(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2013 (27.12.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/192613 A2

(51) International Patent Classification:
A01H 5/00 (2006.01) *C12N 15/82* (2006.01)

(21) International Application Number:
PCT/US2013/047329

(22) International Filing Date:
24 June 2013 (24.06.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/663,467 22 June 2012 (22.06.2012) US
61/783,342 14 March 2013 (14.03.2013) US

(71) Applicant: **J.R. SIMPLOT COMPANY** [US/US]; 999 Main Street, Suite 1300, Boise, Idaho 83702 (US).

(72) Inventors: **BARBEY, Chris**; c/o J.R. SIMPLOT COMPANY, 999 Main Street, Suite 1300, Boise, Idaho 83702 (US). **ROMMENS, Caius**; c/o J.R. SIMPLOT COMPANY, 999 Main Street, Suite 1300, Boise, Idaho 83702 (US).

(74) Agents: **VEITENHEIMER, Erich E.** et al.; Cooley LLP, 1299 Pennsylvania Avenue, N.W., Suite 700, Washington, District of Columbia 20004-2400 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2013/192613 A2

(54) Title: PVY RESISTANCE

(57) Abstract: The present invention concerns new compositions and methods for conferring resistance to Potato virus Y (PVY) in plants by expressing nucleotide sequences that share homology to sequences in both PVY and Solanum.

PVY RESISTANCE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/663,467, filed June 22, 2012, and U.S. Provisional Patent Application Serial No. 61/783,342, filed March 14, 2013, both of which are hereby incorporated by reference in their entireties for all purposes.

FIELD

The present inventive technology relates to the expression of recombined genetic elements from potato plants, e.g., *Solanum phureja*, and/or recombined genetic elements from Potato Virus Y (PVY) to confer broad-spectrum Potato Virus Y (PVY) resistance to intragenic plants.

BACKGROUND

Potato virus Y (PVY), a member of the family Potyviridae, is a virus that affects potato production worldwide. Necrotic ringspot disease symptoms and reduced tuber yields caused by PVY infection results in substantial production losses. Some PVY resistant and tolerant varieties have been developed through traditional plant breeding, but none of these varieties were widely adopted because they lack important traits required by the industry. Efforts to introgress PVY resistance into the most popular cultivars are complicated by complex autotetraploid genetics, numerous prezygotic and postzygotic barriers, and inbreeding depression.

Previous efforts to develop PVY resistance in transgenic potato plants focused on the expression of pathogen-derived sequences, such as the viral coat protein gene (Lawson et al., 1990, Biotechnol J 8:127-134) and the helper component proteases gene (Arif et al., 2011, Transgenic Res 21: 303-11). The permanent introduction of such foreign DNA in the food supply raises public concerns.

SUMMARY

One aspect of the present invention is an isolated polynucleotide comprising multiple plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y. In one embodiment, the isolated polynucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 plant DNA sequences. In

one embodiment, each plant DNA sequence of the polynucleotide shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, sequence homology with a PVY DNA sequence. In one embodiment, the plant DNA sequences are potato plant DNA sequences. In one embodiment, each plant DNA sequence of the polynucleotide shares at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, sequence homology to potato, and is selected from the group consisting of Ranger Russet, Russet Burbank, Atlantic, Phureja and Shepody. In another embodiment, the plant DNA sequences within the polynucleotide are sequences from the genome of the same potato variety. In another embodiment, the plant DNA sequences within the polynucleotide are sequences from multiple genomes of different potato varieties. In one embodiment, the polynucleotide comprises potato plant DNA sequences selected from one or more of sequences endogenous to Ranger Russet, Russet Burbank, Atlantic, and Shepody potato varieties. In another embodiment the DNA source is a wild potato species such as *Solanum phureja*, *Solanum microdontum*, *Solanum bulbocastanum*. In one embodiment, the polynucleotide in the vector comprises one or more sequences selected from the group consisting of SEQ ID NOs. 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103.

Another aspect of the present invention is an isolated polynucleotide comprising multiple viral DNA sequences. In one embodiment, each viral DNA sequence is derived from the same and/or different strain of Potato Virus Y, wherein each viral DNA sequence shares high sequence homology to a DNA sequence from the genome(s) of a plant, for example, a potato plant. In one embodiment, each viral DNA sequence of the polynucleotide shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, sequence homology with a plant DNA sequence. In one embodiment, the isolated polynucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 viral DNA sequences. In another embodiment, the viral DNA sequences share high homology to the DNA sequences from the genome of the same potato variety. In another embodiment, the viral DNA sequences share high homology to sequences from multiple genomes of different potato varieties. In one embodiment, the potato is selected from the group consisting of Ranger Russet, Russet Burbank, Atlantic, Phureja and Shepody. In another embodiment the potato plant is a wild potato species such as *Solanum phureja*, *Solanum microdontum*, or *Solanum bulbocastanum*.

Another aspect of the present invention is an expression vector comprising a promoter operably linked to a polynucleotide comprising multiple plant DNA sequences, wherein each plant

DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y. In one embodiment, the polynucleotide in the vector comprises one or more sequences selected from the group consisting of SEQ ID NOs. 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103.

Another aspect of the present invention is an expression vector comprising a promoter operably linked to a polynucleotide comprising multiple viral DNA sequences, wherein viral DNA sequence shares high sequence homology to a DNA sequence from the genome(s) of one or more plant species/varieties, such as one or more potato species/varieties. In one embodiment, the polynucleotide in the vector comprises one or more sequences selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57.

Another aspect of the present invention is a method for conferring full or partial resistance in a plant to at least one strain of Potato Virus Y infection, comprising expressing in a plant a polynucleotide that comprises multiple plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y, wherein the plant is fully or partially resistant to PVY infection compared to a plant of the same species that does not express the polynucleotide. In one embodiment, the plant is a potato plant. In one embodiment, the potato is selected from the group consisting of Ranger Russet, Russet Burbank, Atlantic, Phureja and Shepody. In another embodiment, the plant DNA sequences within the polynucleotide are sequences from the genome of the same potato variety. In another embodiment, the plant DNA sequences within the polynucleotide are sequences from multiple genomes of different potato varieties. In another embodiment the sequences are from a wild potato species such as *Solanum phureja*, *Solanum microdontum*, *Solanum bulbocastanum*. In one embodiment, the polynucleotide comprises potato plant DNA sequences selected from one or more of sequences endogenous to Ranger Russet, Russet Burbank, Atlantic, Shepody potato varieties, or a wild potato species. In one embodiment of this method, the plant DNA sequences are potato plant DNA sequences. In another embodiment of this method, the plant is a potato plant and the plant DNA sequences are potato plant DNA sequences. In one embodiment, the polynucleotide in the vector comprises one or more sequences selected from the group consisting of SEQ ID NOs. 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103.

Another aspect of the present invention is a method for conferring full or partial resistance in a plant to at least one strain of Potato Virus Y infection, comprising expressing in a plant a polynucleotide that comprises multiple viral DNA sequences, wherein each viral DNA sequence shares sequence homology to a DNA sequence from the genome(s) of a plant, wherein the plant is fully or partially resistant to PVY infection compared to a plant of the same species that does not express the polynucleotide. In one embodiment, the plant is a potato plant. In one embodiment, the potato is selected from the group consisting of Ranger Russet, Russet Burbank, Atlantic, Phureja and Shepody. In another embodiment, the viral DNA sequences within the polynucleotide are sequences sharing homology to the genome of the same potato species/variety. In another embodiment, the viral DNA sequences within the polynucleotide are sequences sharing homology to multiple genomes of different potato species/varieties. In another embodiment the sequences share homology to the genome of a wild potato species such as *Solanum phureja*, *Solanum microdontum*, *Solanum bulbocastanum*. In one embodiment, the polynucleotide shares homology to a sequence comprises potato plant DNA sequences selected from one or more of sequences endogenous to Ranger Russet, Russet Burbank, Atlantic, Shepody potato varieties, or a wild potato species. In one embodiment of this method, the plant DNA sequences are potato plant DNA sequences. In one embodiment of this method, the polynucleotide comprises one or more sequences selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57.

In one embodiment, a polynucleotide of the present invention comprises at least two sequences selected from the SEQ ID NOs.: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. In another embodiment, the polynucleotide comprises the sequence of SEQ ID NO: 13.

In one embodiment, a polynucleotide of the present invention comprises at least two sequences selected from the SEQ ID NOs.: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, and 71.

In one embodiment, a polynucleotide of the present invention comprises at least two sequences selected from the SEQ ID NOs.: 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25. In another embodiment, the polynucleotide comprises the sequence of SEQ ID NO: 26.

