGCTTGACATCTTTTTCAATTTCCTGA
AAAGTTCAAAGCTCGTCATAGAGAATGCGTGCTTTTCTGCTTACGAAAGGATTGA
AAGGAACATGAAACTATACTTTTTTCCTTTAAATTCTTCGGAAGAAGAAGCTCGC
CGCTTAATTCGGTGCGCGGGAGACTTCGACTACTTAAGCGATAGCGCTTTCGACG
AAGATGAAATGTTACGGCAAGCTTTCGAACAATACTATTCCAGTGACGACGAGA
GTGTAACTTACGATGGAAAACCCACAGTTCTCCGTAGTTACTTGAACGTGAGTAG
AAGGTTCTTGGAAACGTTCTGTAACGGCCCGAAGTTTTTCGTCAAAGTTTCGAAT
TACTTTAAAGCGCTGTATAGTCGTTTGCTCCGTGTTCTACCGTGGGTAGACAGAA
ATCTTTCTGACTCGCCAGGACTTAAAGGAGGTAACGAGAAAGCTCTTCTTGCGAA
GTTCCTTAAAACCTGCGTCATAACCGCTTGCGAATGTGTGTCGCAGATATGCTGC
CTGCGTCTTATTCGCTTATGTTGGGGGACACCAGCGTGTGGTTTAGTTAGGTTATT
TTACATAACTTATTCCGGTACTCGCGTGTTGTCGCGTGTTGTGGTCGCGGTGGCTG
TATGCCCTCTTTTGGTCAGAAACGAACTGGATGGCTTGAGTGATGGATTAACCAA
CATGGGCGTTTCGGTTTTTCGTCGCTTGTTCGTCGCCCTTCGGCGCGCGCTTTCGG
CGTACTCTAATTCCGCCCTACGACGGAAGATTTTCGAATTCATTTTCGGAAACAT
TCACCACCCTTTTGATGTCGCAGTAATCGAAACAAACGAAGTTGCGCCGGAACCT
CTTTCACCGGAAGTGGACATTGATGTCGACTGCGACTTTGGTTCCGATTCAGAAT
CGGTTTCTTCAGATGAAGTCGCGTCGATTCCCCGTCCAGGCTTACACGGTGGGAA
TAGGCGCTCTTCCAACTTTCTAACCTCTCTCGTGAAGGTTGTTTTTAAGTTGGCTG
GGCGCATTCCGCGTTTGCTTTTCCGTTTACGCAATTTTGTGGCGTACTTTGTTGAA
CGACGGCTGGCTTCAAAAAGGTTGAAGACGTTCATCGGTCTGGCCAGGCTGTTTG
ATAATTTCTCACTCACTTCGGTAGTTTACCTTCTCCAAGAGTACGATTCAGTGCTG
AACGCATTTATAGACGTTGAGCTAGTCCTACTTAACTCGGGTAGCGTGAATGTGC
TACCTTTAGTTTCTTGGGTCAGGGGATCTCTAACGAAGTTAGCGGAAGTGATCGT
TGGTTCAGGCTTCGCTTCGTTTCTAGGAAGGATGTGCTGTCGTGTGTCCGACTGGT
GCTCCTCGTCTTCGAACGCCGGTTGTAACTTTATGAGTCCGGTTCGTACGAAAGG
GAAGTTCGTTCCCCCTTCGTCTTCCGGTTCAACCGCTTCGATGTATGAACGTCTCG
AAGCTCTCGAGAGCGATATCCGCGAACACGTGCTTTCCACGTGTCGTGTAGGAAG
CGACGAAGAAGAGGAAAGGCCGAAAGAAGTGACAGAACCAGGAATTGAACATA
CTTCTGAAGATGTTGTTCCCATTCGTTCACACTCGCAACCCTTATCTGGAGGTGAA
TGTTCGT

Figure 12D

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

ATTCTGAAGATCGTGAAGAGAATGAACGAGCGAACCTGTTACCGCACGTTAGCA
AAATCGTCAGCGAACGAAGGGGTTTGGAGACCGCCCGTCGAAACAAACGTACTC
TACGTGGTGTAAGCGAGTTTCTCAACGCTATTAATACTAGCAATGAGCAACCTAG
GCCGATAATCGTTGACCACTCTCCTGAATCTCGCGCGTTGACCAACTCCGTGAGG
GAATTCTATTACCTCCAGGAACTCGCTCTTTTTGAGTTGAGTTGCAAACTTCGTGA
GTATTACGATCAATTGAAGGTTGCGAATTTTAACAGACAAGAGTGTTTGTGCGAC
AAAGACGAAGACATGTTTGTTCTACGAGCCGGACAAGGTGTAGTTTCCGGCAGA
AACTCGAGGTTGCCTCTTAAGCATTTCAAGGATCACGAATTTTGTTTTCGCTCTGG
AGGGTTGGTCCCTTACGACGGTACCAGCAGAGTGGACACCATTTTTCACACGCAA
ACGAATTTCGTTTCCGCGAACGCGCTTCTTTCGGGCTATCTCTCCTATAGAACTTT
CACTTTCACTAATTTGAGCGCTAACGTACTGCTGTACGAAGCTCCTCCAGGTGGT
GGAAAGACCACGACTTTGATAAAGGTTTTTTGTGAAACTTTTTCAAAAGTTAATT
CGCTGATTTTAACGGCGAATAAGAGTTCGCGAGAAGAAATACTTGCGAAGGTGA
ATCGCATCGTACTTGACGAAGGCGATACGCCTCTTCAGACGCGTGACAGGATTTT
AACTATCGATTCTTATCTAATGAACAACAGAGGTTTGACGTGTAAGGTTTTGTAC
CTCGATGAGTGTTTCATGGTTCACGCTGGAGCTGCTGTAGCTTGCATCGAATTCA
CCAAATGCGATTCAGCCATCTTATTTGGAGACAGCAGGCAAATTCACTACATAGA
CCGTAACGAATTGGATACTGCTGTTCTTTCAGATTTGAACCGTTTTGTCGATGATG
AATCGAGAGTATATGGTGAAGTCTCATACAGGTGTCCTTGGGACGTTTGTGCTTG
GTTGTCAACTTTCTACCCGAAAACTGTGGCCACTACCAACTTGGTTTCAGCCGGT
CAATCTTCGATGCAAGTACGCGAGATTGAAAGCGTAGACGACGTCGAATATTCC
AGTGAATTCGTCTACTTGACTATGTTACAGTCAGAGAAGAAAGATCTGCTGAAAT
CTTTCGGCAAGAGGTCTCGTTCGAGCGTTGAAAAACCCACGGTCTTAACAGTCCA
TGAAGCTCAAGGTGAAACCTACCGCAAAGTTAACCTCGTCAGAACGAAATTTCA
AGAGGACGATCCTTTTCGTAGCGAGAACCACATCACAGTGGCCTTGTCTAGGCAT
GTCGAGAGTCTGACCTATTCGGTCCTGAGTAGTAAACGTGACGACGCAATAGCTC
AAGCTATAGCGAAGGCGAAACAACTTGTGGATGCCTATCGCGTTTACCCCACGTC
ATTTGGTGGGAGTACTCTTGATATTAGTGTTAACCCTTCTACATCTGACAGGAGC
AAATGTAAAGCTTCCTCTGCCCCTTACGAAGTTATAAACAGCTTCTTGGAGAGCG
TGGTTCCGGGCACTACTTCAGTAGACTTTGGGGACGTTTCCGAAGAGATGGGCAC
TCAGGTTTTTGAGTCCGGTGCTGATAACGTTGTTATTCGTGATTCCGCACCTGTTA
ACAAGTCGACGGATCACGACCCGCAGCGGGTTTAGCTCGATTCGCTCGCAGGCG
ATTCCTAAGAGGAAACCGTCGCTGCAAGAGAATTTATACTCTTATGAGTCGCGTA
ATTACAACTTTACCGTTTGTGAACGTTTTTCCGGACCGCAGGAGTTCGGACAGGC
GATGGCGATGGTTATGTTGGAACGAAGCTTTGACTTAGAGAAAGTTGCTAAAGTT
AGAAGCGATGTGATCGCCATAACAGAAAAAGGGGTGCGAACATGGATGTCAAA
ACGTGAACCTTCTCAGCTTAGGGCTCTTAGTAGTGACTTACAAAAGCCTCTAAAC
TTGGAAGAGGAAATAACGACTTTTAAGTTGATGGTTAAGCGGGACGCGAAAGTC
AAACTCGATTCGTCGTGTTTGGTGAAACACCCACCAGCGCAGAATATAATGTTCC
ATCGCAAGGCGGTGAACGCGATTTTCTCGCCGTGTTTCGACGAGTTTAAAAATAG
AGTCATTA

