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# PVY$^{NTN-NW}$, a novel recombinant strain of *Potato virus Y* predominating in potato fields in Syria

M. Chikh Ali[ab]*, T. Maoka[c], T. Natsuaki[b] and K. T. Natsuaki[d]

[a]*Laboratory of Plant Pathology, Faculty of Agriculture, Utsunomiya University, Mine-machi 350, Utsunomiya 321-8505, Japan;* [b]*General Organization for Seed Multiplication (GOSM), Aleppo, Syria;* [c]*National Agricultural Research Center for Hokkaido Region (NARCH), Hitsujigaoka 1, Toyohira-ku, Sapporo, Hokkaido, 062-8555, Japan; and* [d]*Graduate School of Agriculture, Department of International Agricultural Development, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156-8502, Japan*

Detailed characterization of a number of isolates of PVY$^{SYR}$, a novel recombinant strain of *Potato virus Y* (PVY) from Syria, was conducted to elucidate their origin, assess their significance and achieve a final classification of PVY$^{SYR}$. Recombination analysis grouped isolates of PVY$^{SYR}$ into three recombination patterns, SYR-I, SYR-II and SYR-III, which varied in the first 700 nucleotides of their genomes, with the second recombination pattern, SYR-II, the most frequent. PVY$^{SYR}$ isolates shared highest genomic identity and close phylogenetic relationships with PVY$^{NTN}$ and PVY$^{N}$W isolates from Syria, suggesting a common origin and local emergence of these isolates in Syria. All PVY$^{SYR}$ isolates (total of 20) induced tobacco veinal necrosis, but reacted to a PVY$^{O}$ monoclonal antibody, typical characteristics of the previously reported PVY$^{N}$W (or PVY$^{N:O}$). In potato, however, four isolates tested (one of SYR-I and three of SYR-II) induced potato tuber necrotic ringspot disease (PTNRD), which is the characteristic phenotype of PVY$^{NTN}$. Given the shared properties of SYR-I and SYR-II isolates with PVY$^{NTN}$ and PVY$^{N}$W, it was decided that they represent a new recombinant strain of the PVY$^{N}$ strain group, with the proposed name PVY$^{NTN-NW}$. The classification of SYR-III will be possible only after testing the phenotype in potato. The high prevalence of PVY$^{NTN-NW}$ in potatoes and weeds, as well as its ability to induce PTNRD, demonstrates its importance and the necessity for its control.

*Keywords*: potato tuber necrosis, PVY$^{SYR,}$ recombination pattern, *Solanum tuberosum*

## Introduction

*Potato virus Y* (PVY) is one of the most common and destructive viruses found in potato (Singh *et al.*, 2008). It is the type species of the genus *Potyvirus*, family *Potyviridae*, with a single-stranded positive-sense genomic RNA of approximately 9·7 kb (Fauquet *et al.*, 2005). According to the reaction of potato cultivars carrying different resistance genes, potato isolates of PVY are traditionally classified into three main strain groups, PVY$^{O}$, PVY$^{C}$ and PVY$^{N}$. The ordinary (PVY$^{O}$) and stipple streak (PVY$^{C}$) strain groups induce hypersensitive resistance reaction (HR) in potato cultivars with the *Ny* and *Nc* resistance genes, respectively, and mosaic in *Nicotiana tabacum* (Jones, 1990). PVY$^{N}$ induces severe tobacco veinal necrosis and mild mosaic in most potato cultivars (Singh *et al.*, 2008). A new strain group of PVY found in the UK induced mosaic in tobacco and potato cultivars with the resistance genes *Ny* and *Nc*, but caused top necrosis in potato cultivars carrying a hypothetical resistance gene *Nz* and was referred to as PVY$^{Z}$ (Jones, 1990). Another strain group, PVY$^{E}$, induced mosaic in tobacco and potato cultivars carrying the resistance genes *Ny*, *Nc* and the proposed *Nz* (Kerlan *et al.*, 1999; Singh *et al.*, 2008).

In the last two decades, new strains, namely PVY$^{NTN}$ and PVY$^{N}$W (or PVY$^{N:O}$ in North America) have been reported from different geographic regions (Beczner *et al.*, 1984; Chrzanowska, 1991; McDonald & Singh, 1996a,b; Ohshima *et al.*, 2000). PVY$^{NTN}$ and PVY$^{N}$W were classified as new strains within the PVY$^{N}$ strain group (Singh *et al.*, 2008). Although PVY$^{NTN}$ isolates have a serotype and tobacco phenotype identical to those of PVY$^{N}$, they induced potato tuber necrotic ringspot disease (PTNRD; Beczner *et al.*, 1984; Le Romancer *et al.*, 1994; Van den Heuvel *et al.*, 1994). In addition, the majority of PVY$^{NTN}$ isolates have recombinant genomes with variable recombination patterns (Boonham *et al.*, 2002; Glais *et al.*, 2002; Lorenzen *et al.*, 2006a; Schubert *et al.*, 2007; Ogawa *et al.*, 2008), although non-recombinant PVY$^{NTN}$ isolates were also reported from North America and were prevalent in Japan (Ohshima *et al.*, 2000; Nie & Singh, 2003a; Ogawa *et al.*, 2008). According to their sequences, Japanese and North American

*E-mail: mhmdsaidsyr@hotmail.com

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$PVY^{N/NTN}$ differed from the conventional $PVY^{N}$ (Ogawa *et al.*, 2008).