In one embodiment, a polynucleotide of the present invention comprises at least two sequences selected from the SEQ ID NOs.: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, and 83, or at least two sequences selected from the SEQ ID NOs.: 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, and 95, or at least two sequences selected from the SEQ ID NOs.: 96, 97, 98, 99, 100, 101, 102, and 103. In one

embodiment, a polynucleotide of the present invention comprises at least two sequences selected from the SEQ ID NOs.: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, and 38. In another embodiment, the polynucleotide comprises the sequence of SEQ ID NO: 39.

In one embodiment, a polynucleotide of the present invention comprises at least two sequences selected from the SEQ ID NOs.: 45, 48, 53, and 57. In another embodiment, the polynucleotide comprises the sequence of SEQ ID NO: 58.

In one embodiment, a polynucleotide of the present invention comprises one or more sequences selected from the group consisting of: (1) complements of a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57; (2) reverse complements of a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57; (3) reverse sequences a sequence of1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57; (4) a portion of a nucleotide sequence comprising at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of a nucleotide sequence recited in (1) –(3); (5) a nucleotide sequence having at least 95% identity to a nucleotide sequence recited in (1) –(3); (6) a nucleotide sequence having an E value of 0.01 when aligned with a sequence recited in (1) –(3); and (7) a nucleotide sequences that hybridize to a sequence recited in (1) –(3) under stringent conditions, wherein said stringent conditions are hybridization in 0.25 M Na2HPO4 buffer (pH 7.2) containing 1 mM Na2EDTA, 20% sodium dodecyl sulfate at 45°C, followed by a wash in 5×SSC, containing 0.1% (w/v) sodium dodecyl sulfate, at 55°C to 65°C.

In one embodiment, a polynucleotide of the present invention comprises one or more sequences selected from the group consisting of: (1) complements of a sequence of SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103; (2) reverse complements of a sequence of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103; (3) reverse sequences a sequence of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103; (4) a portion of a nucleotide sequence comprising at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of a nucleotide sequence recited in (1) –(3); (5) a nucleotide sequence having at least 95% identity to a nucleotide sequence

recited in (1)--(3); (6) a nucleotide sequence having an E value of 0.01 when aligned with a sequence recited in (1)--(3); and (7) a nucleotide sequence that hybridizes to a sequence recited in (1)--(3) under stringent conditions, wherein said stringent conditions are hybridization in 0.25 M Na2HPO4 buffer (pH 7.2) containing 1 mM Na2EDTA, 20% sodium dodecyl sulfate at 45°C, followed by a wash in 5×SSC, containing 0.1% (w/v) sodium dodecyl sulfate, at 55°C to 65°C.

In one embodiment, a polynucleotide of the present invention comprises (1) one or more plant DNA sequence, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of a virus, such as Potato Virus Y; and (2) one or more viral DNA sequences, wherein each viral DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one plant, such as a potato plant. In one embodiment, each plant DNA sequence shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more sequence homology with a PVY DNA sequence. In one embodiment, the polynucleotide comprises at least one sequence selected from SEQ ID NOs.: 60-83, or reverse complements thereof. In one embodiment, each viral DNA sequence shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more sequence homology with a potato plant DNA sequence. In one embodiment, the polynucleotide comprises at least one sequence selected from SEQ ID NOs. 1-38, 45, 48, 53, 57, and 58, or reverse complements thereof.

The present invention also provides expression vectors. In one embodiment, an expression vector of the present invention comprises a polynucleotide described herein.

The present invention also provides transgenic cells comprising a polynucleotide described herein, or organisms comprising said transgenic cells. In one embodiment, provided are transgenic plants, plant parts, or plant cells. Also provided are methods of making and using said transgenic plants, plant parts, or plant cells. In one embodiment, the plant parts include potato tubers or microtubers.

In one embodiment, seeds of the transgenic plants are provided, wherein said seed comprises said isolated polynucleotide.

In one embodiment, progeny plants of the transgenic plants are provided, wherein the progeny plants have an altered pathogen tolerance and/or resistance as a result of inheriting the polynucleotide.

The present invention also provides methods for producing hybrid seeds. In one embodiment, the methods comprise crossing at least one transgenic plant or a progeny plant of the present invention with a second plant. In one embodiment, the second plant is a different plant of the same species, or a related species. In one embodiment, the second plant is a different plant of the same variety, or a different variety. In one embodiment, the methods comprise harvesting the resultant seed.

The present invention also provides methods for producing a plant having altered pathogen tolerance and/or resistance. In one embodiment, the methods comprise transforming a plant cell with an expression vector to provide a transgenic cell. In one embodiment, the expression vector comprises: (i) a promoter sequence; (ii) an isolated polynucleotide sequence of the present invention; and (iii) a gene termination sequence. In one embodiment, the methods further comprise cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth of a plant having altered pathogen tolerance and/or resistance.

The present invention also provides methods for modifying a phenotype of a target organism. In one embodiment, the methods comprise stably incorporating into the genome of the target organism a genetic construct. In one embodiment, the genetic construct comprises: (a) a promoter sequence; (b) an isolated polynucleotide sequence of the present invention; and (c) a gene termination sequence. In one embodiment, the target organism is a plant.

The present invention also provides processes of determining the presence or absence of an isolated polynucleotide sequence of the present invention, or fragments and variations thereof in a plant. In one embodiment, the processes are based on DNA hybridization, RNA hybridization, and/or protein hybridization. In one embodiment, the processes comprise at least one of: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; (b) isolating nucleic acid molecules from said plant and performing a hybridization to detect the polynucleotide; and (c) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

The present invention also provides methods for breeding plants to produce altered pathogen tolerance and/or resistance. In one embodiment, the methods comprise making a cross between a first plant with an isolated polynucleotide sequence of the present invention with a second plant to produce a F1 plant. In one embodiment, the methods further comprise backcrossing the F1 plant to the second plant. Still in one embodiment, the methods further comprise repeating the backcrossing step to generate a near isogenic or isogenic line, wherein the isolated

polynucleotide sequence of the present invention is integrated into the genome of the second plant and the near isogenic or isogenic line derived from the second plant with the isolated polynucleotide sequence has conferred or enhanced pathogen tolerance and/or resistance compared to that of the second plant without the isolated polynucleotide sequence.

DETAILED DESCRIPTION

A new approach for conferring resistance to Potato virus Y (PVY) in plants is described herein and is based upon the expression of nucleotide sequences that are endogenous to the genomes of both PVY and species of *Solanum*. Genetically engineered plants that are resistant to PVY and which express polynucleotides obtained from within the plant's own gene pool, as is the case for conventional plant breeding are highly desirable (Rommens CM, Haring MA, Swords K, Davies HV, Belknap WR (2007) The intragenic approach as a new extension to traditional plant breeding. Trends Plant Sci 12: 397-403, which is incorporated herein by reference in its entirety).

According to the present invention, therefore, multiple discontiguous regions of intergenic and/or intragenic DNA from the wild potato species *Solanum phureja* are recombined, operably linked to a promoter and terminator, and transformed into a cultivated plant susceptible to potato virus Y (PVY). In one embodiment, the promoter is selected from the group consisting of constitutive promoters, non-constitutive promoters, inducible promoters, tissue-specific promoters, and developmental stage-specific promoters. In one embodiment, the tissue-specific promoter is a leaf-specific promoter or a shoot-specific promoter, such as the promoters associated with ribulose 1,5-bisphosphate carboxylase (RUBISCO), chlorophyll A/B binding protein (CAB), rubisco activase (RA), early light inducible protein (ELIP), glyoxysomal malate dehydrogenase (gMDH), and those described in U.S. Pat. No. 7534934 and U.S. Pat. Appl. Publ. Nos. 20080301837 and 20090220670, each of which is incorporated by reference in its entirety. In one embodiment, the tissue-specific promoter does not drive gene expression in the tuber.

Alternatively, another way to introduce resistance to virus into plants while reducing the public concerns of permanent introduction of foreign DNA into plants is to use viral sequences which share high homology to plant genome sequences. Due to the high homology to plant genome sequences, such viral sequences are more likely to be deemed safe and therefore be perceived as being more acceptable to the public. Therefore, multiple discontiguous regions of DNA from a virus that share high homology to the DNA of potato plants, such as the wild potato species *Solanum*

phureja can be recombined, operably linked to a promoter and terminator, and transformed into a cultivated plant susceptible to potato virus Y (PVY). In one embodiment, the promoter is selected from the group consisting of constitutive promoters, non-constitutive promoters, inducible promoters, tissue-specific promoters, and developmental stage-specific promoters. In one embodiment, the tissue-specific promoter is a leaf-specific promoter or a shoot-specific promoter, such as the promoters associated with ribulose 1,5-bisphosphate carboxylase (RUBISCO), chlorophyll A/B binding protein (CAB), rubisco activase (RA), early light inducible protein (ELIP), glyoxysomal malate dehydrogenase (gMDH), and those described in U.S. Pat. No. 7534934 and U.S. Pat. Appl. Publ. Nos. 20080301837 and 20090220670, each of which is incorporated by reference in its entirety. In one embodiment, the tissue-specific promoter does not drive gene expression in the tuber.