Figure 12E

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

CCTGTACGAATTCAAATATTGTTTTCTTTACCGAAATGACTAACTCTACTCTCGCG
TCGATAGCGAAAGAGATGCTGGGGAGCGAACACGTTTACAACGTTGGGGAAATA
GACTTTTCGAAATTCGACAAATCCCAAGACGCTTTCATTAAGTCGTTTGAACGAA
CCTTGTATTCAGCGTTTGGTTTCGACGAAGACCTGCTTGACGTGTGGATGCAAGG
TGAATACACCAGCAACGCTACAACTCTGGACGGTCAACTTTCTTTTTCCGTCGAC
AACCAGAGGAAATCGGGCGCTTCTAATACGTGGATTGGTAATTCCATCGTGACTC
TTGGCATTTTGAGCATGTTCTATTACACCAATCGATTTAAGGCTCTTTTCGTGTCC
GGGGACGACTCTTTGATTTTCTCCGAATCTCCTATTAGAAATTCAGCCGATGCGA
TGTGCACAGAACTCGGTTTTGAGACTAAGTTTCTCACTCCGAGCGTCCCGTATTTC
TGCTCAAAGTTTTTCGTTATGACCGGTCACGACGTTTTCTTCGTGCCCGACCCTTA
TAAACTTTTAGTGAAATTAGGAGCTTCTAAGGATGAAGTGGACGATGAGTTTCTG
TTTGAAGTGTTCACCTCTTTTCGCGATTTAACGAAAGATTTAGTCGATGAAAGAG
TGATCGAACTCTTGACGCATTTGGTTCACAGTAAGTACGGGTACGAAAGTGGTGA
TACGTACGCCGCCCTGTGTGCTATTCATTGTATTCGTTCAAACTTTTCATCGTTCA
AGAAATTGTACCCTAAAGTTAAGGGCTGGGTCGTTCACTACGGTAAACTGAAGTT
TGTGCTGCGCAAATTCGCGAACTGTTTTCGCGAGAAGTTTGACACTGCTTTCGGC
GAAGCGTACTTTCTTACTTACGACGAAACTTGAGACTGTGTGGTAACTCGGTTGT
TGTTTTGTTTGTTCGTGTGTCTGTAGTCCTACTTCGCGGTGATGGACTGTGTACTC
CGCTCGTATTTATTACTCGCATTCGGGTTTTTGATTTGCTTGTTTCTTTTCTGCTTA
GTGGTTTTTATTTGGTTCGTGTATAAACAAATACTTTTTCGAAACACCCCGCCTTC
GAACGAAGCGCGTTTCAACCGTTCCACGGTCGTGTGATGGTTGTTTTCGGATTAG
ATTTCGGCACTACTTTCTCGAGCGTTTGCGCTTATGTTGGAGAAGAACTCTATTTG
TTCAAACAAAGAGATAGTGCTTACATACCCACTTTTGTGTTTTTACATTCCGATAC
TCAAGAAGTGGCTTTCGGTTACGATGCCGAAGTTCTTTCTAACGACCCTTCGGTT
CGTGGAGGTTTCTATCGCGACTTAAAACGCTGGATAGGATGTGACGAAGAGAAT
TACGGGGATTACCTGGAAAAACTCAAGCCGCACTACAAGACGGAATTGCTTAAA
GTTGCGCAAAGTTCGAAATCCACCGTGAGGTTAGACTGTTACTCAGGTACCGTGC
CGCAGAACGCCACTCTACCGGGTTTGATAGCGACTTTCGTCAAAGCTCTCATTTC
CACAGCCAGTGAAGCTTTCAAGTGTCAGTGCACCGGCGTTATTTGTTCGGTCCCT
GCCAACTATAACTGTCTGCAAAGGTCTTTTACCGAAAGCTGCGTCAACCTAAGCG
GCTACCCCTGTGTTTACATGGTTAACGAACCGTCGGCTGCGGCGCTCTCAGCTTG
TTCCAGAATCAAAGGCGCTACTTCGCCCGTTCTCGTGTATGATTTCGGGGGTGGA
ACGTTTGATGTTTCCGTGATTTCAGCGCTCAACAACACTTTTGTGGTGCGCGCTTC
TGGGGGCGACATGAATTTGGGAGGTCGTGATATCGATAAGGCTTTTGTTGAACAC
TTGTACAAAAAGGCTCAACTGCCCGTGAACTACAAAATTGACATTTCCTTCTTGA
AAGAATCGCTCTCAAAGAAGGTTTCCTTCTTAAACTTCCCGGTGGTCAGTGAACA
GAACGTAAAAGTTGACGTTTTGGTTAATGTCAGCGAACTGGCTGAAGTGGCGGCT
CCTTTCGTAGAAAGAACGATCAAGATCGTTAAAGAAGTTTACGAAAAATATCGT
GGTAGCATGCGATTGGAACCGAGTGTTAAAGCTAAACTGCTTATGGTAGGTGGTT
CTTCCTACTTGCCAGGTCTTTTATCGCGTCTTTCCTCCGTGCCTTTCGTGGAAGAA
TGCCTTGTG

Figure 12F

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

CTGCCCGATGCTCGAGCGGCGGTTGCCGGGGGGTGTGCTTTGTACTCAGCTTGTC
TTCGAAACGATTCTCCGATGTTGCTGGTCGACTGTGCAGCTCACAATCTTAGCAT
TAGTAGCAAATATTGCGAATCCATCGTTTGCGTTCCGGCTGGTTCACCCATCCCG
TTTACGGGAGTTCGAACAGTTAACATGGCTGGTTCCAACGCTTCCGCTGTTTACA
GTGCGGCGCTTTTCGAAGGAGACTTCGTCAAATGTCGCTTAAACAAAAGGATTTT
CTCTGGTGACGTCGCCTTAGGAGACGTCGGGGTGTTCGGTTCAAACACTAGGACG
GTCCCGTTAACACTCGAAATTAATGTTTCGAGCGTCGGAACAATCACGTTTTCCC
TCGTCGGACCGACAGGTGTTAAGAAGTTGGTGGGTGGAAATGCTGCTTACGATTT
CTCGAGTTATCAGCTCGGGGAGCGCGTCGTCGCAGACTTGCACAAACATAATTCT
GATAAAGTGAAACTTATTCATGCGTTGACGTATAAGCCTTTTCAAAGGAAGAAAT
TGACTGACAGCGACAAAGCTTTGTTCTTAAAGAGACTAAGTGCAGACTACCGAC
GTGAAGCGGGAAAGTTCAGTTCATATGACGACGCGGTTCTCAACTCCAGCGAAC
TACTACTGGGGAGAGTTATTCCGAAGATTCTTCGGGGGTCGAGAGTGGAAAAAC
TTGATGTCTGAAGCTGCTTCGGTTAGGCGGCCTGTGTATGATCGCGACTTTCGTTT
TTCAAACGGTGAAGTATTAAGTCGCAAGAATTTCAGCGACTCAACCGGCGAATC
ATTTGTGCGCGAGTTTTCATTGCTGCTGACTTTCCCAAAGACTTATGAAGTTTGCA
AGTTATGCGGTGTGGCTATGGAACAAGCTCTGAGCGGCATGAATCGTCTATCCGA
TTATAACGTCTCCGAACTTAACGTCGTCGACGTGAAAACCGTGGGTTGCAAATTT
AATATACAAACCGTCACTGAATTCGTGAGAAAGCTCAACGGGAATCTGGCCGAA
CCTTCTCTCGTCGAGCACTGTTGGTCTCTGTCTAATTCTTGCGGCGAACTGATCAA
TCCGAAAGACACGAAGCGGTTCGTTTCCCTCATCTTTAAAGGGAAGGACGTCGTC
GAAAGCACCGACGAAGCCGTAGTTTCCTCTTCTTACTTAGATTACCTTTCCCACTG
TTTGAACTTGTACGAAACGTGTAATCTTTCGTCTAACTCAGGGAAGAAAGCTTTG
TACGACGAATTTCTAAAGTACGTGATCGTTTATCTTGAAACTAGTGACTTAGAAT
ACCGTTCACTTTCCGACAACCCTTTGGTTGCCGGTGTTCTTTACGATATGTGTTTT
GAATATAACACGCTCAAGTCGACGTACCTAAAGAACATAGAGTCATTCGATTGTT
TTTTGAGTCTGTACTTACCGTTGCTCAGTGAAATCTTTTCGATGAATTGGGAACAA
CCAGCGCCTGACGTCCGGTTGCTTTTCGAACTCGATACAACCGAACTTCTTTTGA
AAATTCCGACTATCAACACGCACGACTCAACTTTTTTGTATAAGAATAAGTTGAG
GTATCTGGAATCCTACTTTGAAGACGACTCTAATGAGCTCATAAAGGTGAAAGTT
GATTCACTCTTAACCCGAGACAATCCCGAACTGAAACTAGCTCAGAGGTGGGTA
GGTTTCCATTGTTACTACGGGGTTTTTAGGACCGCTCAGACTAGGAAAGTGAAAA
GAGATGCGGAGTACAAACTGCCTCCAGCTCTCGGTGAATTCACAATAAACATGA
GTGGAGTGGAAGAATTCTTTGATGAGCTGCAGAAGAAAATGCCTTCCGTTTCTGT
TCGTCGCAGATTTTGTGGCAGTTTGTCTCACGAAGCTTTTTCCATTTTCAAGAGAT
TCGGGGTGGGTTTTCCTCCGATCACTAGATTAAACGTTCCCGTGAAATACTCCTA
TCTAAACGTTGACTATTACAGACACGTGAAGAGAGCGGGCTTGACGCAGGACGA
GTTGACTATTCTCAGTAACATCGAATTCGACGTCGCTGAAATGTGCTGCGAGAGG
GAAGTGGCTTTACAAGCCCGTCGCGCTCAGCGCGGCGAAAAACCATTTCAGGGT
TGGAAAGGTGTTAAAAACGAAGTTTCTCCGCACGCGCGTTCCTCAATTCGAGTGA
AAAAGAGC