$PVY^{N}W$ induced veinal necrosis in tobacco, but reacted to $PVY^{O}$ monoclonal antibodies (McDonald & Singh, 1996a; Chrzanowska & Doroszewska, 1997). These combined characteristics resulted from the recombinant genome of this strain (Glais *et al.,* 2002; Schubert *et al.,* 2007).

The emergence of $PVY^{N}W$ and $PVY^{NTN}$ by recombination between the classical strains $PVY^{O}$ and $PVY^{N}$ was followed by a dramatic increase in their incidence. Within a comparatively short time they have reached predominance in most potato production areas (Kerlan *et al.*, 1999; Piche *et al.*, 2004; Glais *et al.*, 2005; Crosslin *et al.*, 2006; Lorenzen *et al.*, 2006a; Chikh Ali *et al.*, 2007; Schubert *et al.*, 2007). The emergence of new strains of PVY would complicate PVY management strategies (Gray *et al.,* 2008), hence increasing the necessity of studying PVY populations in potato production areas. In a previous study on PVY, the main virus infecting potatoes in Syria, a novel recombinant isolate group of PVY, provisionally designated $PVY^{SYR}$, was reported (Chikh Ali *et al.,* 2007). Isolates of $PVY^{SYR}$ were prevalent in Syria, which highlights their significance (Chikh Ali *et al.*, 2007, 2008a). The representative isolate of $PVY^{SYR}$, SYR-NB-16 shared properties with $PVY^{NTN}$ and $PVY^{N}W$, complicating the classification of $PVY^{SYR}$ (Chikh Ali *et al.,* 2007). Moreover the phenotype in potato, which is a classification criterion of PVY strains (Singh *et al.*, 2008), is needed to achieve final classification of $PVY^{SYR}$. The objectives of the present study were to elucidate the origin, assess the significance and achieve final classification of $PVY^{SYR}$.

## Materials and methods

### $PVY^{SYR}$ isolates

A total of 20 $PVY^{SYR}$ isolates from potato and weed plants were sampled from potato fields in Syria from 2002 to 2007 (Table 1). Original samples were stored as silica-dried samples at 4°C. Among the 20 $PVY^{SYR}$ isolates, 15 were maintained in *N. tabacum* cv. Samsun, from which samples were stored as fresh and/or freeze-dried at −80°C.

### Identification of $PVY^{SYR}$ isolates by RT-PCR

TRIzol Reagent (Invitrogen) was used to extract total RNA from fresh tobacco or dried potato leaves according to the manufacturer's instructions. The total RNA extract was dissolved in 125 μL RNase-free water. To identify $PVY^{SYR}$ isolates and test for possible mixed infections with other PVY strains, three polymerase chain reaction (PCR) assays were conducted. $PVY^{SYR}$ isolates were identified by detecting the three recombination points at HC-Pro/P3, 6K2/VPg and NIb/CP previously reported for the genome of SYR-NB-16 (Chikh Ali *et al.*, 2007). These recombination points were detected using two PCR assays. Multiplex PCR to detect the three recombination points at HC-Pro/P3, 6K2/VPg and the C terminus of the CP coding region of PVY genome (Nie & Singh, 2003b) was performed to scan all PVY samples as a first step (hereafter referred to as MultiRec-PCR). Then, PVY isolates with two recombination points at HC-Pro/P3 and 6K2/VPg were tested with the primer pair YN5-7350 and YO3-8648 (Schubert *et al.*, 2007) in a uniplex PCR (hereafter referred to as UniRec-PCR) to detect the third recombination point at NIb/CP. To check that $PVY^{SYR}$ did not contain a mix of PVY genotypes including those of $PVY^{O}$, $PVY^{N}W$ and $PVY^{N:O}$, the multiplex PCR assay reported by Lorenzen *et al.* (2006b) was conducted. For this purpose, a primer set consisting of five primers (o2172, o2439c, o6266c, S5585m and A6032m; Lorenzen *et al.,* 2006b) was used (hereafter referred to as Mix-PCR).

First-strand cDNA was synthesized using ReverTra Ace Kit (Toyobo Co., Ltd.) according to the manufacturer's instructions using an Oligo-dT primer as a reverse primer for the UniRec- and Mix-PCR assays (Schubert *et al.,* 2007; Lorenzen *et al.,* 2006b) and the specific reverse primers for the MultiRec-PCR assays (Nie & Singh, 2003b).

PCR assays were carried out using a TaKaRa Ex Taq™ kit (Takara Bio Inc.) according to the manufacturer's instructions. Amplification was performed in a PTC-100™ thermal cycler (MJ Research, Inc.). Thermocycle programmes and primer concentrations were used according to the original report of each assay. PCR products were separated in 2% agarose gel in TAE buffer, stained in a solution of ethidium bromide, and viewed under UV illumination.

### Biological characterization

To investigate the pathotype of $PVY^{SYR}$ isolates in tobacco, two plants per isolate of *N. tabacum* cv. Samsun were manually inoculated at the 3- to 4-leaf stage. Inoculated plants were allowed to grow in an incubator at 20–23°C with a 14-h photoperiod.