Expression of the cassette into plants results in the production of RNA transcripts that target the PVY genome for degradation through various mechanisms, such as RNA interference. The development of PVY resistance through expression of several discontiguous endogenous DNA elements as single DNA element to target multiple and specific regions of a viral polyribonucleotide while reducing the public's concern of permanent introduction of foreign DNA in to plants is a novel and inventive approach to conferring PVY resistance to a plant.Accordingly, the present invention encompasses methods for identifying in a plant genome endogenous one or more nucleotide sequences that share sequence identity with a corresponding sequence or sequences endogenous to the PVY genome. Alternatively, the present invention encompasses methods for identifying one or more viral genome nucleotide sequences that share high (e.g., ≥90%) or very high (e.g., ≥95%) homology with a sequence or sequences endogenous to the plant genome. One way this can be done is by aligning publicly available PVY sequences against plant DNA databases, such as GenBank. The alignment can then be analyzed for regions of conservation among the aligned sequences. The genome sequence information of potato species, such as *S. tuberosum* and *S. phureja,* is publicly available and can be obtained through the Potato Genome Sequencing Consortium database. The tomato genome sequence is recently released by the Tomato Genome Consortium (Nature, 2012, 485:635–641). The genome sequence information for PVY is described in Thole et al. (Gene, Volume 123, Issue 2, 30 January 1993, Pages 149–156), Robaglia et al. (J. gen. Virol. 1989, 70:935-947), and Lorenzen et al. (Arch. Virol. 2006, 151:1055-1074), each of which is incorporated by reference in its entirety.

In one embodiment, the endogenous nucleotide sequences in the plant genome are intergenic sequences. In one embodiment, the intergenic sequences do not encode any functional

polypeptides. In some embodiments, the endogenous nucleotide sequences in the plant genome are intragenic sequences (e.g., cis-elements, exons, introns, 5' or 3' terminal sequences). In some embodiments, the endogenous nucleotide sequences in the plant genome encode functional polypeptides which when suppressed do not bring any undesired plant phenotypes.

Regions of high genetic conservation among PVY strains can also be searched for PVY sub-regions of high homology, e.g., greater than, for instance, at least 90% homology, to the sequenced and publicly available plant genome, such as to the *Solanum Phureja* genome provided by the Potato Genome Sequence Consortium. The skilled person knows that alignment programs such as those provided by the NCBI BLAST analysis, is useful in this regard. These highly identical PVY sub-regions can then be analyzed for homology with potato cDNAs, and desired sequences sharing homology to any cDNA can be identified and prioritized.

These prioritized sequences therefore are sequences that are highly identical to sequences represented by the genomes of the various PVY strains, and also highly (e.g., ≥90%) or very highly (e.g., ≥95%) identical to sequences represented by the genomes of the various plant species/varieties. In one aspect of the present invention, one or more of these sequences can be used in an expression vector. In one embodiment, two or more expression vectors each comprising at least one of the prioritized sequences can be introduced into a plant. In one embodiment, a binary vector system is used, such as those described in U.S. Pat. No. 7923600, which is incorporated by reference in its entirety.

In one aspect of the present invention, two or more of these sequences can be joined together to yield a single polynucleotide within which two or more plant-derived, PVY-homologous sub-sequences, or two or more virus-derived, plant homologous sub-sequences, or at least one plant-derived, PVY-homologous sub-sequence and at least one virus-derived, plant homologous sub-sequence. Accordingly, in one polynucleotide there may be sequences derived from plants that are identical to, or highly or very highly homologous to, sequences present in one or more strains of PVY. Alternatively, in one polynucleotide there may be sequences derived from virus that are identical to, or highly or very highly homologous to, sequences present in one or more plant species/varieties. In other embodiments, in one polynucleotide there may be sequences derived from plants that are identical to, or highly or very highly homologous to, sequences present in one or more strains of PVY, and sequences derived from virus that are identical to, or highly or very highly homologous to, sequences present in one or more plant species/varieties.

In one embodiment, the two or more sequences can be joined together in any order, in any way, by any method as suitable. In one embodiment, one or more copies of each sequence are joined. In one embodiment, each sequence is directly joined. In one embodiment, an "insulator" sequence is included between the joined sequences. In one embodiment, the "insulator" sequence does not share any homology or very little homology to any sequence of the plant and/or PVY. In one embodiment, the single polynucleotide produces at least one interference RNA against PVY when transcribed. In one embodiment, the single polynucleotide comprises one or more inverted repeats, antisenses, and/or hairpin structures.

"Resistance": a resistant plant is able to limit PVY proliferation and/or PVY-caused disease symptom development when compared to a plant that is not resistant. Thus, "resistance" is a relative concept. The transgenic resistant plants described in this invention are able to limit PVY proliferation and/or PVY-caused disease symptom development compared to their untransformed counterparts. For example, the plants show no symptoms or show some symptoms but that are still able to produce marketable product with an acceptable yield. As defined by the International Seed Federation (ISF), the recognition of whether a plant is affected by or subject to a pest or pathogen can depend on the analytical method employed. Resistant plant types may still exhibit some disease symptoms or damage. The resistance can be either high (e.g., the growth and development of the specified pest or pathogen is highly restricted under normal pest or pathogen pressure when compared to susceptible varieties), or moderate/intermediate (e.g., the growth and development of the specified pest or pathogen is restricted, but the plants exhibit a greater range of symptoms or damage compared to plant types with high resistance). A plant is resistant to the virus strain if it has a virus RNA and/or protein density that is about 50%, about 40%, about 30%, about 20%, about 10%, about 5%, about 2%, about 1%, about 0.1%, about 0.01%, about 0.001%, or about 0.0001% of the RNA and/or protein density in a control plant.

The term "full resistance" in the context of the present invention means that, after being infected with the PVY virus, no viral proliferation is detected and/or no PVY-caused symptoms are observed the entire length of an experiment, which is usually 2-4 weeks.

The term "partial resistance" in the context of the present invention means that, the plants have reduced multiplication of the virus in the cell, as reduced (systemic) movement of the virus, and/or as reduced symptom development after infection compared to susceptible plants. The resistance is often referred to as "intermediate resistance".

The term "delayed disease progression" or "delayed disease symptoms" or "to resist the onset of one or more symptoms of PVY disease," or "partial resistance" in the context of the present invention means that PVY proliferation and/or PVY-caused disease symptom development is limited in a plant compared to another plant, whereby limited should be interpreted here to mean that PVY proliferation and/or PVY-caused disease symptom development is not fully prevented. For example, the transformed plant may display disease symptoms 1-3 days, 3-5 days, 5-7 days, 7-9 days, 9-11 days, 11-13 days, 13-15 days, 2-3 weeks, 3-4 weeks, 4-5 weeks, 5-7 weeks, 7-10 weeks, 2-3 months and 3-5 months later than the untransformed plant. Transformed plants with delayed disease progression typically carry the PVY virus protein upon infection. The PVY virus protein can be detected via ELISA assay. The length of the symptom delay varies.

Orthologs and paralogs of the sequences disclosed herein are also part of the invention. The term "ortholog" refers to genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes. The term "paralog" refers to genes related by duplication within a genome. While orthologs generally retain the same function in the course of evolution, paralogs can evolve new functions, even if these are related to the original one.

PVY: Potato virus Y (PVY) is one of the most prevalent and important viruses in potatoes. Recently, strains of PVY which can cause necrosis (dead spots on leaves and in tubers) have been discovered, creating more concern about this widespread virus. PVY is a Potyvirus, the type member of the largest group of plant viruses. It is transmitted by aphids in a nonpersistent manner, by sticking to aphid mouthparts (stylet). The virus can be acquired from the infected plant within seconds, and transmitted to a healthy plant just as fast. PVY can also be transmitted mechanically by machinery, tools, and damaging plants while walking through the field. Aphids are by far the most efficient means of transmission. Several strains of PVY have been identified that differ by the symptoms they cause in potatoes and tobacco. PVY^{O} is the common strain, and causes mosaic symptoms. PVY^{C} causes stipple streak. PVY^{N}, the necrotic strain, generally causes mild foliage symptoms, but necrosis in the leaves of susceptible potato varieties. Mixed infections of common strains and the necrotic strain are common, and the genomes (genetic material) can mix, producing hybrid strains (i.e. $PVY^{N:O}$ and PVY^{NTN}). PVY^{NTN} strains can cause tuber necrosis, and are of increasing importance in New York. Diagnosis can be difficult, because there are antibodies to PVY^{O} and PVY^{N}, but immunological methods (ELISA, Enzyme Linked Immunosorbent Assay) cannot distinguish PVY^{NTN} from these two virus strains.