Figure 12G

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

AACGAATCGTTACTAAACGTTTTGTGGAAAGATGTTGGCGCCCGAAGGCAGGGG
AGACTTAATCCACTTCACCGAAAACACTAGAGATGCCATGGAAACTTTCTTCAAC
AGCTACGATTTAGCTGAATACTCCGAAGTGAACCCTAACAAACTTAATCGGAAA
GAGACCGACGAGTTGTTAGGTGTTATTCGAGAGAGATTTAAATCCGAATTGGTGA
TAACAGATGAAGATTTTGTGAAACATTTAGCCTTCGCGTTGATTCGCGCGGCCAA
TATCACCACTAGTACGAAAGTCAACTACGTCGGAGCGTACGAGTATACGATAGG
GGGAAAGAAGTTCCTGGTCAAGGACGCTTGGGTTTTCCCTTTGATAAAGGAGTGT
ATGAAAAAGTTCAACAAACCCAATCCTGTGAGGACGTTTTGTGCTACTTTCGAAG
ACGCTTACATAGTGATAGCTCGTTCGTTGCCTAAACTGTTCCTGAATAGAACCAT
TGGCAAACGTGGGATCCCGTCAGGGTATGAGTTCCTCGGGGCAGATTTTCTAACT
GCGACCAGCGTGTGTTTGAACGATCACGAAAAAGCTATCGTACTACAGGCCTCA
AGAGCTGCCATTGATAGAGCAGTCTCTTCGTCGGTCGACGGGAAGATCGTCAGTC
TTTTCGACCTCGGTCGTCTTAGTTAACACAGTTACTAAGGTTCCATTTTATTATTG
CATTGTTTTTCATTTAGTGTAATCGTACTTGAG**TTAATTAAGGCGCGCCTGTAC**
AGCTAGCTAT**GGCCGGCC**TCAACGTGTCCGTTAATGTCCGACCACGATCGTGCT
GTAGCTTTGAGTGCATCTAGGAACGCACTCGACCGATCGGCGGCGTCTCAAATTG
ATAAAAAGATGGTTAGTTTGTACGACTTCGGTAAGGTAGTGTATACCTAACAGCT
TGCTTCTACCTGACACAGTTAAGAAGCGGCATAAATCGAAGCCAAACCCTAAATT
TTGCAACTCGATCAATTGTAACCTAGAGCGAAGTGCAATCAATGGGATCAGCCG
AACCTATAAGTGCAATCGCGACTTTTGAAAACGTGAGTCTCGCAGACCAAACGT
GTTTGCACGGTGAAGACTGCGACAAACTACGGAGGGATTTCGAAGAGTGTTTGA
AATTGAAAGGGGTTCCGGAAGATAAACTCGGTCTCGCGTTAGGACTTTGTTTGTA
CTCCTGTGCGACGATAGGTACTTCTAATAAAGTTAGTGTCCAACCGACGTCTACT
TTCATCAAAGCTTCGTTCGGTAGTGGGAAGGAATTGTTCCTCACTCACGGTGAAC
TGAGGTCCTTTCTGGACTCTCAGAAACTTTTAGAGGGAAAGCCTAACAAATTGCG
GTGTTTCTGCCGCACTTTTCAGAAGGATTACATATCCTTCGCGAAGGAATACCGA
GGAAGACTGCCTCCGATTGCTAGAGCCAACCGTCACGGTCTACCTGCTGAAGATC
ACTACTTAGCTGCTGATTTCATATCGACATCAACAGAACTTACTGACCTACAACA
AGGTCGTCTGCTGTTGGCGCGCGAAAACGCCACTCACACAGAATTCTCGTCTGAA
TCACCAGTAACTAGTTTGAAACAGCTGGGTCGTGGTCTAGCCACCGGAAAATGA
CTAGCTCTGTCGAACTAGCTCAGACGAAACCCCTTTTTAGAGTGTTGCTATTAAA
GGGTTTCGTTTTTTATATTGTTGCAATCGAAACAGAAGAAGAATCGCCTGAAGTT
GAACTTCCTTTGGTTTACCTCCACGATCTCGAGTTGAATGTTAACAAAAAGGGGA
AGATCGAATCTTCGTACATCGACTTTAAATCTTGTATGACTAGGTTGAAACCCAG
TTCGGTCTCTTACACTCGAGTGAGTTCTGGGAAACCTTCAGAAGATTTCTCTTCGT
CATACTCAGGAAAAACTTTCGATTCAAAAATACTTAACCGTAAAGTGACGTTTAC
ATTCGAAAGTGGCATTCAACTCGTGTTCGGGATGTACGGTCGCGATCAGCGGTGT
GTCTCTTCCGAATACTTGTGGTTTGAAAACGTATTCGTCGGCGCGCACTGCGGCA
CCTTACCCTATTGTCTGAATTGCGAATTAGACAAAAGCGGTGGTGAATTGGAAAT
TTTGACTTTTTCAAAGAATGAAGTTTTTCTTTAATGACTGTGAAACTTCTCGAGCC
ATATCTAGGT

Figure 12H

T-DNA Region of a BYV Launch Vector (SEQ ID NO: 1) (cont.)

CTGAATCGCTCCTACGTCGCGTGAAGGAACTTGGAACGAACTCGCCGCAAAGCG
AGGTCTCTGAGTGCATCAACGAATTCAACGAACTAGCAAGGTTTAATCACTTGTT
AGTTACGGTCGAACACAGGGAACGGATGGAAAAACATCCGAAACAGTCGTCGGA
ACTTCGAACTCCATCACGACTTGGCGAAATGCTCAAAGAAATTCGTGCGTTCCTT
AAGGTGCGCGTGGTAACTCCTATGCATAAGGAGACTGCCAGCGAAACTCTGAAC
GCGTTTCTGGAAGAGTACTGCCGGATTACTGGACTCACTCGCGAGGATGCCTTGC
GAGAAAAAATGAGGAAAGTAAGGAGTACGGTGCTGTTTCATCACTCGGAACTTT
TGAAGTTCGAAGTGACTGAAAACATGTTCAGTTTTACTGAACTACTAAAACTAAA
TCTAAGCTTGAGGGTGATTTCTTCTCAAATCCTCGGTATAGCTGTATAGAGAAGT
GACGGAGGTGACCTACCTGCCGTGTAATTTAAGTCGTCACAGAGTGACAACGGC
ACCAAGTGGTGCTTTGTGCGTATGTAAATTGCGAAGTGAAAAAATTTTTTAAAAA
AATTTTTCACACTCCTAGCGAAGTCCCGCTAGGAAGAAAAAATAAGGGCCGTCA
AGACATGATTCACATGTCTTCTGATGAGTCCGTGAGGACGAAACGGCCCGGG*gatc*
gttcaaacatttggcaataaagtttcttaagattgaatcctgttgccggtcttgcgatgattatcatataatttctgttgaattacgttaag
catgtaataattaacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtcccgcaattatacatttaatacgcgatag
aaaacaGaatatagcgcgcaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcaagcttatcgataccgtcga
cctcgagggggggcccggtaccaaaaccacccccagtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgt
ttacaccacaatatatcctgcca

Figure 13A

T-DNA Region of a miniBYV launch vector (SEQ ID NO: 2)

ggtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatctgagctcgcgtgcctgcaggtcaa
catggtggagcacgacacacttgtctactccaaaaatatcaaagatacagtctcagaagaccaaagggcaattgagacttttcaacaaa
gggtaatatccggaaacctcctcggattccattgcccagctatctgtcactttattgtgaagatagtggaaaaggaaggtggctcctaca
aatgccatcattgcgataaaggaaaggccatcgttgaagatgcctctgccgacagtggtcccaaagatggacccccacccacgagga
gcatcgtggaaaaagaagacgttccaaccacgtcttcaaagcaagtggattgatgtgataacatggtggagcacgacacacttgtctac
tccaaaaatatcaaagatacagtctcagaagaccaaagggcaattgagacttttcaacaaagggtaatatccggaaacctcctcggatt
ccattgcccagctatctgtcactttattgtgaagatagtggaaaaggaaggtggctcctacaaatgccatcattgcgataaaggaaaggc
catcgttgaagatgcctctgccgacagtggtcccaaagatggacccccacccacgaggagcatcgtggaaaaagaagacgttccaa
ccacgtcttcaaagcaagtggattgatgtgatatctccactgacgtaagggatgacgcacaatcccactatccttcgcaagacccttcct
ctatataaggaagttcatttcatttggagagcaGTTTTTAACCATCCTTCTACTAGACGTACACTCGTA
CATCCTCTAGTATTTCCTTTATTTATCTTCCCATAAGCCCCTGCCTTTAACGTTTAA
CTCTCGCTATCGACATGGCATTCTTGAACGTTTCTGCTGTCCCCAGCTGTGCTTTC
GCACCTGCTTTCGCACCCCACGCCGGTGCAAGCCCCATTGTGCCCGACAGTTTCC
CTTGCGTACCCCGTTATTCGGACGACATATCCCATTTCCGTTTAACGTTGTCGCTT
GACTTCTCTGTCCCGCGCCCGCTCTTTTTGAACGCGCGTGTGCACTTGAGAGCTTC
TACCGACAACCCCTTACCATCGCTTCCCCTTGGTTTCCACGCTGAAACCTTTGTTT
TGGAATTGAACGGCAGTTCAGCTCCCTTCTCTATACCGTCCCGCCACATTGACTTC
GTTGTGAACCGGCCTTTCTCCGTGTTCCCTACTGAAGTCCTCTCCGTCAGTTCCCT
TCGAACACCTTCTAGGTTGTTCGCTCTTTTATGCGACTTTTTCCTTTACTGTTCCAA
ACCAGGTCCTTGCGTTGAAATTGCTTCTTTTTCAACTCCACCACCCTGTTTAGTTT
CGAACTGCGTTGCACAAATTCCCACGCACGCCGAAATGGAAAGTATTCGTTTTCC
AACGAAGACTCTTCCAGCTGGTCGGCTCTTACAGTTCCACAAACGAAAATACACG
AAACGACCTGAAACTCTAATCATACACGAGAGCGGCTTGGCTCTCAAAACCAGC
GCGTTAGGTGTTACATCGAAACCTAATTCGCGCCCTATCACCGTGAAATCTGCTT
CCGGTGAAAAATATGAAGCTTACGAGATCTCTCGTAAAGACTTCGAACGCTCCCG
CAGGAGGCAACAAACTCCTCGCGTTCGTTCTCACAAGCCCCGGAAGATAAATAA
AGCTGTCGAACCTTTCTTCTTTCCAGAAGAACCAAAGAAAGACAAACGGAAGAG
AGCTTCTCTTCCGACTAAAGATGAAGGTTTCATTACTTTTGGGACCTTGCGCTTTC
CGCTTTCGGAAACCCCTAAGGAAGAACCTCGTCTTCCTAAATTTCGGGAAGTTGA
AATTCCTGTAGTCAAGAAACATGCTGTACCAGCAGTAGTTTCTAAACCAGTTCGA
ACGTTTAGACCGGTCGCTACCACTGGCGCTGAGTATGTCAACGCTCGGACCCAGT
GTTCGCGCCGTCCAAGAAATCATCCGATTTTACGCAGTGCTTCTTATACTTTCGGT
TTTAAGAAAATGCCGCTCCAGCGTTTCATGAAAGAAAAGAAAGATTACTACGTG
AAGCGCTCTAAGGTCGTGAGTAGTTGTAGTGTAACTAAAAGTCCTTTGGAAGCTT
TGACCAGCATACTAAAGAACTTACCACGGTATTCCTACAACTCAGAACGGTTAAA
ATTTTACGATCATTTTATCGGCGACGACTTTGAAATTGAAGTGCATCCGCTGCGA
GGCGGCAAATTAAGTGTGCTTCTTATTTTGCCTAAGGGCGAAGCTTACTGCGTAG
TCACTGCGGCCACGCCGCAGTACCACGCTGCTTTAACTATTGCGCGTGGCGATCG
TCCTCGCGTTGGTGAACTTCTGCAGTACCGACCCGGCGAAGGGTTATGTTACCTC
GCCCACGCTGCTCTTTGTTGCGCTCTTCAGAAGCGCACCTTTCGCGAAGAAGACT
TCTTCGTT

Figure 13B

T-DNA Region of a miniBYV launch vector (SEQ ID NO: 2) (cont.)

GGTATGTACCCGACGAAGTTTGTCTTCGCTAAACGACTCACTGAAAAGTTGGGTC
CTAGTGCTCTCAAACATCCTGTGCGCGGAAGACAAGTCTCTCGTTCACTCTTCCA
CTGTGATGTGGCTTCTGCTTTTTCGAGCCCCTTTTACAGTTTGCCGCGTTTCATCG
GCGGCGTGGAAGAAGAAGCTCCTGAGATCACGTCGTCTCTTAAGCACAAGGCGA
TCGAATCGGTCTACGAACGTGTCAGCATTCACAAAGACAACTTGTTGGCTCGTAG
CGTGGAAAAGGATTTGATCGACTTTAAAGACGAAATCAAGTCGCTGTCGAAAGA
AAAACGTTCCGTTACCGTCCCCTTCTATATGGGGGAGGCCGTACAGAGTGGTTTG
ACGCGCGCGTACCCTCAGTTTAATTTGAGTTTCACTCACAGTGTCTATTCGGACC
ACCCTGCGGCGGCCGGTTCCCGTCTGTTGGAAAACGAAACTTTAGCCTCGATGGC
TAAATCTTCCTTTTCTGATATCGGGGGCTGCCCACTTTTTCACATAAAGAGGGGG
AGCACAGATTACCACGTGTGCAGACCGATTTACGACATGAAGGACGCTCAACGA
AGAGTTTCGAGGGAACTCCAAGCGAGAGGGCTGGTGGAAAACCTTTCCCGCGAA
CAACTTGTTGAAGCTCAGGCGCGCGTTTCGGTGTGCCCTCACACTCTCGGCAATT
GTAACGTGAAAAGCGACGTGCTAATCATGGTGCAGGTTTACGACGCTTCTTTGAA
CGAAATTGCGTCGGCGATGGTCCTAAAGGAATCGAAAGTCGCTTACCTCACCATG
GTGACTCCGGGTGAACTTCTAGATGAACGTGAAGCCTTCGCTATCGACGCTTTGG
GGTGCGACGTCGTCGTGGACACCCGCCGAGACATGGTGCAGTACAAATTCGGCT
CATCTTGTTACTGCCACAAACTGTCGAATATAAAGAATATAATGTTGACTCCCGC
ATTCACTTTCAGCGGGAATCTTTTTTCGGTCGAGATGTACGAAAACCGAATGGGC
GTCAACTACTACAAAATCACTCGTTCAGCGTATTCTCCTGAAATACGCGGCGTTA
AGACGCTGCGGTACCGCCGAGCCTGCACCGAGGTCGTGCAAGTTAAACTGCCAC
GCTTCGATAAAACTCTAAAGACGTTTCTCTCCGGGTATGATTACATATATTTGGA
TGCGAAATTCGTCTCTCGAGTCTTCGATTACGTGGTTAGCAACTGTAGCGTTGTTA
ACAGTAAAACCTTCGAGTGGGTGTGGAGTTACATAAAATCAAGTAAATCGCGCG
TTGTCATAAGTGGGAAAGTGATACATCGAGATGTGCATATCGACCTCAAACACTC
TGAATGTTTTGCGGCTGTGATGCTCGCGGTTGGGGTGCGTTCTCGCACAACTACT
GAATTTTTAGCGAAGAACCTCAATTATTACACTGGCGATGCTTCGTGCTTTGAGA
CTATCCGTTTCCTCTTTCGGGAGTGGAGCCGGAGGGCTTACGCGGAAATAAATCG
CAGTTTTCGAAAGCTTATGAAGAGCATTCTTTCCGCTGGGTTAGATTACGAATTT
CTCGATCTTGACAACTCGCTCCAACACTTGCTTGAATACTCAGAGGTTGAAGTGC
GCGTTTCAATCGCTCAGAATGGTGAGGTGGATTGCAACGAAGAGAACCGTGTTTT
GACGGAGATAATAGCCGAAGCAGCGGACAGGAAATCCATCGCTCAAGGTCTCAG
CGGAGCGTTGAGCTCCGTTCCAACGCAACCGAGAGGCGGTCTGAGAGGGGGTAG
TCGCCGAAGTGGAGTTTTTTTCCTTTATAACTTGGTAGAGGAAGTCGGAAATCTT
TTCTTTTCCGTCGGTGATGCCGTGCGGTTTCTTGTTAAGTTATTTAAAACTTTTTCC
GACTCTCCCATCTTTCGGGTCGTTCGGATGTTCCTGGACTTGGCGGAAGCAGCTT
CACCGTTCGTTTCGGTTGTTTCTTTGTGCGCGTGGTTGCGCGAAGCTGTAAGCGCT
TTCTCAGGCTGGGTTGCCGACAGAACGGTGTCGGAAAGTGTAAAAACCTTTGTAA
ACCGTACGGTAAAAAGGTTTCTAAACTTTATGTCTGCAAAAACCCTGACGAAAA
AATTTTTTAGGTTTTTCTTATCCGCTTCCGCCTTAGCTAAAACCGTTGTGAGGAAG
GCGAAGGT

Figure 13C

T-DNA Region of a miniBYV launch vector (SEQ ID NO: 2) (cont.)

