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- (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.

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**Published:**

- with international search report (Art. 21(3))
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- with sequence listing part of description (Rule 5.2(a))

- (88) **Date of publication of the international search report:**  
3 April 2014

(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. 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.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/047329

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/83 (2014.01)

USPC - 435/468

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)

IPC(8) - C07 14/08; C12N 15/05, 15/09, 15/33, 15/82, 15/83 (2014.01)

USPC - 435/235.1, 320.1, 468; 800/280, 301

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

CPC - C07K 14/005; C12N 15/8216, 15/8218, 15/8282, 15/8283, 2770/34011 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google, PubMed

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                        | Relevant to claim No.                               |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|
| X<br>---  | TANNE et al. "Occurrence of a DNA sequence of a non-retro RNA virus in a host plant genome and its expression: evidence for recombination between viral and host RNAs." Virology, 08 January 2005 (08.01.2005), Vol. 332, Pgs. 614-622. entire document   | 1-3, 5, 50, 52<br>-----<br>4, 16, 18-22, 31, 35, 51 |
| X<br>---  | US 2012/0102589 A1 (ROMMENS et al) 26 April 2012 (26.04.2012) entire document                                                                                                                                                                             | 6-9<br>-----<br>33                                  |
| X<br>---  | US 6,228,637 B1 (KASAOKA et al) 08 May 2001 (08.05.2001) entire document                                                                                                                                                                                  | 14, 15, 17, 41-45, 47-49<br>-----<br>16, 18-22, 51  |
| Y         | HARPER et al. "Viral Sequences Integrated into Plant Genomes," Annu. Rev. Phytopathol., 01 September 2002 (01.09.2002), Vol. 40, Pgs. 119-136. entire document                                                                                            | 4                                                   |
| Y         | ZHU et al. "Analysis of 90 Mb of the potato genome reveals conservation of gene structures and order with tomato but divergence in repetitive sequence composition," BMC Genomics, 13 June 2008 (13.06.2008), Vol. 9, No. 286, Pgs. 1-14. entire document | 31, 33, 35                                          |
| Y         | US 2011/0271409 A1 (BALEY et al) 03 November 2011 (03.11.2011) entire document                                                                                                                                                                            | 51                                                  |
| A         | QIAGEN PCR Cloning Handbook, 01 April 2001 (01.04.2001), Pgs. 1-32, downloaded from the Internet: <http://www.qiagen.com/resources/download.aspx?id=39a59217-944a-4366-9cd0-30ba9cfb3c> on 24 January 2014 (24.01.2014). entire document                  | 1-11, 14-22, 31, 33, 35,<br>41-45, 47-52            |

☒ Further documents are listed in the continuation of Box C.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

24 January 2014

Date of mailing of the international search report

05 FEB 2014

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/047329

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                               | Relevant to claim No.                 |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| A         | SLOAN et al. "Rapid Evolution of Enormous, Multichromosomal Genomes in Flowering Plant Mitochondria with Exceptionally High Mutation Rates," PLoS Biololgy, 17 January 2012 (17.01.2012), Vol. 10, No. 1, e1001241, Pgs. 1-17. entire document                                                   | 1-11, 14-22, 31, 33, 35, 41-45, 47-52 |
| A         | SUN et al. "Bacterially Expressed Double-Stranded RNAs against Hot-Spot Sequences of Tobacco Mosaic Virus or Potato Virus Y Genome Have Different Ability to Protect Tobacco from Viral Infection," Appl Biochem Biotechnol, 02 May 2010 (02.05.2010), Vol. 162, Pgs. 1901-1914. entire document | 1-11, 14-22, 31, 33, 35, 41-45, 47-52 |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/047329

**Box No. I** Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs: 60 and 61 were searched.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/047329

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 37-40, 46, 53-65  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11, 14-22, 31, 33, 35, 41-45, and 47-52 to the extent that they read on SEQ ID NOs: 60 and 61

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

## Continuation of Box III, Lack of Unity

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-36, 41-45, and 47-52 are drawn to an isolated polynucleotide and an expression vector comprising a promoter, comprising one or more plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genomes of at least one strain of Potato Virus Y and methods comprising the same.