Examples of PVY sequences are provided in GenBank accession numbers: X97895, X12456, M95491 D00441, U09509, NC_001616, JN034046, JF928460, JF928459, JF928458, JF795485, HQ912915, HQ912914, HQ912913, HQ912912, HQ912911, HQ912910, HQ912909, HQ912908, HQ912907, HQ912906, HQ912905, HQ912904, HQ912903, HQ912902, HQ912901, HQ912900, HQ912899, HQ912898, HQ912897, HQ912896, HQ912895, HQ912894, HQ912893, HQ912892, HQ912891, HQ912890, HQ912889, HQ912888, HQ912887, HQ912886, HQ912885, HQ912884, HQ912883, HQ912882, HQ912881, HQ912880, HQ912879, HQ912878, HQ912877, HQ912876, HQ912875, HQ912874, HQ912873, HQ912872, HQ912871, HQ912870, HQ912869, HQ912868, HQ912867, HQ912866, HQ912865, HQ912864, HQ912863, HQ912862, HQ631374, HM991454, HM991453, HM590407, HM590406, HM590405, HM367076, HM367075, GQ200836, FJ666337, FJ643479, FJ643478, FJ643477, FJ214726, FJ204166, FJ204165, FJ204164, EU563512, EU482153, EU182576, EF558545, EF026076, EF026075, EF026074, EF016294, DQ309028, DQ157180, DQ157179, DQ157178, DQ008213, AY884985, AY884984, AY884983, AY884982, AY745492, AY745491, AY166867, AY166866, AM268435, AM113988, AJ890350, AJ890349, AJ890348, AJ890347, AJ890346, AJ890345, AJ890344, AJ890343, AJ890342, AJ889868, AJ889867, AJ889866, AJ585342, AJ585198, AJ585197, AJ585196, AJ585195, AJ584851, AJ439545, AJ439544, AF522296, AF463399, AF237963, AB461454, AB461453, AB461452, AB461451, AB461450, AB331519, AB331518, AB331517, AB331516, AB331515, AB270705, AB185833, A08776

The polynucleotides of the present invention can be introduced into a plant by any suitable method, such as *Agrobacterium*-mediated transformation, viral infection, whiskers, electroporation, microinjection, polyethylene glycol-treatment, heat shock, lipofection and particle bombardment. "Transformation" of a plant is a process by which DNA is stably integrated into the genome of a plant cell. "Stably" refers to the permanent, or non-transient retention and/or expression of a polynucleotide in and by a cell genome. Thus, a stably integrated polynucleotide is one that is a fixture within a transformed cell genome and can be replicated and propagated through successive progeny of the cell or resultant transformed plant. Transformation may occur under natural or artificial conditions using various methods well known in the art. See, for instance, METHODS IN PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY, Bernard R. Glick and John E. Thompson (eds), CRC Press, Inc., London (1993); Chilton, Scientific American, 248) (6), pp. 36-45, 1983; Bevan, Nucl. Acids. Res., 12, pp. 8711-8721, 1984; and Van Montague et al., Proc R Soc Lond B Biol Sci., 210 (1180), pp. 351-65, 1980. Plants also may be transformed using "Refined Transformation" and "Precise Breeding" techniques. See, for instance, Rommens et al. in New Zealand Patent 535,395, U.S. Pat. No. 7,250,554, U.S. Pat. No. 7,534,934, WO2005/004585, U.S. Pat. No. 7,598,430, US-2005-

0034188, US-2012-0102589, WO2005/002994, and New Zealand Patent 536,037, which are all incorporated herein by reference.

Any method suitable for gene silencing in plants can be used. Non-limiting examples of gene silencing methods in plants are described in U.S. Patent Nos. 8058509, 7320892, 7754697, 7417180, 7816581, 7902425, 8395023, 7807880, 8362323, 8293977, and 7928289; U.S. Patent Application Publication Nos. 20070011775, 20070298481, 20120246765, 20070220628, 20100199386, 20050214263, 20100058490, and 20070118918; Meyer 2011 (Gene Silencing in Higher Plants and Related Phenomena in Other Eukaryotes, Springer London, Limited, 2011, ISBN 3642791476, 9783642791475), Oksman-Caldente (Plant Biotechnology and Transgenic Plants, CRC Press, 2002, ISBN 0824743784, 9780824743789), and Doran and Helliwell (RNA Interference: Methods for Plants and Animals, CABI, 2009, ISBN 1845934105, 9781845934101), each of which is herein incorporated by reference in its entirety for all purposes. In one embodiment, a silencing vector comprises two or more promoters which flank one or more desired polynucleotides or which flank copies of a desired polynucleotide, such that both strands of the desired polynucleotide are transcribed. That is, one promoter may be oriented to initiate transcription of the 5'-end of a desired polynucleotide, while a second promoter may be operably oriented to initiate transcription from the 3'-end of the same desired polynucleotide. The oppositely-oriented promoters may flank multiple copies of the desired polynucleotide, as described in U.S. Patent Nos. 7713735 and 8158414, each of which is herein incorporated by reference in its entirety for all purposes.

Also provided are methods for identifying plants comprising one or more polynucleotides of the present invention. In one embodiment, the identification is based on detecting the presence or absence of any fragment of the transgene described herein, such as the promoter sequence, the termination sequence, the selection marker, or the polynucleotide sequence having similarity to the PVY sequence. For example, methods based on DNA hybridization (e.g., PCR, Southern blot) can be used. In one embodiment, the identification is based on detecting the presence or absence of any RNA transcript encoded by the transgene described herein. For example, methods based on RNA hybridization (e.g., RT-PCR, Northern blot) can be used. In one embodiment, the identification is based on detecting the presence or absence of resistance to PVY. In one embodiment, two or more methods described above are combined.

Also provided are methods for making and using the plants comprising one or more polynucleotides of the present invention. In one embodiment, classic breeding methods can be included in the present invention to introduce one or more recombinant genes of the present invention into other plant varieties, or other close-related species that are compatible to be crossed

with the transgenic plants of the present invention. Such techniques include, but are not limited to, Open-Pollinated Populations, Mass Selection, Synthetics, Pedigreed varieties, and Hybrids. Additional breeding methods have been known to one of ordinary skill in the art, e.g., methods discussed in Chahal and Gosal (Principles and procedures of plant breeding: biotechnological and conventional approaches, CRC Press, 2002, ISBN 084931321X, 9780849313219), Taji et al. (In vitro plant breeding, Routledge, 2002, ISBN 156022908X, 9781560229087), Richards (Plant breeding systems, Taylor & Francis US, 1997, ISBN 0412574500, 9780412574504), and Hayes (Methods of Plant Breeding, Publisher: READ BOOKS, 2007, ISBN1406737062, 9781406737066), each of which is incorporated by reference in its entirety.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

Example 1

PVY Target Identification and Organization

A potato derived sequence for broad-spectrum PVY control was defined as follows:

1. Full-genome Potato Virus Y (PVY) sequences that are publicly available at GenBank (141 accessions) were aligned using BLAST analyses and other common methods known to those skilled in the art.
2. The alignment was analyzed for candidate conserved regions.
3. The identified regions were validated and ranked for broad-spectrum sequence conservation against a pool of 800+ partial PVY sequence accessions.
4. Regions of high genetic conservation among PVY strains were searched for sub-regions with > 90% homology to the sequenced and publicly available *Solanum Phureja* genome sequence by BLAST analysis.
5. These sub-regions were analyzed for homology with potato cDNAs, and sequences with < 80% homology to any cDNA were prioritized.
6. The prioritized genetic elements, which were highly conserved to both the PVY genetic spectrum and potato intergenic sequence were then joined, yielding a ~425bp molecule comprised of 12 genetic elements.

7. The junctions of flanking potato DNA elements were engineered to share a short region of overlapping homology to PVY sequence. For example, the first 5bp of the potato DNA comprising SEQ ID NO: 3 shares perfect homology to the 5bp of PVY sequence beyond the target region of SEQ ID NO:2.

Alternatively, after step 5 above, one or more plant derived sequences that share high or very high homology to the virus-derived sub-regions can be joined. Such joined sequences can be used to introduce PVY resistance into plants as well.

Exemplary sequences derived from PVY or plants that can be used to introduce PVY resistance into plants are shown in the table below.