GATTTTGGAAGCTTACTGGGAAGTGTGGTTCGAATCGATTCTTTCGGATAGTGGA
GAGTATAGTGCTGTTGAATTTTGTAGCAGCGTTGTGATCACACTGTTAACGAATT
CCGGTCGGCTGCTACCTGGGTTTAGTCCATCCGCTGTCATTACTGAAGTACTGCTT
GACCTAGCCACGAAAATATCCATTGAAGTTCTTCTAAAACAAATTTCTCCTGTCG
ACTCGACAGCTTCTTCGGCCTTATATCGCCGCGTCCTAAGCGAGATTCTGTCGAA
TTTTCGCACAATGGGCGAACACGGGATCTTCACTAAAGTCTTTTTACTCTGCGGG
TTTCTACCCGTGTTTGTTAGGAAGTGCGTCGCTCTGTGCGTTCCTGGTGACATGGC
GACGTACGCCCGGTTTCTCGAATACGGGGTCGACGATCTCTTCTTTTTGGGAAGG
TCCGTGAACTCGATTAAGAACTACCTATGTGTGGTTGCTGCCGGGTTGGTGGACT
CCATTGTCGATTCCGTTGTTTTGAAACTTTCCGGTGTCGCCAAAGAGCGAGTGCTT
GGTTTTAAATCAAAAATCATAAAAAATTTTTTAAACGTCTTTAGGAAGGCGAAAG
TCGTTACACGTACTTCTTCCAGCACGGACTTGTCGGAAGACGAATATTTTTCGTG
CGACGAAAGCAAACCCGGCTTGAGAGGCGGTTCCTCTAGGTTCACGCTTTCGCGA
TTGCTTGACATCTTTTTCAATTTCCTGAAAAGTTCAAAGCTCGTCATAGAGAATGC
GTGCTTTTCTGCTTACGAAAGGATTGAAAGGAACATGAAACTATACTTTTTTCCTT
TAAATTCTTCGGAAGAAGAAGCTCGCCGCTTAATTCGGTGCGCGGGAGACTTCGA
CTACTTAAGCGATAGCGCTTTCGACGAAGATGAAATGTTACGGCAAGCTTTCGAA
CAATACTATTCCAGTGACGACGAGAGTGTAACTTACGATGGAAAACCCACAGTT
CTCCGTAGTTACTTGAACGTGAGTAGAAGGTTCTTGGAAACGTTCTGTAACGGCC
CGAAGTTTTTCGTCAAAGTTTCGAATTACTTTAAAGCGCTGTATAGTCGTTTGCTC
CGTGTTCTACCGTGGGTAGACAGAAATCTTTCTGACTCGCCAGGACTTAAAGGAG
GTAACGAGAAAGCTCTTCTTGCGAAGTTCCTTAAAACCTGCGTCATAACCGCTTG
CGAATGTGTGTCGCAGATATGCTGCCTGCGTCTTATTCGCTTATGTTGGGGGACA
CCAGCGTGTGGTTTAGTTAGGTTATTTTACATAACTTATTCCGGTACTCGCGTGTT
GTCGCGTGTTGTGGTCGCGGTGGCTGTATGCCCTCTTTTGGTCAGAAACGAACTG
GATGGCTTGAGTGATGGATTAACCAACATGGGCGTTTCGGTTTTTCGTCGCTTGTT
CGTCGCCCTTCGGCGCGCGCTTTCGGCGTACTCTAATTCCGCCCTACGACGGAAG
ATTTTCGAATTCATTTTCGGAAACATTCACCACCCTTTTGATGTCGCAGTAATCGA
AACAAACGAAGTTGCGCCGGAACCTCTTTCACCGGAAGTGGACATTGATGTCGA
CTGCGACTTTGGTTCCGATTCAGAATCGGTTTCTTCAGATGAAGTCGCGTCGATTC
CCCGTCCAGGCTTACACGGTGGGAATAGGCGCTCTTCCAACTTTCTAACCTCTCT
CGTGAAGGTTGTTTTTAAGTTGGCTGGGCGCATTCCGCGTTTGCTTTTCCGTTTAC
GCAATTTTGTGGCGTACTTTGTTGAACGACGGCTGGCTTCAAAAAGGTTGAAGAC
GTTCATCGGTCTGGCCAGGCTGTTTGATAATTTCTCACTCACTTCGGTAGTTTACC
TTCTCCAAGAGTACGATTCAGTGCTGAACGCATTTATAGACGTTGAGCTAGTCCT
ACTTAACTCGGGTAGCGTGAATGTGCTACCTTTAGTTTCTTGGGTCAGGGGATCT
CTAACGAAGTTAGCGGAAGTGATCGTTGGTTCAGGCTTCGCTTCGTTTCTAGGAA
GGATGTGCTGTCGTGTGTCCGACTGGTGCTCCTCGTCTTCGAACGCCGGTTGTAA
CTTTATGAGTCCGGTTCGTACGAAAGGGAAGTTCGTTCCCCCTTCGTCTTCCGGTT
CAACCGCTTCGATGTATGAACGTCTCGAAGCTCTCGAGAGCGATATCCGCGAACA
CGTGCTTT

Figure 13D

T-DNA Region of a miniBYV launch vector (SEQ ID NO: 2) (cont.)

CCACGTGTCGTGTAGGAAGCGACGAAGAAGAGGAAAGGCCGAAAGAAGTGACA
GAACCAGGAATTGAACATACTTCTGAAGATGTTGTTCCCATTCGTTCACACTCGC
AACCCTTATCTGGAGGTGAATGTTCGTATTCTGAAGATCGTGAAGAGAATGAACG
AGCGAACCTGTTACCGCACGTTAGCAAAATCGTCAGCGAACGAAGGGGTTTGGA
GACCGCCCGTCGAAACAAACGTACTCTACGTGGTGTAAGCGAGTTTCTCAACGCT
ATTAATACTAGCAATGAGCAACCTAGGCCGATAATCGTTGACCACTCTCCTGAAT
CTCGCGCGTTGACCAACTCCGTGAGGGAATTCTATTACCTCCAGGAACTCGCTCT
TTTTGAGTTGAGTTGCAAACTTCGTGAGTATTACGATCAATTGAAGGTTGCGAAT
TTTAACAGACAAGAGTGTTTGTGCGACAAAGACGAAGACATGTTTGTTCTACGAG
CCGGACAAGGTGTAGTTTCCGGCAGAAACTCGAGGTTGCCTCTTAAGCATTTCAA
GGATCACGAATTTTGTTTTCGCTCTGGAGGGTTGGTCCCTTACGACGGTACCAGC
AGAGTGGACACCATTTTTCACACGCAAACGAATTTCGTTTCCGCGAACGCGCTTC
TTTCGGGCTATCTCTCCTATAGAACTTTCACTTTCACTAATTTGAGCGCTAACGTA
CTGCTGTACGAAGCTCCTCCAGGTGGTGGAAAGACCACGACTTTGATAAAGGTTT
TTTGTGAAACTTTTTCAAAAGTTAATTCGCTGATTTTAACGGCGAATAAGAGTTC
GCGAGAAGAAATACTTGCGAAGGTGAATCGCATCGTACTTGACGAAGGCGATAC
GCCTCTTCAGACGCGTGACAGGATTTTAACTATCGATTCTTATCTAATGAACAAC
AGAGGTTTGACGTGTAAGGTTTTGTACCTCGATGAGTGTTTCATGGTTCACGCTG
GAGCTGCTGTAGCTTGCATCGAATTCACCAAATGCGATTCAGCCATCTTATTTGG
AGACAGCAGGCAAATTCACTACATAGACCGTAACGAATTGGATACTGCTGTTCTT
TCAGATTTGAACCGTTTTGTCGATGATGAATCGAGAGTATATGGTGAAGTCTCAT
ACAGGTGTCCTTGGGACGTTTGTGCTTGGTTGTCAACTTTCTACCCGAAAACTGT
GGCCACTACCAACTTGGTTTCAGCCGGTCAATCTTCGATGCAAGTACGCGAGATT
GAAAGCGTAGACGACGTCGAATATTCCAGTGAATTCGTCTACTTGACTATGTTAC
AGTCAGAGAAGAAAGATCTGCTGAAATCTTTCGGCAAGAGGTCTCGTTCGAGCG
TTGAAAAACCCACGGTCTTAACAGTCCATGAAGCTCAAGGTGAAACCTACCGCA
AAGTTAACCTCGTCAGAACGAAATTTCAAGAGGACGATCCTTTTCGTAGCGAGA
ACCACATCACAGTGGCCTTGTCTAGGCATGTCGAGAGTCTGACCTATTCGGTCCT
GAGTAGTAAACGTGACGACGCAATAGCTCAAGCTATAGCGAAGGCGAAACAACT
TGTGGATGCCTATCGCGTTTACCCCACGTCATTTGGTGGGAGTACTCTTGATATTA
GTGTTAACCCTTCTACATCTGACAGGAGCAAATGTAAAGCTTCCTCTGCCCCTTA
CGAAGTTATAAACAGCTTCTTGGAGAGCGTGGTTCCGGGCACTACTTCAGTAGAC
TTTGGGGACGTTTCCGAAGAGATGGGCACTCAGGTTTTTGAGTCCGGTGCTGATA
ACGTTGTTATTCGTGATTCCGCACCTGTTAACAAGTCGACGGATCACGACCCGCA
GCGGGTTTAGCTCGATTCGCTCGCAGGCGATTCCTAAGAGGAAACCGTCGCTGCA
AGAGAATTTATACTCTTATGAGTCGCGTAATTACAACTTTACCGTTTGTGAACGTT
TTTCCGGACCGCAGGAGTTCGGACAGGCGATGGCGATGGTTATGTTGGAACGAA
GCTTTGACTTAGAGAAAGTTGCTAAAGTTAGAAGCGATGTGATCGCCATAACAG
AAAAAGGGGTGCGAACATGGATGTCAAAACGTGAACCTTCTCAGCTTAGGGCTC
TTAGTAGTGACTTACAAAAGCCTCTAAACTTGGAAGAGGAAATAACGACTTTTAA
GTTGATGGTTAA

Figure 13E

T-DNA Region of a miniBYV launch vector (SEQ ID NO: 2) (cont.)