The first invention of Group I+ is restricted to isolated polynucleotides, expression vectors, and methods comprising the same, comprising one or more plant DNA sequences, wherein the plant DNA sequences are selected to be SEQ ID NOs:60 and 61. It is believed that claims 1-11, 14-22, 31, 33, 35, 41-45, and 47-52 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs: 60 and 61.

Applicant is invited to elect additional plant DNA sequences with specified SEQ ID NO for each isolated polynucleotide, vector, or method to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be isolated polynucleotides, expression vectors, and methods comprising the same, comprising one or more plant DNA sequences, wherein the plant DNA sequences are selected to be SEQ ID NOs:62 and 63. Additional plant DNA sequences will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element conferring resistance to Potato Virus Y, requiring the selection of alternatives for the plant DNA sequences "having at least 90% identity to SEQ ID NO: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71" or "having at least 90% identity to any one of SEQ ID NOs: 72-103."

The Groups I+ share the technical features of 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 genomes of at least one strain of Potato Virus Y; 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; and 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. However, these shared technical features do not represent a contribution over the prior art.

Specifically, "Bacterially Expressed Double-Stranded RNAs against Hot-Spot Sequences of Tobacco Mosaic Virus or Potato Virus Y Genome Have Different Ability to Protect Tobacco from Viral Infection" to Sun et al. discloses an isolated polynucleotide (hairpin RNAs (hpRNAs), Abstract) comprising one or more plant DNA sequences, wherein each plant DNA sequence shares sequence homology to a corresponding DNA sequence from the genomes of at least one strain of Potato Virus Y (posttranscriptional gene silencing (PTGS) mediated by double-stranded RNA (dsRNA) and induced by small interference RNA (siRNA), Pg. 1902, first partial paragraph; posttranscriptional gene silencing, also known as RNA interference, involves degradation of homologous mRNA sequences in organisms. In plants, posttranscriptional gene silencing is part of a defense mechanism against virus infection, Abstract; hpRNA against the PVY Nib, Pg. 1908, partial paragraph); an expression vector (we constructed seven hpRNA vectors against ...the PVY HC-Pro gene, Pg. 1905, first paragraph) comprising a promoter operably linked to a polynucleotide comprising multiple plant DNA sequences (the different vectors were designed to produce the hpRNAs containing TMV or PVY cDNA sequences under the control of the T7 promoter, Pg. 1907, first paragraph), 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 (we constructed seven hpRNA vectors against ...the PVY HC-Pro gene, Pg. 1905, first paragraph; posttranscriptional gene silencing (PTGS) mediated by double-stranded RNA (dsRNA) and induced by small interference RNA (siRNA), Pg. 1902, first partial paragraph; posttranscriptional gene silencing, also known as RNA interference, involves degradation of homologous mRNA sequences in organisms. In plants, posttranscriptional gene silencing is part of a defense mechanism against virus infection, Abstract); and a method for conferring full or partial resistance in a plant to at least one strain of Potato Virus Y infection (method for the control of plant viruses, Pg. 1902, first partial paragraph constructs against different hot-spot sequences of Tobacco mosaic virus (TMV) or Potato virus Y (PVY) genome, Abstract; we constructed seven hpRNA vectors against ...the PVY HC-Pro gene, Pg. 1905, first paragraph), comprising expressing in a plant a polynucleotide that comprises multiple plant DNA sequences (we constructed seven hpRNA vectors against ...the PVY HC-Pro gene, Pg. 1905, first paragraph; expressed dsRNAs, Pg. 1906, second full paragraph), 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 (posttranscriptional gene silencing (PTGS) mediated by double-stranded RNA (dsRNA) and induced by small interference RNA (siRNA), Pg. 1902, first partial paragraph; posttranscriptional gene silencing, also known as RNA interference, involves degradation of homologous mRNA sequences in organisms. In plants, posttranscriptional gene silencing is part of a defense mechanism against virus infection, Abstract); 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 (the PVY Nib hot-spot sequences had stronger resistance. The hpRNA technology is a useful and effective strategy for protecting plants against virus infection, Pg. 1902, first full paragraph).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.