To classify the strain, the PVY isolates SYR-NB-16, SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH were inoculated onto the potato cultivars Desiree (or Pentland Crown), Maris Bard and King Edward, which carry the resistance genes *Ny*, *Nc* and *Nz*, respectively (Jones, 1990). To further investigate the ability to induce PTNRD, potato cv. Nishiyutaka plants were inoculated with PVY isolates SYR-NB-16 (five plants), SYR-II-2-8 (three plants) and SYR-II-DrH (four plants). Potato plants with 4–8 fully developed leaves were manually inoculated and grown at a temperature of 18–20°C and normal light. Symptom observation, which started 3 days after inoculation, was carried out daily for 5 weeks. Harvested potato tubers were kept at room temperature and checked for necrotic symptoms for 6 weeks. Healthy controls were buffer-inoculated potato and tobacco plants from each cultivar.

**Table 1** Isolate name, genomic segments and DDBJ/EMBL/GenBank databases accession numbers of *Potato virus Y* isolates collected in Syria

| Isolate | Sampling year | Host/cultivar | Tobacco reaction[a] | Recombination sites (nucleotide position) | Genotype | Accession No. (sequenced region) |
|---|---|---|---|---|---|---|
| SYR-NB-16 | 2002 | Potato/Unknown | Vn | HC-Pro/P3[b], 6K2/VPg[c], NIb/CP[d] | SYR-I | AB270705 (full) |
| SYR-I-15 | 2002 | Potato/Unknown | Vn | HC-Pro/P3, 6K3/VPg, NIb/CP | SYR-I | AB461455[l], AB461456[m] |
| SYR-II-S1 | 2004 | Potato/Diament | NI | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461483[l], AB461484[m] |
| SYR-II-Be1 | 2004 | Potato/Benilla | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461452 (full) |
| SYR-II-Sn | 2004 | *Solanum nigrum* | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461487[l], AB295475[m] |
| SYR-II-4Z | 2004 | *Physalis* sp. | NI | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461461[l], AB461462[m] |
| SYR-II-A20 | 2004 | *S. nigrum* | NI | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461465[l], AB461466[m] |
| SYR-II-A27 | 2004 | *Physalis* sp. | NI | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461469[l], AB461470[m] |
| SYR-II-Be3 | 2004 | Potato/Benilla | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461471[l], AB461472[m] |
| SYR-II-Be4 | 2004 | Potato/Benilla | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461473[l], AB461474[m] |
| SYR-II-Be5 | 2004 | Potato/Benilla | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461475[l], AB461476[m] |
| SYR-II-Bu5 | 2004 | Potato/Burren | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461477[l], AB461478[m] |
| SYR-II-L1 | 2004 | Potato/Lesita | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461479[l], AB461480[m] |
| SYR-II-L3 | 2004 | Potato/Lesita | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461481[l], AB461482[m] |
| SYR-II-2-8 | 2006 | Potato/Benilla | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461451 (full) |
| SYR-II-DrH | 2007 | Potato/Draga | Vn | P1(499)[e], HC-Pro/P3[f], 6K2/VPg[g], NIb/CP[h] | SYR-II | AB461453 (full) |
| SYR-II-SP8 | 2007 | Potato/Benilla | Vn | P1(499), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-II | AB461485[l], AB461486[m] |
| SYR-III-A26 | 2004 | *Physalis* sp. | NI | P1(686)[i], HC-Pro/P3[j], 6K2/VPg[j], NIb/CP[k] | SYR-III | AB461467[l], AB461468[m] |
| SYR-III-2-5 | 2006 | Potato/Benilla | Vn | P1(686), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-III | AB461459[l], AB461460[m] |
| SYR-III-2-4 | 2006 | Potato/Benilla | Vn | P1(686), HC-Pro/P3, 6K2/VPg, NIb/CP | SYR-III | AB461457[l], AB461458[m] |