GCGGGACGCGAAAGTCAAACTCGATTCGTCGTGTTTGGTGAAACACCCACCAGC
GCAGAATATAATGTTCCATCGCAAGGCGGTGAACGCGATTTTCTCGCCGTGTTTC
GACGAGTTTAAAAATAGAGTCATTACCTGTACGAATTCAAATATTGTTTTCTTTA
CCGAAATGACTAACTCTACTCTCGCGTCGATAGCGAAAGAGATGCTGGGGAGCG
AACACGTTTACAACGTTGGGGAAATAGACTTTTCGAAATTCGACAAATCCCAAG
ACGCTTTCATTAAGTCGTTTGAACGAACCTTGTATTCAGCGTTTGGTTTCGACGAA
GACCTGCTTGACGTGTGGATGCAAGGTGAATACACCAGCAACGCTACAACTCTG
GACGGTCAACTTTCTTTTTCCGTCGACAACCAGAGGAAATCGGGCGCTTCTAATA
CGTGGATTGGTAATTCCATCGTGACTCTTGGCATTTTGAGCATGTTCTATTACACC
AATCGATTTAAGGCTCTTTTCGTGTCCGGGGACGACTCTTTGATTTTCTCCGAATC
TCCTATTAGAAATTCAGCCGATGCGATGTGCACAGAACTCGGTTTTGAGACTAAG
TTTCTCACTCCGAGCGTCCCGTATTTCTGCTCAAAGTTTTTCGTTATGACCGGTCA
CGACGTTTTCTTCGTGCCCGACCCTTATAAACTTTTAGTGAAATTAGGAGCTTCTA
AGGATGAAGTGGACGATGAGTTTCTGTTTGAAGTGTTCACCTCTTTTCGCGATTT
AACGAAAGATTTAGTCGATGAAAGAGTGATCGAACTCTTGACGCATTTGGTTCAC
AGTAAGTACGGGTACGAAAGTGGTGATACGTACGCCGCCCTGTGTGCTATTCATT
GTATTCGTTCAAACTTTTCATCGTTCAAGAAATTGTACCCTAAAGTTAAGGGCTG
GGTCGTTCACTACGGTAAACTGAAGTTTGTGCTGCGCAAATTCGCGAACTGTTTT
CGCGAGAAGTTTGACACTGCTTTCGGCGAAGCGTACTTTCTTACTTACGACGAAA
CTTGAGACTGTGTGGTAACTCGGTTGTTGTTTTGTTTGTTTGTGTGTCTGTAGTCC
TACTTCGCGGTGATG**GGATCCTTAATTAAGGCGCGCCTGTACAGCTAGC**TAT**G**
GCCGGCCTCTAGAGAAGTGACGGAGGTGACCTACCTGCCGTGTAATTTAAGTCG
TCACAGAGTGACAACGGCACCAAGTGGTGCTTTGTGCGTATGTAAATTGCGAAGT
GAAAAAATTTTTTAAAAAAATTTTTCACACTCCTAGCGAAGTCCCGCTAGGAAGA
AAAAATAAGGGCCGTCAAGACATGATTCACATGTCTTCTGATGAGTCCGTGAGG
ACGAAACGGCCCGGG*gatcgttcaaacatttggcaataaagtttcttaagattgaatcctgttgccggtcttgcgatgatt*
atcatataatttctgttgaattacgttaagcatgtaataattaacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtc
ccgcaattatacatttaatacgcgatagaaaacaGaatatagcgcgcaaactaggataaattatcgcgcgcggtgtcatctatgtt
actagatcaagcttatcgataccgtcgacctcgaggggggcccggtaccaaaaccaccccagtacattaaaaacgtccgcaat
*gtgttattaagttgtctaagcgtcaatt*tgtttacaccacaatatatcctgcca

Figure 14

A. BYV CP Promoter (SEQ ID NO: 3)

CGTCAGGGTATGAGTTCCTCGGGGCAGATTTTCTAACTGCGACCAGCGTGTGTTT
GAACGATCACGAAAAAGCTATCGTACTACAGGCCTCAAGAGCTGCCATTGATAG
AGCAGTCTCTTCGTCGGTCGACGGGAAGATCGTCAGTCTTTTCGACCTCGGTCGT
CTTAGTTAACACAGTTACTAAGGTTCCATTTTATTATTGCATTGTTTTTCATTTAGT
GTAATCGTACTTGAG

B. BYSV CP Promoter (SEQ ID NO: 4)

AACCAAATGGTTTAAGGGCTTTTTGCGCCTCTTTAGAAGGAATGTACCTTTCTGT
AGCTCGACTGGGCCCGGACGCGTTCGGCACTAGGTCCGTTGGGAAGCGTGGTGC
GCCTTCAGGAAGCGAGTATTTAGGCGCCGATTTTCTGACCTCAACGTGTCCGTTA
ATGTCCGACCACGATCGTGCTGTAGCTTTGAGTGCATCTAGAAACGCACTCGACC
GATCTGCGGCGTCTCAAATTGATAAAAAGATGGTTAGTTTGTACGACTTCGGTAA
GGTAGTGTATACCTAGCAGCTTGCTTCTACCTGACACAGTTAAGAAGCGGCATAA
ATCGAAGCCAAACCCTAAATTTTGCAACTCGATCAATTGTAACCTAGAGCGAAGT
GCAATCAT

C. GLRaV2 CP Promoter (SEQ ID NO: 5)

GATGAAAAGTCTCACGAAGAAGTACAAACGAGTGAATGGTCTGCGTGCGTTCTG
TTGCGCGTGCGAAGATCTATATCTAACCGTCGCACCAATAATGTCAGAACGCTTT
AAGACTAAAGCCGTAGGGATGAAAGGTTTGCCTGTTGGAAAGGAATACTTAGGC
GCCGACTTTCTTTCGGGAACTAGCAAACTGATGAGCGATCACGACAGGGCGGTCT
CCATCGTTGCAGCGAAAAACGCTGTCGATCGTAGCGCTTTCACGGGTGGGGAGA
GAAAGATAGTTAGTTTGTATGATCTAGGGAGGTACTAAGCACGGTGTGCTATAGT
GCGTGCTATAATAATAAACACTAGTGCTTAAGTCGCGCAGAAGAAAACGCT

Figure 15

A. Lc Amino Acid Sequence (SEQ ID NO: 6)

MGFVLFSQLPSFLLVSTLLLFLVISHSCRAEIVLTQSPGTLSLSPGERATLSCRASQSVS
YSSLAWYQQKPGQAPSLLIYGASSRATGIPDRFSGSGSGPDFTLTISRLEPEDFAVYYC
QHYGNSPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC

B. Lc Nucleic Acid Sequence (SEQ ID NO: 7)

ATGGGATTTGTTCTCTTTTCACAATTGCCTTCATTTCTTCTTGTCTCTACACTTCTC
TTATTCCTAGTAATATCCCACTCTTGCCGTGCCGAAATTGTGCTTACTCAGTCTCC
AGGAACTCTTTCTCTTTCTCCAGGTGAAAGGGCTACTCTTTCTTGCAGGGCTTCTC
AGTCTGTGTCTTACTCTTCTCTTGCTTGGTATCAGCAAAAGCCAGGACAAGCTCC
ATCTCTTCTTATTTACGGTGCTTCTTCTAGGGCTACTGGTATTCCAGATAGGTTCT
CTGGATCTGGATCTGGTCCAGATTTCACTCTTACTATTTCTAGGCTTGAGCCAGAG
GATTTCGCTGTTTACTACTGCCAGCACTACGGAAATTCTCCATACACTTTCGGAC
AGGGAACTAAGCTTGAGATTAAGAGGACTGTGGCTGCTCCATCTGTGTTTATTTT
CCCACCATCTGATGAGCAACTTAAGTCTGGAACTGCTTCTGTTGTGTGCCTTCTTA
ACAACTTCTACCCAAGGGAAGCTAAGGTTCAGTGGAAAGTGGATAACGCTCTTC
AGTCTGGAAACTCTCAAGAGTCTGTGACTGAGCAGGATTCTAAGGATTCAACTTA
CTCTCTTTCTTCTACTCTTACTTTGTCTAAGGCTGATTACGAGAAGCACAAGGTTT
ACGCTTGCGAAGTTACTCATCAGGGACTTTCTTCTCCAGTGACTAAGTCTTTCAAC
AGGGGAGAGTGCTGA

Figure 16

A. Hc Amino Acid Sequence (SEQ ID NO: 8)

MGFVLFSQLPSFLLVSTLLLFLVISHSCRAQVQLVQSGAEVKKPGASVKVSCKASGY
TFTSNAIQWVRQAPGQRLEWVGWINGGDGNTKYSQKFQGRVTISRDISASTAYMEL
SSLRSEDTAVYYCARHRLQRGGFDPWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