SEQ ID	Sequences in pSIM2201	Homology to PVY (%)	*S.phujera* genome homology (%)	*S. phureja* Chromosome	Start	Stop	Sequence in *S. phureja*	SEQ ID
1	CATCCATCATAACCCAAACTC	100%	90.5	5	5562979	5562998	CATCCATCATAACC-AATCTC	60
2	CGTTGATGTTTGGCGAGGTT	100%	90.0	12	34296651	34296632	CGTTGATGTGGGGCGAGGTT	61
3	CCATTTTCAATGCACCAAACCA	100%	90.9	3	38265887	38265866	CCATTTTTAATGCACTAAACCA	62
4	TAAGCCCATTCATCACAGTT	100%	90.0	6	7383439	7383420	TAAGCCCATTCATGACGGTT	63
5	GGGCTGATTTCAATGCTGCG	100%	90.0	2	52886128	52886109	GGGCTCATTTCAATGATGCG	64
6	GCCTTCATTTGAATGTGCGCTT	100%	90.9	4	42796985	42796965	GCCTTCATTTGAATTTG-GCTT	65
7	ATTTGTTTAATTCTTGAACTTCAA GTACTCACACTCCACCACATAAAC CCCCAAGGGTGGACCAACTGTTT ACCAAAACAACACATCCCTTTATG TATTTTTCAATCAAATAGTTTGTT GTTTCATCAACTTTCTAATCTTCAA TACAGGATGACTCCAATACATCA GTTGCCTAAAGCGCACATTCAAAT GAAGGC	175 first nucl. = 100% hom to *S. phujera*; last 22 nucl. = 100% to PVY	99.0	4	42796790	42796985	ATTTGTTTAATTCTTGAACTTCAAGTA CTCACACTCCACCACATAAACCCCCAA GGGTGGACCAACTGTTTACCAAAACA ACACATCCCTTTATGTATTTTTCAATC AAATAGTTTGTTGTTTCATCAACTTTC TAATCTTCAATACAGGATGACTCCAA TACATCAGTTGCCTAaagc-caaattcaaatgaaggc	66
8	CGCAGCATTGAAATCAGCCC	100%	90.0	2	52886109	52886128	CGCATCATTGAAATGAGCCC	67
9	AACTGTGATGAATGGGCTTA	100%	90.0	6	7383420	7383439	AACCGTCATGAATGGGCTTA	68
10	TGGTTTGGTGCATTGAAAATGG	100%	90.9	3	38265866	38265887	TGGTTTAGTGCATTAAAAATGG	69
11	AACCTCGCCAAACATCAACG	100%	90.0	12	34296632	34296651	AACCTCGCCCCACATCAACG	70
12	GAGTTTGGGTTATGATGGATG	100%	90.5	5	5562998	5562979	GAGATT-GGTTATGATGGATG	71
13	Elements [1-12]							

SEQ ID	Sequences in pSIM2202	Homology to PVY (%)	*S.phujera* genome homology (%)	*S. phureja* Chromosome	Start	Stop	Sequence in *S. phureja*	SEQ ID
14	AGTGATAGAGCGTTTTGCTCTGT	100%	91.3	1	15757842	15757821	AGTGA-AGAGGGTTTTGCTCTGT	72
15	ATGAGTACCATGTTGCG	100%	94.1	7	5866954	5866938	ATGAGGACCATGTTGCG	73
16	CATTTCTATATACGCTTCTG	100%	95.0	11	15720258	15720239	CATTTCTATATATGCTTCTG	74
17	CAACATCTGAGAAATGTGCCAT	100%	90.9	2	56165745	56165725	CAA-ATCTGAGAAATGTGTCAT	75
18	CAACCTTTGTGACCATCAT	100%	94.7	7	28428553	28428570	CAA-CTTTGTGACCATCAT	76
19	ATTCTTGTCTGTTAGGAGCTT	100%	90.5	10	42302832	42302813	ATT-TTATCTGTTAGGAGCTT	77

20	AATGTTCCTTGTAGAATGAAAGG GAAAGAAATTTAAAAATAGGGCC GGGCCTTGAAACGCTGCCTACGT ATCTCCCAAGGAGAAATCAGGCC CTACGTAGTTCGAATACAAAGGA AATTTTCATTTTCGTTCATTCATTT CTGGTGATTACAAGGAAAGTGAA AAACAGACAATAAAGCTCCTAAC AGACAAGAAT	175 first nucl. = 100% hom to *S. phujera*; last 21 nucl. = 100% to PVY	99.0	10	42302638	42302832	AATGTTCCTTGTAGAATGAAAGGGAA AGAAATTTAAAAATAGGGCCGGGCC TTGAAACGCTGCCTACGTATCTCCCA AGGAGAAATCAGGCCCTACGTAGTTC GAATACAAAGGAAATTTTCATTTTCG TTCATTCATTTCTGGTGATTACAAGGA AAGTGAAAAACAGACAATAaagctccta acagataa-aat	78
21	ATGATGGTCACAAAGGTTG	100%	94.7	7	28428570	28428553	ATGATGGTCACAAAG-TTG	79
22	ATGGCACATTTCTCAGATGTTG	100%	90.9	2	56165725	56165745	ATGACACATTTCTCAGAT-TTG	80
23	CAGAAGCGTATATAGAAATG	100%	95.0	11	15720239	15720258	CAGAAGCATATATAGAAATG	81
24	CGCAACATGGTACTCAT	100%	94.1	7	5866938	5866954	CGCAACATGGTCCTCAT	82
25	ACAGAGCAAAACGCTCTATCACT	100%	91.3	1	15757821	15757842	ACAGAGCAAAACCCTCT-TCACT	83
26	Elements [14-25]							

SEQ ID	Sequences in Vector pSIM2203	Homology to PVY (%)	*S.phujera* genome homology (%)	*S. phureja* Chromosome	Start	Stop	Sequence in *S. phureja*	SEQ ID
27	TATATATCGTCCGGAGAGACAC	100%	90.9	12	37486531	37486552	TACATATCGTCGGGAGAGACAC	84
28	TACATCACATGTTCTTGACTC	100%	90.5	1	29931323	29931304	TAC-TCACATGTTCTTGATTC	85
29	AATGTAC-TCATCCCATGTGAAATC	100%	96	4	34983808	34983784	AATGTACTTCATCCCATGTGAAATC	86
30	GCGTTCAAGTTTGCGC	100%	93.7	5	35308576	35308591	GCGTTCAAGTTTGTGC	87
31	AGATTTCGAATTAAACCATATC	100%	86.4	11	39303698	39303677	AGATTTCGAACTTAACCAAATC	88
32	GTGGCATATATGGTTCCTT	100%	94.7	8	15219778	15219796	GTGGCATATATGGTGCCTT	89
33	TCATTAAAGGATGTACCACAGAG TATGCATCAGTACTTGGAATGTAC TGAGCATACAGGATATAGATATT AAGCTGAACAAATATATATAAGC TAAACAAAATATAATTGAGCAAT GACATAATGACACGACCCAGGGG TACACCATAGATGTAACAAGGCA CATAGAACCTCGAAAGGAACCAT ATATGCCAC	175 first nucl. = 100% hom to *S. phujera*; last 19 nucl. = 100% to PVY	99.5	8	15219971	15219778	TCATTAAAGGATGTACCACAGAGTAT GCATCAGTACTTGGAATGTACTGAGC ATACAGGATATAGATATTAAGCTGAA CAAATATATATAAGCTAAACAAAATA TAATTGAGCAATGACATAATGACACG ACCCAGGGGTACACCATAGATGTAAC AAGGCACATAGAACCTCGAaaggcacc atatatgccac	90
34	GATATGGTTTAATTCGAAATCT	96%	86.4	11	39303677	39303698	GATTTGGTTAAGTTCGAAATCT	91
35	GCGCAAACTTGAACGC	100%	93.7	5	35308591	35308576	GCACAAACTTGAACGC	92

36	GATTTCACATGGGATGA-GTACATT	100%	96	4	34983784	34983808	GATTTCACATGGGATGAAGTACATT	93
37	GAGTCAAGAACATGTGATGTA	100%	90.5	1	29931304	29931323	GAATCAAGAACATGTGA-GTA	94
38	GTGTCTCTCCGGACGATATATA	100%	90.9	12	37486552	37486531	GTGTCTCTCCCGACGATATGTA	95
39	[Elements 27-38]							