[a]Vn, veinal necrosis; NI, no infection after inoculation of tobacco.
[b]Detected by *P*-values using RDP (R; $5{\cdot}364 \times 10^{-35}$), GENECONV (G; $2{\cdot}912 \times 10^{-24}$), BOOTSCAN (B; $7{\cdot}662 \times 10^{-33}$), MAXCHI (M; $1{\cdot}103 \times 10^{-22}$), CHIMAERA (C; $9{\cdot}867 \times 10^{-13}$) and SISCAN (S; $5{\cdot}732 \times 10^{-26}$).
[c]Detected by *P*-values using R ( $\leq 2{\cdot}8 \times 10^{-6}$), G ($7{\cdot}939 \times 10^{-12}$), B ($1{\cdot}578 \times 10^{-16}$), M ($6{\cdot}166 \times 10^{-6}$), C ($4{\cdot}506 \times 10^{-6}$) and S ($2{\cdot}752 \times 10^{-8}$).
[d]Detected by *P*-values using R ( $\leq 2{\cdot}8 \times 10^{-6}$), G ($7{\cdot}939 \times 10^{-12}$), B ($1{\cdot}578 \times 10^{-16}$), M ($5{\cdot}023 \times 10^{-6}$), C ($4{\cdot}506 \times 10^{-6}$) and S ($2{\cdot}752 \times 10^{-8}$).
[e]Detected by *P*-values using R ($1{\cdot}138 \times 10^{-29}$), G ($2{\cdot}121 \times 10^{-59}$), B ($2{\cdot}194 \times 10^{-28}$), M ($4{\cdot}813 \times 10^{-23}$), C ($6{\cdot}674 \times 10^{-24}$) and S ($2{\cdot}946 \times 10^{-30}$).
[f]Detected by *P*-values using R ($1{\cdot}297 \times 10^{-34}$), G ($8{\cdot}114 \times 10^{-23}$), B ($3{\cdot}807 \times 10^{-34}$), M ($4{\cdot}607 \times 10^{-15}$), C ($1{\cdot}691 \times 10^{-8}$) and S ($1{\cdot}556 \times 10^{-33}$).
[g]Detected by *P*-values using R ( $\leq 2{\cdot}045 \times 10^{-6}$), G ($2{\cdot}124 \times 10^{-5}$), B ($1{\cdot}459 \times 10^{-15}$), M ($1{\cdot}661 \times 10^{-5}$), C ($5{\cdot}755 \times 10^{-05}$) and S ($6{\cdot}280 \times 10^{-8}$).
[h]Detected by *P*-values using R ( $\leq 2{\cdot}045 \times 10^{-6}$), G ($2{\cdot}124 \times 10^{-5}$), B ($1{\cdot}459 \times 10^{-15}$), M ($5{\cdot}153 \times 10^{-7}$), C ($1{\cdot}549 \times 10^{-7}$) and S ($6{\cdot}280 \times 10^{-8}$).
[i]Detected by *P*-values using R ($1{\cdot}754 \times 10^{-65}$), G ($1{\cdot}217 \times 10^{-49}$), B ($4{\cdot}158 \times 10^{-64}$), M ($7{\cdot}724 \times 10^{-34}$), C ($4{\cdot}590 \times 10^{-16}$) and S ($3{\cdot}028 \times 10^{-22}$).
[j]Detected by MultiRec-PCR.
[k]Detected by *P*-values using R ($1{\cdot}754 \times 10^{-65}$), G ($1{\cdot}217 \times 10^{-49}$), B ($4{\cdot}158 \times 10^{-64}$), M ($3{\cdot}452 \times 10^{-34}$), C ($8{\cdot}116 \times 10^{-18}$) and S ($3{\cdot}028 \times 10^{-22}$).
[l]5′ NTR, P1 and partial HC-Pro.
[m]Partial NIb and CP.

## Serological characterization

The serotype of $PVY^{SYR}$ was investigated by compound direct ELISA using two PVY monoclonal antibodies, MAb2, which is specific for $PVY^{O+C}$, and 1F5, which is specific for $PVY^{N}$ (Agdia). In addition, $PVY^{SYR}$ isolates were tested using *Potato virus X* (PVX) antiserum (National Center for Seeds and Seedlings, Japan). The assays were conducted in duplicate according to manufacturer's instructions.

## Nucleic acid sequencing

The nucleotide sequences of three $PVY^{SYR}$ isolates, SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH, were fully determined excluding the primer sites at the 5′ and 3′ termini according to Chikh Ali *et al.* (2007). In addition, two nucleotide segments were determined for 16 $PVY^{SYR}$ isolates from potato and weed hosts. The first segment covered the 5′ nontranslated region (NTR) and the full P1 gene, and was amplified by the primers A (5′-CATT TGTGCCCAATTGCC-3′; Nie & Singh, 2002) and Nie 1 (5′-GGATCCAATTAAAACAACTCAATA-3′; Nie & Singh, 2003a). The second segment included the partial NIb and CP coding regions, and was amplified by the primers YN5-7350 (5′-TGCATGGCTTCCAGAAATG CAAT-3′) and YO3-8648 (5′-CTTTTCCTTTGTTCGG GTTTGAC-3′; Schubert *et al.*, 2007). Amplification was performed using a PTC-100 thermal cycler with the following programme: one cycle of 5 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 58°C and 90 s at 72°C, followed by a final extension for 10 min at 72°C. The amplification products (15 μL) were separated in 2% agarose gel in TAE buffer, stained in a solution of ethidium bromide and viewed under UV illumination. DNA purification, cloning and sequencing were carried out according to

Chikh Ali *et al.* (2007). The nucleotide sequences of 5′ NTR-P1 and partial NIb-CP were combined for each isolate to form a concatenated sequence (concat) of 2778bp.

### Sequence analysis

Sequence analysis was carried out using DNASIS software (Hitachi Software Engineering Co.). For the multiple alignment, the program CLUSTAL-X ver. 1·81 (Thompson *et al.,* 1997) was used with the default parameters. Alignments were conducted based on the whole genome and the concat of all PVY isolates with the complete genome available in GenBank (as of January 2009). In both cases the corresponding sequence of an isolate of *Pepper mottle virus* (PepMoV) was used as an outgroup. The aligned sequences were checked for recombination, using RDP (Martin & Rybicki, 2000), GENECONV (Sawyer, 1999), BOOTSCAN (Salminen *et al.,* 1995), MAXCHI (Maynard-Smith, 1992), CHIMAERA (Posada & Crandall, 2001) and SISCAN methods in RDP3 software (Martin *et al.,* 2005). The RDP3 analyses were done using default settings and a Bonferroni-corrected *P*-value cutoff of 0·05. Only recombination points detected using the six methods in the RDP3 program were taken into consideration. The identified recombination points were then tested by the program SISCAN version 2 (Gibbs *et al*., 2000) and confirmed visually. A homology search for the non-recombinant sequences was performed with other PVY isolates available in the GenBank database using the BLAST program provided by the National Center for Biotechnology Information (NCBI). Phylogeny inference was conducted using the neighbour-joining (NJ) method. The NJ trees were calculated with 1000 bootstrap replicates using the neighbour-joining option in CLUSTAL-X. Phylograms were displayed using TREEVIEW (Page, 1996). Isolate names and GenBank accession numbers are shown in Fig. 3a.