B. Hc Nucleic Acid Sequence (SEQ ID NO: 9)

ATGGGATTTGTTCTCTTTTCACAATTGCCTTCATTTCTTCTTGTCTCTACACTTCTC
TTATTCCTAGTAATATCCCACTCTTGCCGTGCCCAGGTTCAGCTTGTTCAATCTGG
TGCTGAGGTTAAGAAACCTGGTGCTTCTGTTAAGGTGTCATGCAAGGCTTCAGGA
TACACTTTCACTTCTAACGCTATTCAGTGGGTTAGGCAAGCTCCAGGACAAAGAC
TTGAATGGGTTGGCTGGATTAACGGTGGAGATGGAAACACTAAGTACTCTCAGA
AGTTCCAGGGAAGGGTTACAATTTCTAGGGATATTTCTGCTTCTACTGCTTACAT
GGAACTTTCTTCTCTTAGATCTGAGGATACTGCTGTGTATTACTGCGCTAGGCATA
GACTTCAAAGGGGAGGATTTGATCCTTGGGGACAGGGAACTCTTGTGACTGTGTC
ATCTGCTTCAACTAAGGGACCATCTGTGTTTCCACTTGCTCCATCTTCTAAGTCTA
CTTCAGGTGGAACTGCTGCTCTTGGATGCCTTGTGAAGGATTACTTCCCAGAGCC
AGTTACTGTTTCTTGGAACTCTGGTGCTCTTACTTCTGGTGTTCACACTTTCCCAG
CTGTGCTTCAGTCATCTGGACTTTACTCACTTTCTTCAGTGGTGACTGTGCCTTCT
TCTTCTCTTGGAACTCAGACTTACATCTGCAACGTGAACCACAAGCCATCTAACA
CAAAAGTGGATAAGAGGGTGGAGCCAAAGTCTTGCGATAAGACTCATACTTGTC
CACCATGTCCAGCTCCAGAACTTCTTGGAGGACCTTCTGTGTTCCTTTTCCCACCA
AAGCCAAAGGATACTCTTATGATTTCTAGGACTCCAGAGGTTACATGCGTTGTGG
TTGATGTGTCTCATGAGGATCCAGAGGTGAAGTTCAACTGGTACGTGGATGGTGT
TGAGGTTCACAACGCTAAGACTAAGCCAAGGGAAGAGCAGTACAACTCTACTTA
CAGGGTTGTGTCTGTGCTTACTGTGCTTCACCAGGATTGGCTTAACGGAAAGGAA
TACAAGTGCAAGGTGTCAAACAAGGCTCTTCCAGCTCCAATTGAAAAGACTATTT
CTAAGGCTAAGGGACAACCTAGAGAGCCACAGGTTTACACTCTTCCACCATCTAG
GGAAGAGATGACTAAGAACCAGGTGTCACTTACTTGCTTGGTGAAGGGATTCTA
CCCATCTGATATTGCTGTTGAGTGGGAGTCTAATGGACAACCAGAGAACAACTAC
AAGACTACTCCACCAGTGCTTGATTCTGATGGATCTTTCTTCCTTTACTCTAAGCT
TACTGTGGATAAGTCTAGGTGGCAGCAGGGAAATGTTTTCTCTTGCTCTGTGATG
CATGAGGCTCTTCACAATCACTACACTCAGAAGTCTTTGTCTCTTTCTCCTGGAAA
GTGA

Figure 17A

A. PA83 Amino Acid Sequence (SEQ ID NO: 10)

MGFVLFSQLPSFLLVSTLLLFLVISHSCRAEVKQENRLLNESESSSQGLLGYYFSDLNF
QAPMVVTSSTTGDLSIPSSELENIPSENQYFQSAIWSGFIKVKKSDEYTFATSADNHVT
MWVDDQEVINKASNSNKIRLEKGRLYQIKIQYQRENPTEKGLDFKLYWTDSQNKKE
VISSDNLQLPELKQKSSNSRKKRSTSAGPTVPDRDNDGIPDSLEVEGYTVDVKNKRTF
LSPWISNIHEKKGLTKYKSSPEKWSTASDPYSDFEKVTGRIDKNVSPEARHPLVAAYP
IVHVDMENIILSKNEDQSTQNTDSETRTISKNTSTSRTHTSEVHGNAEVHASFFDIGGS
VSAGFSNSNSSTVAIDHSLSLAGERTWAETMGLNTADTARLNANIRYVNTGTAPIYN
VLPTTSLVLGKNQTLATIKAKENQLSQILAPNNYYPSKNLAPIALNAQDDFSSTPITM
NYNQFLELEKTKQLRLDTDQVYGNIATYNFENGRVRVDTGSNWSEVLPQIQETTARI
IFNGKDLNLVERRIAAVNPSDPLETTKPDMTLKEALKIAFGFNEPNGNLQYQGKDITE
FDFNFDQQTSQNIKNQLAELNATNIYTVLDKIKLNAKMNILIRDKRFHYDRNNIAVG
ADESVVKEAHREVINSSTEGLLLNIDKDIRKILSGYIVEIEDTEGLKEVINDRYDMLNIS
SLRQDGKTFIDFKKYNDKLPLYISNPNYKVNVYAVTKENTIINPSENGDTSTNGIKKIL
IFSKKGYEIGKL**HHHHHH***KDEL*

B. PA83 Nucleic Acid Sequence (SEQ ID NO: 11)

ATGGGATTTGTTCTCTTTTCACAATTGCCTTCATTTCTTCTTGTCTCTACACTTCTC
TTATTCCTAGTAATATCCCACTCTTGCCGTGCCGAGGTGAAGCAAGAGAATAGGC
TTCTTAATGAGTCTGAGTCATCTTCTCAGGGATTGCTTGGTTACTACTTCTCTGAT
CTTAATTTCCAGGCTCCTATGGTGGTTACTTCTTCTACTACTGGTGATCTTTCTATT
CCTTCTTCTGAGCTTGAGAATATTCCTTCTGAGAATCAGTACTTCCAGTCTGCTAT
TTGGAGTGGTTTCATTAAGGTGAAGAAATCTGATGAGTACACTTTCGCTACTTCA
GCTGATAATCATGTGACTATGTGGGTGGACGATCAGGAGGTGATCAATAAGGCT
TCTAATTCTAATAAGATTAGGCTTGAGAAGGGAAGGCTTTACCAGATTAAGATTC
AGTACCAGAGGGAAAATCCTACTGAGAAGGGTCTTGATTTCAAGTTGTACTGGA
CTGATTCACAGAATAAGAAAGAAGTGATTTCTTCTGATAATCTTCAGCTTCCTGA
GCTTAAGCAGAAGTCATCTAATTCTAGGAAGAAGAGGTCTACTTCTGCTGGTCCT
ACTGTTCCTGATAGGGATAATGATGGTATTCCTGATTCTCTTGAGGTGGAGGGTT
ACACTGTGGATGTGAAGAATAAGAGGACTTTCCTTTCTCCTTGGATTTCTAATATT
CATGAGAAGAAGGGTCTTACTAAGTACAAGTCATCTCCTGAGAAGTGGTCTACTG
CTTCTGATCCTTACTCTGATTTCGAGAAGGTGACAGGAAGGATTGATAAGAATGT
GTCTCCTGAGGCTAGACATCCTCTTGTTGCTGCTTACCCTATTGTGCATGTGGATA
TGGAGAATATTATTCTTTCTAAGAATGAGGATCAGTCTACTCAGAATACTGATTC
TGAGACTAGGACTATTTCTAAGAATACTTCTACTTCTAGGACTCATACTTCTGAA
GTGCATGGAAATGCTGAAGTTCATGCTTCTTTCTTCGATATTGGTGGTTCTGTGTC
TGCTGGTTTCTCTAATTCAAATTCTTCTACTGTGGCTATTGATCATTCTCTTTCTCT
TGCTGGTGAAAGGACTTGGGCTGAGACTATGGGACTTAATACTGCAGATACTGCT
AGGCTTAATGCTAATATTAGATACGTGAATACTGGTACTGCTCCTATCTACAATG
TGCTTCCTACTACTTCTTTGGTGCTTGGAAAGAATCAGACTCTTGCTACTATTA

Figure 17B

B. PA83 Nucleic Acid Sequence (SEQ ID NO: 11) (cont.)

AGGCTAAAGAGAATCAGCTTTCTCAGATTCTTGCTCCTAACAATTACTACCCTTCT
AAGAATCTTGCTCCAATTGCTCTTAATGCTCAGGATGATTTCTCTTCTACTCCTAT
TACTATGAATTACAATCAGTTCCTTGAGCTTGAAAAGACTAAGCAGCTTAGGCTT
GATACTGATCAGGTGTACGGTAATATTGCTACTTACAATTTCGAGAATGGTAGAG
TGAGAGTGGATACTGGTTCTAATTGGAGTGAGGTGTTGCCTCAGATTCAAGAGAC
TACTGCTAGGATTATTTTCAATGGAAAGGATCTTAATCTTGTGGAGAGAAGGATT
GCTGCTGTGAATCCTTCTGATCCTCTTGAGACTACTAAGCCTGATATGACTCTTAA
AGAGGCTCTTAAGATTGCTTTCGGTTTCAATGAGCCTAATGGAAATCTTCAGTAC
CAGGGAAAGGATATTACTGAGTTCGATTTCAATTTCGATCAGCAGACTTCTCAGA
ACATTAAGAACCAGCTAGCTGAGTTGAATGCTACTAATATCTACACTGTGTTGGA
TAAGATTAAGTTGAATGCAAAGATGAATATTCTTATTAGGGATAAGAGGTTCCAT
TACGATAGGAACAATATTGCTGTGGGTGCTGATGAGTCTGTTGTGAAAGAGGCTC
ATAGGGAAGTTATCAATTCTTCAACTGAGGGACTTCTTCTTAATATTGATAAGGA
TATTAGGAAGATTCTTTCTGGTTACATTGTGGAGATTGAGGATACTGAGGGTCTT
AAAGAAGTGATCAATGATAGATACGATATGTTGAATATTTCTTCTCTTAGGCAGG
ATGGAAAGACTTTCATTGATTTCAAAAAGTACAATGATAAGTTGCCTCTTTACAT
TTCTAATCCTAATTACAAAGTGAATGTGTACGCTGTGACAAAAGAGAACACTATT
ATCAATCCATCTGAGAATGGTGATACTTCTACTAATGGTATTAAGAAGATTTTGA
TTTTCTCTAAGAAGGGTTACGAGATTGGAAAGCTTCACCACCATCATCATCATAA
GGATGAACTTTGA