SEQ ID	Sequences in Vector pSIM2204	Homology to PVY (%)	*S.phujera* genome homology (%)	*S. phureja* Chr.	Start	Stop	Sequence in *S. phureja*	SEQ ID
40	TTAACATGAAGTGATGAAGA	NA	100	12	64851983	64851964		
41	TCATGGCATATTAATGGTTGCAAA AACTATGCCAAATGATATGATGA	NA	100	12	64851942	64851896		
42	AATTCATAACTACACAATT	NA	100	12	64851874	64851856		
43	TGGTTTGGTGCATTGAAAATT	95.2	90.5	11	27434679	27434699	TGGTTTGGTGCATTAAAAATA	96
44	TATTTTCAATGCACCAAACCA	95.2	95.2	11	27434699	27434679	TATTTTTAATGCACCAAACCA	97
45	[Elements 40,43,41,44,42]							
46	CTGAGTGTTTATCATCAAGCA	100	90.5	4	20942557	20942537	CTGAGTGTTTATCAGCTAGCA	98
47	TGCTTGATGATAAACACTCAG	100	90.5	4	20942537	20942557	TGCTAGCTGATAAACACTCAG	99
48	[Elements 40,46,41,47,42]							
49	TTAACATGAAGTGATGAAGAT	NA	100	12	64851983	64851963		
50	AAATTCATAACTACACAATT	NA	100	12	64851875	64851856		
51	TTCTATATACGCTTCTGCAA	100	90.9	9	29947442	29947461	TTCTATATACGCTGCTGGAA	100
52	TTGCAGAAGCGTATATAGAA	100	90.9	9	29947461	29947442	TTCCAGCAGCGTATATAGAA	101
53	[Element 49,51,41,51,50]							
54	GTGTGTCAGGCAATAATTTTT	95.2	90.5	5	31054475	31054456	ATGTGTCAGGAAATAATTTTA	102
55	TAAAATTATTGCCTGACACAC	100	90.5	5	31054456	31054475	TAAAATTATTTCCTGACACAT	103
56	[Elements 40, 54, 41, 55, 42]							
57	[Elements 45,48,53, 56]							

Citation for elements 40,41,42,49, and 50: Zhang W, Luo Y, Gong X, Zeng W, Li S. "Computational identification of 48 potato microRNAs and their targets." Comput Biol Chem. 2009 Feb;33(1):84-93. Epub 2008 Jul 15.; Yang W, Liu X, Zhang J, Feng J, Li C, Chen J. "Prediction and validation of conservative microRNAs of Solanum tuberosum L." Mol Biol Rep. 2010 Oct;37(7):3081-7. Epub 2009 Oct 31.; Xie F, Frazier TP, Zhang B. "Identification, characterization and expression analysis of MicroRNAs and their targets in the potato (Solanum tuberosum)." Gene. 2011 Feb 15;473(1):8-22. Epub 2010 Oct 7.

NA: Not Applicable

Example 2

In one embodiment, polynucleotide SEQ ID NO: 13, comprising SEQ ID NO: 1-12, is operably linked to promoter Ubi7 SEQ ID NO: 58 and terminator Ubi3 SEQ ID NO: 59. The resulting expression cassette is positioned within a T-DNA region of a binary vector to produce pSIM2201. This transformation vector will be introduced into an Agrobacterium strain such as LBA4404 or AGL-1 as follows.

Competent LB4404 cells (50 μL) are incubated for 5 min on ice in the presence of 1μg of vector DNA, frozen for about 15 s in liquid nitrogen, and incubated at 37°C for 5 min. After adding 1 mL of liquid broth, the treated cells are grown for 3 h at 28°C and plated on liquid broth/agar containing streptomycin (100 mg/L) and kanamycin (100 mg/L). The vector DNAs are then isolated from overnight cultures of individual LBA4404 colonies and examined by restriction analysis to confirm the presence of intact plasmid DNA.

Ten-fold dilutions of overnight-grown Agrobacterium cultures can be grown for 5-6 hours, precipitated for 15 minutes at 2,800 RPM, washed with MS liquid medium (Phytotechnology) supplemented with sucrose (3%, pH 5.7), and resuspended in the same medium to 0.2 OD/600nm. The resuspended cells are mixed and used to infect 0.4-0.6 mm internodal segments of the potato varieties Ranger Russet, Russet Burbank, Atlantic, Phureja and Shepody.

Infected stems are incubated for two days on co-culture medium (1/10 MS salts, 3% sucrose, pH 5.7) containing 6 g/L agar at 22°C in a Percival growth chamber (16 hrs light) and subsequently transferred to callus induction medium (CIM, MS medium supplemented with 3% sucrose 3, 2.5 mg/L of zeatin riboside, 0.1 mg/L of naphthalene acetic acid, and 6g/L of agar) containing timentin (150 mg/L) and kanamycin (100 mg/L). After one month of culture on CIM, explants are transferred to shoot induction medium (SIM, MS medium supplemented with 3% sucrose, 2.5 mg/L of zeatin riboside, 0.3 mg/L of giberellic acid GA3, and 6g/L of agar) containing timentin and kanamycin (150 and 100 mg/L respectively) until shoots arise. Shoots arising at the end of regeneration period are transferred to MS medium with 3% sucrose, 6 g/L of agar and timentin (150mg/L). Transgenic plants are transferred to soil and placed in a growth chamber.

The transformed plants are challenged by PVY as follows. First, an inoculum of PVY strain "NTN" or "O" is prepared fresh for each infection by grinding 5 g of systemically infected tobacco tissue in 15 ml ice-cold phosphate buffer. The resulting suspension is filtered through miracloth, and kept on ice. One drop of inoculum is gently rubbed on a single carborundum-dusted leaf from each plant transferred from tissue culture to soil six weeks earlier. Plants are grown for an additional 2-3 weeks to facilitate systemic viral spread and symptom development. All experiments are carried out in triplicate. Plants are assessed for the presence of PVY using the "ImmunoStrip" affinity assay that relies on previously characterized antibodies (Ellis et al., 1996) and was developed by Agdia (Elkhart, IN).

In another embodiment, polynucleotide SEQ ID NO: 26, consisting of SEQ ID NO: 14-25, is operably linked to promoter Ubi7 SEQ ID NO: 58 and terminator Ubi3 SEQ ID NO: 59 to produce pSIM2202.

In another embodiment, polynucleotide SEQ ID NO: 39, consisting of SEQ ID NO: 27-38, is operably linked to promoter Ubi7 SEQ ID NO: 58 and terminator Ubi3 SEQ ID NO: 59 to produce pSIM2203.

In another embodiment, polynucleotide SEQ ID NO: 57, consisting of SEQ ID NO: 45, 48, 53, & 56 to produce pSIM2204, is operably linked to promoter Ubi7 SEQ ID NO: 58 and terminator Ubi3 SEQ ID NO: 59. Element 57 is not constructed the same way as 13, 26 and 39. In this case it is an miRNA approach and it contains much more potato sequences than PVY sequences.

Example 3

pSIM2201, pSIM2202, pSIM2203, and pSIM2204 were made according to the method described in Example 2, and transformed into Russet Burbank variety to produce transgenic potato plants RB-2201, RB2202, RB 2203, and RB2204, respectively. RB-2201 plants contain a construct pSIM2201 with SEQ ID 13 (combination of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12). RB-2202 plants contain a construct pSIM2202 with SEQ ID 26 (combination of SEQ ID NOs: 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 & 25). RB-2203 plants contain a construct pSIM2203 with SEQ ID 39 (combination of SEQ ID NOs: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 & 38), and RB-2204 plants contain a construct pSIM2204 with SEQ ID 57 (combination of SEQ ID NOs: 45, 48, 53 & 56). PVY virus was rub-inoculated to the transgenic plants. PVY immuno detection was performed by using ImmunoStrip®ISK 20001/0025 from Agdia, 20 days post rub inoculation on Russet Burbank transgenic lines. The initial test result is shown in the table below.