## Results

### Identification of $PVY^{SYR}$ isolates by RT-PCR

In the MultiRec-PCR, all $PVY^{SYR}$ isolates produced two bands of 641 and 448 bp, indicating recombination points at HC-Pro/P3 and 6K2/VPg. $PVY^{SYR}$ isolates produced bands of 1337 bp in the UniRec-PCR, revealing recombination points in the NIb/CP region. $PVY^{SYR}$ isolates tested by the Mix-PCR produced bands of 452 bp, indicating a recombination point at 6K2/VPg, with no mixed infections with isolates of $PVY^{N}W$ and/or $PVY^{O}$.

### Biological characterization

A total of 15 isolates of $PVY^{SYR}$ inoculated onto tobacco induced veinal necrosis (Table 1). No infection in tobacco was detected for five isolates (Table 1). In potato, isolates SYR-NB-16, SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH induced systemic mottle and HR displayed as necrotic stipple streak and veinal necrosis in cvs Desiree and Pentland Crown. SYR-NB-16, SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH induced mosaic but not necrosis in King Edward. In Maris Bard, SYR-NB-16, SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH induced upper mottle and mosaic followed by systemic HR displayed as necrotic stipple streak and veinal necrosis.

Regarding tuber symptoms, SYR-II-Be-1 induced tuber necrosis on potato cvs Pentland Crown and Maris Bard (Fig. 1). SYR-II-2-8 induced cracks on Nishiyutaka and necrotic arcs and rings on Pentland Crown, while SYR-II-DrH induced deep cracks and necrosis on Nishiyutaka (Fig. 1). SYR-NB-16 induced milder and less frequent tuber necrosis on Nishitytaka, Desiree and Pentland Crown than the above isolates (Fig. 1).

### Serological characterization

All $PVY^{SYR}$ isolates reacted with MAb2, but not with 1F5, revealing O serotype. No specific reaction was detected with PVX antiserum for all samples tested.

### Molecular characterization

The fully determined sequences of SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH were 9660 nucleotides long, excluding primers, and encoded a polyprotein of 3061 amino acids. The DDBJ/EMBL/GenBank databases accession numbers for complete and partial sequences are shown in Table 1.

According to the structure of the sequences of the whole genome and concat, three recombination patterns in $PVY^{SYR}$ isolates were detected, designated SYR-I, SYR-II and SYR-III (Table 1; Fig. 2). Recombination points of SYR-I, SYR-II and SYR-III were detected using RDP, GENECONV, BOOTSCAN, MAXCHI, CHIMAERA and SISCAN methods in RDP3 software (examples of the *P*-values are shown in Table 1). All recombination points had *Z* scores >3 in SISCAN version 2 (data not shown). It is worth mentioning that the recombination event at NIb/CP of SYR-I and SYR-II occurred at various nucleotide positions in the segment from 8314 to 8598.

Most of the genome of SYR-I, SYR-II and SYR-III isolates shared highest identity with Syrian isolates of $PVY^{NTN}$ (PVY-12; AB185833; Chikh Ali *et al*., 2008b) and $PVY^{N}W$ (SYR-Wi-11; AB185832; Chikh Ali *et al.,* 2007; Fig. 2). The genomic segments from 2420 to 8590 of SYR-NB-16 showed highest identity with PVY-12 (99·5%). The genomic segment 8600–9660 had highest identity with that of SYR-Wi-11 (99·4%). Similarly, the sequences flanked by the recombination points in the P1 and NIb/CP regions of isolates SYR-II-2-8, SYR-II-Be1 and SYR-II-DrH shared highest identity with those of SYR-NB-16 and PVY-12 (99·1–99·7%; Fig. 2), whereas the genomic segment from the recombination point at NIb/CP to nucleotide position 9660 shared highest identity (99·5–99·7%) with that of isolate SYR-Wi-11 (Fig. 2). The homology search of the concat showed similar results to those for the complete genomes. As for SYR-III, the first genomic segment (19–680 nt) shared highest identity


Figure 1 Potato tuber necrosis caused by *Potato virus Y* (PVY$^{NTN-NW}$) isolates. (a, b, c) SYR-NB-16 in cvs Desiree, Pentland Crown and Nishiyutaka, respectively; (d) SYR-II-DrH in cv. Nishiyutaka; (e, f) SYR-II-2-8 in cvs Pentland Crown and Nishiyutaka, respectively; (g, h) SYR-II-Be1 in cvs Pentland Crown and Maris Bard, respectively.

of 97·2% with that of isolate SASA110 (PVY$^{O}$; UK), while the rest of the partial genome showed similar identity results to those of SYR-II (Fig. 2).