Figure 18

A. Pfs48 Amino Acid Sequence (SEQ ID NO: 12)

MGFVLFSQLPSFLLVSTLLLFLVISHSCRANNDFCKPSSLNSEISGFIGYKCNFSNEGVH
NLKPDMRERRSIFCTIHSYFIYDKIRLIIPKKSSSPEFKILPEKCFQKVYTDYENRVETDI
SELGLIEYEIEENDTNPNYNERTITISPFSPKDIEFFCFCDNTEKVISSIEGRSAMVHVRV
LKYPHNILFTNLTNDLFTYLPKTYNESNFVSNVLEVELNDGELFVLACELINKKCFQE
GKEKALYKSNKIIYHKNLTIFKAPFYVTSKDVNTECTCKFKNNNYKIVLKPKYEKKVI
HGCNFSSNVSSKHTFTDSLDISLVDDSAHISCNVHLSEPKYNHLVGLNCPGDIIPDCFF
QVYQPESEELEPSNIVYLDSQINIGDIEYYEDAEGDDKIKLFGIVGSIPKTTSFTCICKK
DKKSAYMTVTIDSA**HHHHHH***KDEL*

B. Pfs48 Nucleic Acid Sequence (SEQ ID NO: 13)

ATGGGATTCGTGCTTTTCTCTCAGCTTCCTTCTTTCCTTCTTGTGTCTACTCTTCTT
CTTTTCCTTGTGATTTCTCACTCTTGTAGGGCTAACAACGATTTCTGCAAGCCATC
TTCTCTTAACTCTGAGATTTCTGGATTCATTGGATACAAGTGCAACTTCTCTAACG
AGGGTGTTCACAACCTTAAGCCAGATATGAGAGAGAGAAGATCAATTTTCTGCA
CTATTCACTCTTACTTCATTTACGATAAGATTAGGCTTATTATTCCAAAGAAGTCA
TCTTCTCCAGAGTTCAAGATTCTTCCAGAGAAGTGCTTCCAGAAGGTGTACACTG
ATTACGAGAACAGGGTGGAGACTGATATTTCTGAGCTTGGACTTATTGAGTACGA
GATTGAAGAGAACGATACAAACCCAAACTACAACGAGAGGACTATTACTATTTC
TCCATTCTCTCCAAAGGATATTGAGTTCTTCTGCTTCTGCGATAACACTGAGAAA
GTGATTTCTTCTATTGAGGGAAGATCAGCTATGGTTCATGTGAGGGTGTTGAAGT
ACCCACACAACATTCTTTTCACTAACCTTACTAACGATCTTTTCACTTACTTGCCA
AAGACTTACAACGAGTCTAACTTCGTGTCTAACGTGCTTGAGGTGGAGCTTAATG
ATGGTGAGTTGTTCGTTCTTGCTTGCGAGCTTATTAACAAGAAGTGTTTCCAAGA
GGGAAAAGAGAAGGCTCTTTACAAGTCTAACAAGATTATTTACCACAAGAACCT
TACTATTTTCAAGGCTCCATTCTACGTGACTTCTAAGGATGTGAACACTGAGTGC
ACTTGCAAGTTCAAGAACAACAACTACAAGATTGTGCTTAAGCCAAAGTACGAG
AAGAAAGTGATTCACGGATGCAACTTCTCATCTAACGTGTCATCTAAGCACACTT
TCACTGATTCTCTTGATATTTCTCTTGTGGATGATTCTGCTCACATTTCTTGCAAC
GTGCACCTTTCTGAGCCAAAGTACAACCACCTTGTGGGACTTAATTGCCCAGGTG
ATATTATTCCAGATTGCTTCTTCCAGGTTTACCAACCAGAGTCTGAAGAACTTGA
GCCATCTAACATTGTGTACCTTGATTCTCAGATTAACATTGGAGATATTGAGTAC
TACGAGGATGCTGAGGGTGATGATAAGATTAAGTTGTTCGGAATTGTGGGATCTA
TTCCAAAGACTACTTCTTTCACTTGCATCTGCAAGAAGGATAAGAAATCTGCTTA
CATGACTGTGACTATTGATTCAGCTCATCACCATCACCACCACAAGGATGAGCTT
TGA

Figure 19

A. PNGaseF Amino Acid Sequence (SEQ ID NO: 14)

MGFVLFSQLPSFLLVSTLLLFLVISHSCRAAPADNTVNIKTFDKVKNAFGDGLSQSAE
GTFTFPADVTTVKTIKMFIKNECPNKTCDEWDRYANVYVKNKTTGEWYEIGRFITPY
WVGTEKLPRGLEIDVTDFKSLLSGNTELKIYTETCLAKGREYSVDFDIVYGTPDYKYS
AVVPVIQYNKSSIDGVPYGKAHTLGLKKNIQLPTNTEKAYLRTTISGWGHAKPYDAG
SRGCAEWCFRTHTIAINNANTFQHQLGALGCSANPINNQSPGNWAPDRAGWCPGMA
VPTRIDVLNNSLTGSTFSYEYKFQSWTNNGTNGDAFYAISSFVIAKSNTPISAPVVTN**D**
YKDDDD*KDEL*

B. PNGaseF Nucleic Acid Sequence (SEQ ID NO: 15)

ATGGGTTTCGTGCTGTTCTCTCAGCTTCCATCTTTCCTTTTGGTGTCTACCCTTCTT
CTGTTCCTTGTGATTTCTCATTCTTGCAGAGCTGCTCCAGCTGATAACACCGTGAA
CATTAAGACCTTCGATAAGGTGAAGAACGCTTTCGGTGATGGTCTTTCTCAATCT
GCTGAGGGAACTTTTACCTTCCCTGCTGATGTGACTACCGTTAAGACCATCAAGA
TGTTCATCAAGAACGAGTGCCCTAACAAGACTTGTGATGAGTGGGATAGGTACG
CTAATGTGTACGTGAAGAACAAGACTACTGGTGAGTGGTATGAGATCGGTAGAT
TCATTACTCCTTACTGGGTGGGAACTGAGAAGCTTCCTAGAGGTCTTGAGATTGA
TGTGACCGATTTCAAGTCTCTGCTGTCTGGTAATACCGAGCTTAAGATCTACACC
GAGACTTGTCTTGCTAAGGGTAGAGAGTACTCCGTTGATTTCGATATTGTGTACG
GAACCCCTGATTACAAGTACTCAGCTGTTGTTCCTGTGATCCAGTACAACAAGTC
TAGCATTGATGGTGTGCCATACGGTAAGGCTCATACTCTTGGTCTGAAGAAGAAC
ATTCAGTTGCCTACTAACACCGAGAAGGCTTATCTTAGGACCACTATTTCTGGTT
GGGGTCATGCTAAGCCTTATGATGCTGGTTCTAGAGGTTGTGCTGAGTGGTGTTT
TAGGACTCATACCATTGCTATCAACAACGCTAACACTTTCCAGCATCAGCTTGGT
GCTCTTGGTTGTTCTGCTAACCCTATTAACAACCAGTCTCCTGGTAATTGGGCTCC
TGATAGAGCTGGTTGGTGTCCTGGTATGGCTGTTCCTACTAGGATTGATGTGCTG
AACAACTCTCTTACCGGTTCTACATTCAGCTACGAGTACAAGTTCCAGTCTTGGA
CTAACAACGGTACTAACGGTGATGCTTTCTACGCTATTAGCTCTTTCGTGATCGCT
AAGTCTAATACCCCTATTTCTGCTCCTGTGGTGACCAATGATTACAAGGATGATG
ATGATAAGGATGAGCTTTAG

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/055365

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	V. V. PEREMYSLOV: "HSP70 homolog functions in cell-to-cell movement of a plant virus", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 96, no. 26, 21 December 1999 (1999-12-21), pages 14771-14776, XP55015320, ISSN: 0027-8424, DOI: 10.1073/pnas.96.26.14771	1-5,13, 15-22
Y	paragraph bridging column 1 and 2; page 14772	6-12,14, 23-42

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[X] Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 December 2011	29/12/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Blanco Urgoiti, B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/055365

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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