Accession	Nbr of plants per line	PVY ImmunoStrip® results	Observation
Russet Burbank	3	Positive	Control plant is susceptible to PVY^{NTN}
RB-2201-1	3	Negative	1 accession susceptible to PVY^{NTN} out of 25 tested. Element 13 bring resistance against PVY^{NTN}.
RB-2201-2	3	Negative	
RB-2201-3	3	Negative	
RB-2201-4	3	Negative	
RB-2201-5	3	Negative	
RB-2201-6	3	Positive	
RB-2201-7	3	Negative	
RB-2201-8	3	Negative	
RB-2201-9	3	Negative	
RB-2201-10	3	Negative	
RB-2201-11	3	Negative	
RB-2201-12	3	Negative	
RB-2201-13	3	Negative	
RB-2201-14	3	Negative	
RB-2201-15	3	Negative	
RB-2201-16	3	Negative	
RB-2201-17	3	Negative	
RB-2201-18	3	Negative	
RB-2201-19	3	Negative	
RB-2201-20	3	Negative	
RB-2201-21	3	Negative	
RB-2201-22	3	Negative	
RB-2201-23	3	Negative	
RB-2201-24	3	Negative	
RB-2201-25	3	Negative	
RB-2202-1	3	Positive	13 accessions susceptible to PVY^{NTN} out of 25 tested. Element 26 bring resistance against PVY^{NTN}.
RB-2202-2	3	Negative	
RB-2202-3	3	Positive	
RB-2202-4	3	Negative	
RB-2202-5	3	Negative	
RB-2202-6	3	Negative	
RB-2202-7	3	Positive	
RB-2202-8	3	Negative	
RB-2202-9	3	Positive	
RB-2202-10	3	Positive	
RB-2202-11	3	Negative	

RB-2202-12	3	Negative	
RB-2202-13	3	Positive	
RB-2202-14	3	Negative	
RB-2202-15	3	Positive	
RB-2202-16	3	Positive	
RB-2202-17	3	Positive	
RB-2202-18	3	Positive	
RB-2202-19	3	Negative	
RB-2202-20	3	Negative	
RB-2202-21	3	Positive	
RB-2202-22	3	Negative	
RB-2202-23	3	Positive	
RB-2202-24	3	Negative	
RB-2202-25	3	Positive	
RB-2203-1	3	Positive	25 accessions susceptible to PVY^{NTN} out of 25 tested. Element 39 does not bring resistance against PVY^{NTN}.
RB-2203-2	3	Positive	
RB-2203-3	3	Positive	
RB-2203-4	3	Positive	
RB-2203-5	3	Positive	
RB-2203-6	3	Positive	
RB-2203-7	3	Positive	
RB-2203-8	3	Positive	
RB-2203-9	3	Positive	
RB-2203-10	3	Positive	
RB-2203-11	3	Positive	
RB-2203-12	3	Positive	
RB-2203-13	3	Positive	
RB-2203-14	3	1 plant Pos. 2 Neg.	
RB-2203-15	3	2 plants Pos. 1 Neg.	
RB-2203-16	3	Positive	
RB-2203-17	3	Positive	
RB-2203-18	3	Positive	
RB-2203-19	3	Positive	
RB-2203-20	3	Positive	
RB-2203-21	3	Positive	
RB-2203-22	3	Positive	
RB-2203-23	3	Positive	
RB-2203-24	3	Positive	
RB-2203-25	2	Positive	

RB-2204-1	3	Positive	5 accessions susceptible to PVY^{NTN} out of 5 tested. Element 58 does not bring resistance against PVY^{NTN}.
RB-2204-2	3	Positive	
RB-2204-3	3	Positive	
RB-2204-4	3	Positive	
RB-2204-5	3	Positive	

RB-2201 plants contain a construct with SEQ ID 13 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12)
RB-2202 plants contain a construct with SEQ ID 26 (14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 & 25)
RB-2203 plants contain a construct with SEQ ID 39 (27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 & 38)
RB-2204 plants contain a construct with SEQ ID 57 (45, 48, 53 & 56)

Although transgenic plants RB-2203 and RB-2204 did not show obvious resistance to PVY in the initial trial, it does not mean that the silencing sequences targeting PYV in these vectors, i.e., SEQ ID NOs: 39 and 57, do not have the ability to induce PVY resistance in plants. It does not mean that every single sub-sequence within SEQ ID NOs: 39 or 57 could not be combined with other sequences to make additional vectors capable of inducing PVY resistance in plants. Many reasons may cause a false negative result. For example, there may be a particular mistake in pSIM2203 and pSIM2204 vetors; the vectors may have been suppressed in the plant genome; the vectors may not be particularly suitable for expressing SEQ ID NOs: 39 or 57 in plants since such sequences are much longer compared to other sequences; there may be a particular sub-sequence in the vectors that caused suppression of the expression of the whole vectors, etc. In addition, this is just one-time experiment so the negative data should not lead to a final conclusion that the vectors do not work.

Example 4

In one embodiment, a polynucleotide comprising at least two potato sequences selected from SEQ ID NO: 60-103, is operably linked to promoter Ubi7 SEQ ID NO: 58 and terminator Ubi3 SEQ ID NO: 59. The resulting expression cassette is positioned within a T-DNA region of a binary vector. This transformation vector will be introduced into an Agrobacterium strain such as LBA4404 or AGL-1 as described in Example 2 above.

In another embodiment, a polynucleotide comprising at least one viral sequence selected from SEQ ID NOs: 1-58, and at least one potato sequence selected from SEQ ID NOs: 60-103, is operably linked to promoter Ubi7 SEQ ID NO: 58 and terminator Ubi3 SEQ ID NO: 59. This transformation vector

will be introduced into an Agrobacterium strain such as LBA4404 or AGL-1 as described in Example 2 above.

The Agrobacterium strains are used to transform the polynucleotides into a plant, such as a potato plant to confer resistance to PVY.

Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising one or more plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y.

2. The isolated polynucleotide of claim 1, comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 plant DNA sequences.

3. The isolated polynucleotide of claim 1, wherein each plant DNA sequence shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100%, sequence homology with a PVY DNA sequence.

4. The isolated polynucleotide of claim 1, wherein the plant DNA sequences are potato plant DNA sequences.

5. An expression vector comprising a promoter operably linked to a polynucleotide comprising multiple plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y.

6. A method for conferring full or partial resistance in a plant to at least one strain of Potato Virus Y infection, comprising expressing in a plant a polynucleotide that comprises multiple plant DNA sequences, wherein each plant DNA sequence shares sequence homology, in the sense or antisense orientation, to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y, wherein the plant is fully or partially resistant to PVY infection compared to a plant of the same species that does not express the polynucleotide.

7. The method of claim 6, wherein the plant is a potato plant.

8. The method of claim 6, wherein the plant DNA sequences are potato plant DNA sequences.

9. The method of claim 6, wherein the plant is a potato plant and the plant DNA sequences are potato plant DNA sequences.

10. The isolated polynucleotide of claim 1, wherein the polynucleotide comprises at least two sequences selected from the SEQ ID NOs.: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, and 71.

11. The isolated polynucleotide of claim 10, wherein the polynucleotide comprises at least two sequences having at least 90% identity to SEQ ID NO: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71.

12. The isolated polynucleotide of claim 1, wherein the polynucleotide comprises at least two sequences selected from the SEQ ID NOs.: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, and 83, or at least two sequences selected from the SEQ ID NOs.: 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, and 95, or at least two sequences selected from the SEQ ID NOs.: 96, 97, 98, 99, 100, 101, 102, and 103, or at least two sequences selected from the SEQ ID NO.s 72-103.

13. The isolated polynucleotide of claim 12, wherein the polynucleotide comprises at least two sequences having at least 90% identity to SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, or 83, or at least two sequences having at least 90% identity to SEQ ID NOs.: 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, or 95, or at least two sequences having at least 90% identity to SEQ ID NOs.: 96, 97, 98, 99, 100, 101, 102, or 103, or at least two sequences having at least 90% identity to any one of SEQ ID NO.s 72-103.

14. An isolated polynucleotide comprising one or more viral DNA sequences, wherein each viral DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one plant.

15. The isolated polynucleotide of claim 14, comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 plant DNA sequences.

16. The isolated polynucleotide of claim 14, wherein each viral DNA sequence shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100%, sequence homology with a potato plant DNA sequence.

17. The isolated polynucleotide of claim 14, wherein the viral DNA sequences are Potato Virus Y DNA sequences.

18. An expression vector comprising a promoter operably linked to a polynucleotide comprising multiple viral DNA sequences, wherein each viral DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one potato plant.

19. A method for conferring full or partial resistance in a plant to at least one strain of Potato Virus Y infection, comprising expressing in a plant a polynucleotide that comprises multiple viral DNA sequences, wherein each viral DNA sequence shares sequence homology, in the sense or antisense orientation, to a corresponding DNA sequence from the genome(s) of at least one potato plant, wherein the plant is fully or partially resistant to PVY infection compared to a plant of the same species that does not express the polynucleotide.

20. The method of claim 19, wherein the plant is a potato plant.

21. The method of claim 19, wherein the viral DNA sequences are Potato Virus Y DNA sequences.

22. The method of claim 19, wherein the plant is a potato plant and the viral DNA sequences are Potato Virus Y DNA sequences.

23. The isolated polynucleotide of claim 14, wherein the polynucleotide comprises at least two sequences selected from the SEQ ID NOs.: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.

24. The isolated polynucleotide of claim 23, wherein the polynucleotide comprises the sequence of SEQ ID NO: 13.

25. The isolated polynucleotide of claim 14, wherein the polynucleotide comprises at least two sequences selected from the SEQ ID NOs.: 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25.

26. The isolated polynucleotide of claim 25, wherein the polynucleotide comprises the sequence of SEQ ID NO: 26.

27. The isolated polynucleotide of claim 14, wherein the polynucleotide comprises at least two sequences selected from the SEQ ID NOs.: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, and 38.