In the phylogenetic tree of the genomic segment from nucleotides 37 to 484, isolates of SYR-I grouped in the N lineage (Fig. 3a). Isolates of SYR-II and SYR-III grouped

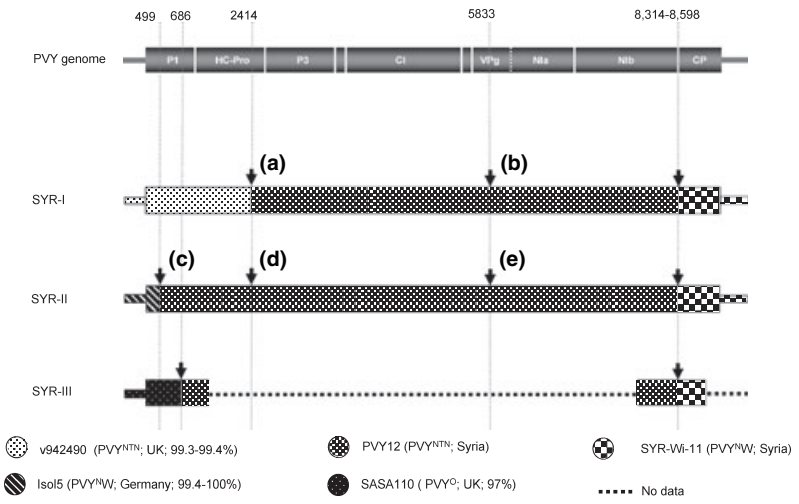


Figure 2 Sequence identity of *Potato virus Y* ($PVY^{SYR}$) genotypes SYR-I, SYR-II and SYR-III as revealed by the identity search. Arrows indicate recombination points: (a) a common recombination point in SYR-I, PVY-12 and v942490; (b) a common recombination point in SYR-I and PVY-12; (c) a common recombination point in SYR-II, PVY-12 and Isol5; (d, e) common recombination points in SYR-II and PVY-12.

in the O lineage along with PVY-12 and SYR-Wi-11 isolates (Fig. 3a). All $PVY^{SYR}$ isolates and SYR-Wi-11 grouped in the N lineage in the phylogenetic tree of the nucleotide segment 689–1077 (data not shown). $PVY^{SYR}$ moved to the O lineage in the phylogenetic tree of the nucleotide segment 2415–5715 (Fig. 3b). $PVY^{SYR}$ isolates regrouped in the N lineage of the phylogenetic tree of the segment 7348–8364 along with PVY-12 (data not shown). In the tree of the partial CP coding region (8600–9058) all Syrian isolates except for PVY-12 clustered in the O lineage along with SYR-Wi-11 (Fig. 3c).

## Discussion

In previous studies (Chikh Ali *et al.*, 2007, 2008a), a new recombinant strain group of PVY, namely $PVY^{SYR}$, was found to be prevalent in Syria. The present study on $PVY^{SYR}$ supports the following conclusions. First, $PVY^{SYR}$ consists of at least three recombination patterns, SYR-I, SYR-II and SYR-III. Secondly, $PVY^{SYR}$ is closely related to $PVY^{NTN}$ and $PVY^{N}W$ isolates from Syria, suggesting a common origin and possibly indicating local emergence of these isolates, i.e. in Syria. Thirdly, because of their ability to induce PTNRD, $PVY^{SYR}$ isolates represent a real challenge to potato production in Syria. Fourthly, considering their novel combination of characteristics and the shared properties with $PVY^{NTN}$ and $PVY^{N}W$, SYR-I and SYR-II isolates of $PVY^{SYR}$ are to be classified as a new recombinant strain within the $PVY^{N}$ strain group with the proposed name $PVY^{NTN\text{-}NW}$. Isolates of SYR-III were not included in the $PVY^{NTN\text{-}NW}$ strain since their phenotype in potato was not tested.

To investigate the origin of $PVY^{SYR}$ and the driving forces that led to their emergence, genetic analysis was performed for these isolates. In the recombination analysis, three recombination patterns were identified, namely SYR-I, SYR-II and SYR-III, which varied according to the 5′ NTR and the N terminus of the P1 coding region. This indicates that $PVY^{SYR}$ isolates represent heterogeneous genotypes and the isolate SYR-NB-16 represents the recombination pattern SYR-I, but not all isolates of $PVY^{SYR}$, as thought previously (Chikh Ali *et al.*, 2007). The central genomic portion of SYR-I (flanked by nucleotide position 2400 and the recombination point at NIb/CP) and that of the Syrian $PVY^{NTN}$ isolate PVY-12 were closely related. The CP and 3′ NTR of SYR-I were closely related to those of the Syrian $PVY^{N}W$ isolate SYR-Wi-11. The same held true for the isolates of SYR-II, except for the first 500-nucleotide segment which shared highest identity with those of $PVY^{NTN}$ and $PVY^{N}W$ from Europe, reaching 100% for some isolates. This would suggest that the recombination event in the P1 region might have followed that of the NIb/CP and resulted in the emergence of SYR-II. Isolates of SYR-II were more frequent than those of SYR-I, which were detected in only two potato samples collected in 2002, but not later. Isolates of SYR-III also seem to have emerged in the same way as those of SYR-II, except for the first 700-nucleotide sequence. The high sequence identity and close phylogenetic relationship of $PVY^{SYR}$ with $PVY^{NTN}$ and $PVY^{N}W$ isolates from Syria suggests a common origin and may indicate a local emergence of these isolates, i.e. in Syria. The recombination point at NIb/CP was not at an identical nucleotide position. These variable recombination sites could be explained by a low selection pressure at this site of the PVY genome.

The high incidence (Chikh Ali *et al.*, 2007, 2008a) and ability of $PVY^{SYR}$ isolates tested so far to induce PTNRD highlight the significance of these isolates and the necessity for their control. Although the tuber necrosis caused by SYR-NB-16 (SYR-I) was notably milder and less frequent than those caused by isolates of SYR-II it still affected tuber quality (Fig. 1). The emergence of a new genotype by recombination might be followed by a dramatic increase in the frequency of the emerged recombinants as a result of increased fitness (Nagy, 2008) and would consequently complicate management strategies.

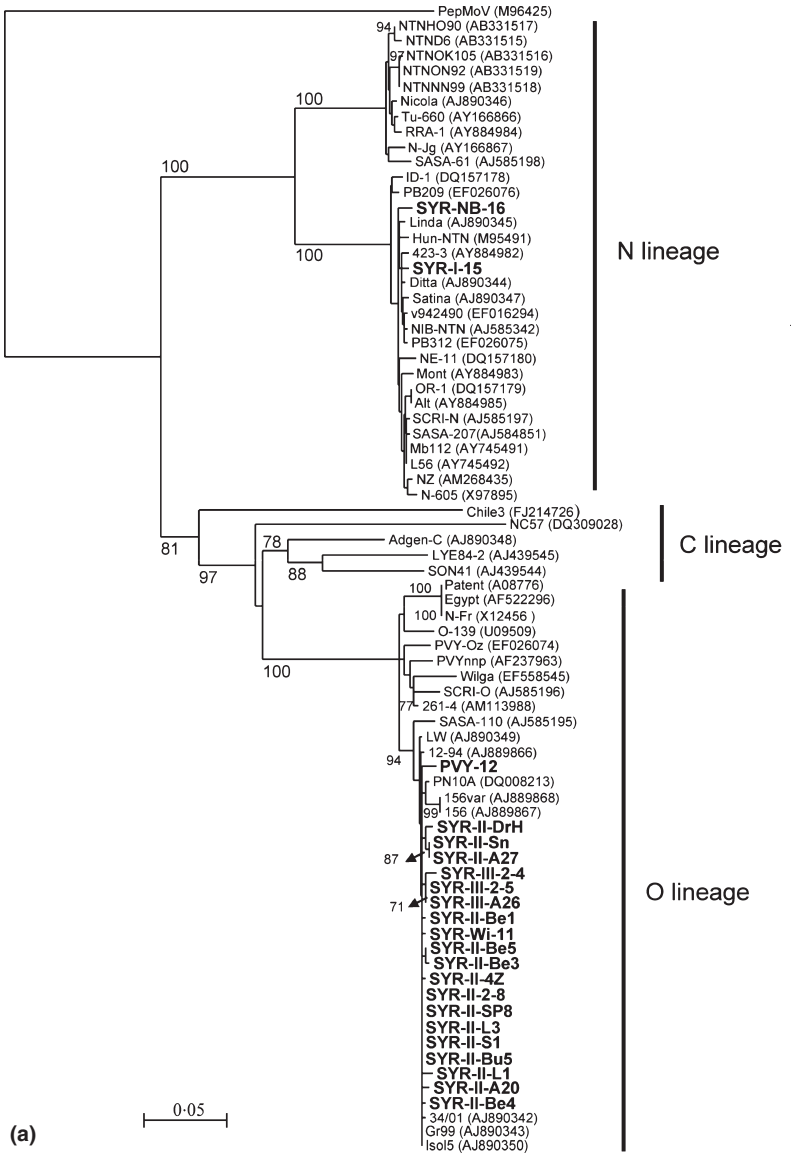


Figure 3 Neighbour-joining trees of all *Potato virus Y* (PVY) isolates available in the DDBJ/EMBL/GenBank databases (January 2009) based on nucleotide sequences (37–484; a), (2415–5715; b) and (8600–9058; c). Sequences were numbered according to the genome of PVY-12. The scale bar shows the number of substitutions per residue. Numbers at nodes indicate cluster bootstrap values (%). Syrian PVY isolates are shown in bold.

This would increase the significance of $PVY^{SYR}$ isolates as newly emerged genotypes that reflect the dynamic nature of PVY.

To classify $PVY^{SYR}$, their characteristics were compared with those of previously recognized PVY strains from potato (Singh *et al.*, 2008). $PVY^{SYR}$ shared common biological, serological and molecular properties with both $PVY^{N}W$ (or $PVY^{N:O}$) and $PVY^{NTN}$, which complicates their classification. In addition, no information on the reaction caused by $PVY^{NTN}$ and $PVY^{N}W$ in differential potato cultivars is available (Singh *et al.*, 2008), which prevents comparison with $PVY^{SYR}$. In general, PVY strains induce either HR or mosaic in potato cultivars with the *Ny*, *Nc* and *Nz* resistance genes (Singh *et al.*, 2008). In the present study, however, isolates of $PVY^{SYR}$ tested induced both reactions, i.e. mosaic and HR, in some cultivars, particularly in Desiree/Pentland Crown and Maris Bard. The recombinant nature of these isolates may have resulted in this double reaction. However, the validation of such a conclusion awaits the results for similar isolates from other parts of the world, or the discovery of the genomic determinants of HR. All $PVY^{SYR}$ isolates tested caused similar phenotypes in the potato cultivars carrying the *Ny*, *Nc* and *Nz* resistance genes used in this study.