28. The isolated polynucleotide of claim 27, wherein the polynucleotide comprises the sequence of SEQ ID NO: 39.

29. The isolated polynucleotide of claim 14, wherein the polynucleotide comprises at least two sequences selected from the SEQ ID NOs.: 45, 48, 53, and 57.

30. The isolated polynucleotide of claim 29, wherein the polynucleotide comprises the sequence of SEQ ID NO: 58.

31. The expression vector of claim 5, comprising a polynucleotide that comprises one or more sequences selected from the group consisting of SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103.

32. The expression vector of claim 18, comprising a polynucleotide that comprises one or more sequences selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57.

33. The method of claim 6, wherein the polynucleotide comprises one or more sequences selected from the group consisting of SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103.

34. The method of claim 19, wherein the polynucleotide comprises one or more sequences selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57.

35. The isolated polynucleotide of claim 1, wherein the isolated polynucleotide comprising comprises one or more sequences selected from the group consisting of:

(1) complements of a sequence of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103 ;

(2) reverse complements of a sequence of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103;

(3) reverse sequences a sequence of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,86,87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103;

(4) a portion of a nucleotide sequence comprising at least 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of a nucleotide sequence recited in (1) –(3);

(5) a nucleotide sequence having at least 95% identity to a nucleotide sequence recited in (1) –(3);

(6) a nucleotide sequence having an E value of 0.01 when aligned with a sequence recited in (1) –(3); and

(7) a nucleotide sequences that hybridize to a sequence recited in (1) –(3) under stringent conditions, wherein said stringent conditions are hybridization in 0.25 M Na2HPO4 buffer (pH 7.2) containing 1 mM Na2EDTA, 20% sodium dodecyl sulfate at 45°C, followed by a wash in 5×SSC, containing 0.1% (w/v) sodium dodecyl sulfate, at 55°C to 65°C.

36. The isolated polynucleotide of claim 14, wherein the isolated polynucleotide comprising comprises one or more sequences selected from the group consisting of:

(1) complements of a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57;

(2) reverse complements of a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57;

(3) reverse sequences a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 45, 48, 53, and 57;

(4) a portion of a nucleotide sequence comprising at least 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of a nucleotide sequence recited in (1) –(3);

(5) a nucleotide sequence having at least 95% identity to a nucleotide sequence recited in (1) –(3);

(6) a nucleotide sequence having an E value of 0.01 when aligned with a sequence recited in (1) –(3); and

(7) a nucleotide sequences that hybridize to a sequence recited in (1) –(3) under stringent conditions, wherein said stringent conditions are hybridization in 0.25 M Na2HPO4 buffer (pH 7.2) containing 1 mM Na2EDTA, 20% sodium dodecyl sulfate at 45°C, followed by a wash in 5×SSC, containing 0.1% (w/v) sodium dodecyl sulfate, at 55°C to 65°C.

37. The expression vector of claim 5, comprising a polynucleotide that comprises one or more sequences selected from the isolated polynucleotide of claim 35.

38. The expression vector of claim 18, comprising a polynucleotide that comprises one or more sequences selected from the isolated polynucleotide of claim 36.

39. The method of claim 6, wherein the polynucleotide comprises one or more sequences selected from the isolated polynucleotide of claim 35.

40. The method of claim 19, wherein the polynucleotide comprises one or more sequences selected from the isolated polynucleotide of claim 36.

41. A transgenic cell comprising the isolated polynucleotide of claim 1, 14, 35, or 36.

42. An organism comprising the transgenic cell of claim 41.

43. A transformed plant, plant part or plant cell comprising the isolated polynucleotide of claim 1, 14, 35, or 36.

44. A seed of the transformed plant of claim 43, wherein said seed comprises said isolated polynucleotide.

45. Progeny plants of the plant of claim 43, wherein the progeny plants have an altered pathogen tolerance and/or resistance as a result of inheriting the polynucleotide.

46. A method of producing hybrid seed comprising crossing the plant of claim 43 or a progeny plant of claim 45 with a different plant of the same species, and harvesting the resultant seed.

47. A method for producing a plant having altered pathogen tolerance and/or resistance comprising: (a) transforming a plant cell with an expression vector to provide a transgenic cell, wherein the expression vector comprises: (i) a promoter sequence; (ii) an isolated polynucleotide sequence of claim 1, 14, 35, or 36; and (iii) a gene termination sequence; and (b) cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth of a plant having altered pathogen tolerance and/or resistance.

48. A method for modifying a phenotype of a target organism, comprising stably incorporating into the genome of the target organism a genetic construct comprising: (a) a promoter sequence; (b) an isolated polynucleotide sequence of claim 1, 14, 35, or 36; and (c) a gene termination sequence.

49. The method of claim 48, wherein the target organism is a plant.

50. A process of determining the presence or absence of an isolated polynucleotide sequence of claim 1, 14, 35, or 36 and fragments and variations thereof in a plant, wherein the process comprises at least one of:

(a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; and/or

(b) isolating nucleic acid molecules from said plant and performing a hybridization to detect the polynucleotide; and/or

(c) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

51. A method of breeding plants to produce altered pathogen tolerance and/or resistance comprising:

i) making a cross between a plant with an isolated polynucleotide sequence of claim 1, 14, 35, or 36 with a second plant to produce a F1 plant;

ii) backcrossing the F1 plant to the second plant; and

iii) repeating the backcrossing step to generate a near isogenic or isogenic line, wherein the isolated polynucleotide sequence of claim 1, 14, 35, or 36 is integrated into the genome of the second plant and the near isogenic or isogenic line derived from the second plant with the isolated polynucleotide sequence has conferred or enhanced pathogen tolerance and/or resistance compared to that of the second plant without the isolated polynucleotide sequence.

52. An isolated polynucleotide comprising one or more plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one strain of Potato Virus Y, and one or more viral DNA sequences, wherein each viral DNA sequence shares sequence homology to a corresponding DNA sequence from the genome(s) of at least one potato plant.

53. The isolated polynucleotide of claim 52, wherein the plant DNA sequences are selected from the sequences of claims 1-4, 10-13, and 35, and wherein the viral DNA sequences are selected from the sequences of claims 14-17, 23-30, and 36.

54. An expression vector comprising a promoter operably linked to a polynucleotide comprising the isolated polynucleotide of claim 52 or 53.

55. A method for conferring full or partial resistance in a plant to at least one strain of Potato Virus Y infection, comprising expressing in a plant a polynucleotide of claim 52 or 53.

56. A transgenic cell comprising the isolated polynucleotide of claim 52 or 53.

57. An organism comprising the transgenic cell of claim 56.

58. A transformed plant, plant part or plant cell comprising the isolated polynucleotide of claim 52 or 53.

59. A seed of the transformed plant of claim 58, wherein said seed comprises said isolated polynucleotide.

60. Progeny plants of the plant of claim 58, wherein the progeny plants have an altered pathogen tolerance and/or resistance as a result of inheriting the polynucleotide.

61. A method of producing hybrid seed comprising crossing the plant of claim 58 or a progeny plant of claim 59 with a different plant of the same species, and harvesting the resultant seed.

62. A method for producing a plant having altered pathogen tolerance and/or resistance comprising: (a) transforming a plant cell with an expression vector to provide a transgenic cell, wherein the expression vector comprises: (i) a promoter sequence; (ii) an isolated polynucleotide sequence of claim 52 or 53; and (iii) a gene termination sequence; and (b) cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth of a plant having altered pathogen tolerance and/or resistance.

63. A method for modifying a phenotype of a target organism, comprising stably incorporating into the genome of the target organism a genetic construct comprising: (a) a promoter sequence; (b) an isolated polynucleotide sequence of claim 52 or 53; and (c) a gene termination sequence.

64. A process of determining the presence or absence of an isolated polynucleotide sequence of claim 52 or 53 and fragments and variations thereof in a plant, wherein the process comprises at least one of:

(a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; and/or

(b) isolating nucleic acid molecules from said plant and performing a hybridization to detect the polynucleotide; and/or

(c) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

65. A method of breeding plants to produce altered pathogen tolerance and/or resistance comprising:

i) making a cross between a plant with an isolated polynucleotide sequence of claim 52 or 53 with a second plant to produce a F1 plant;

ii) backcrossing the F1 plant to the second plant; and

iii) repeating the backcrossing step to generate a near isogenic or isogenic line, wherein the isolated polynucleotide sequence of claim 52 or 53 is integrated into the genome of the second plant and the near isogenic or isogenic line derived from the second plant with the isolated polynucleotide sequence has conferred or enhanced pathogen tolerance and/or resistance compared to that of the second plant without the isolated polynucleotide sequence.