According to Singh *et al.* (2008), new PVY strains should not be named based on geographic region, but on

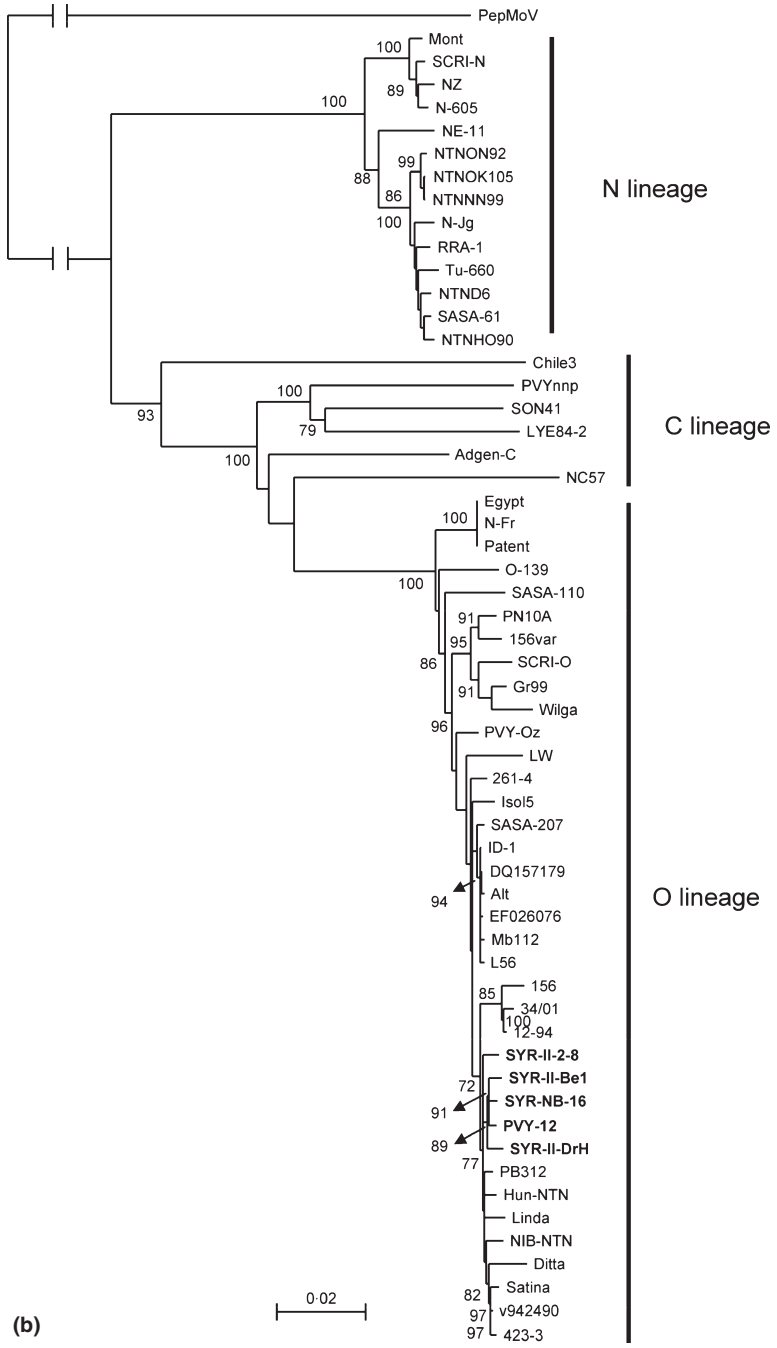


Figure 3 *Continued.*

the characteristics of the strain. Similar to $PVY^{NTN}$ and $PVY^{N}W$, $PVY^{SYR}$ therefore represents a new strain within the $PVY^{N}$ strain group, with the proposed name $PVY^{NTN\text{-}NW}$. This classification is to be applied only for SYR-I and SYR-II, since the phenotype of SYR-III in potato was not tested and the ability to induce PTNRD has yet to be examined. Although the novel strain $PVY^{NTN\text{-}NW}$ is composed of more than one genotype (at least two) it can still be considered as a single strain. In fact, variable genotype would not affect the classification of PVY strains unless linked to a distinct phenotype (Singh *et al.*, 2008). Such is the case for many PVY strains which consist of various genotypes, such as $PVY^{C}$ (C1 and C2; Blanco-Urgoiti *et al.*, 1998), $PVY^{N}$ (N-North America and N-Europe; Ogawa *et al.*, 2008), $PVY^{NTN}$ and $PVY^{N}W$, with variable recombination patterns (Schubert *et al.*, 2007; Ogawa *et al.*, 2008). The recombination pattern of SYR-II seems to be similar to that of

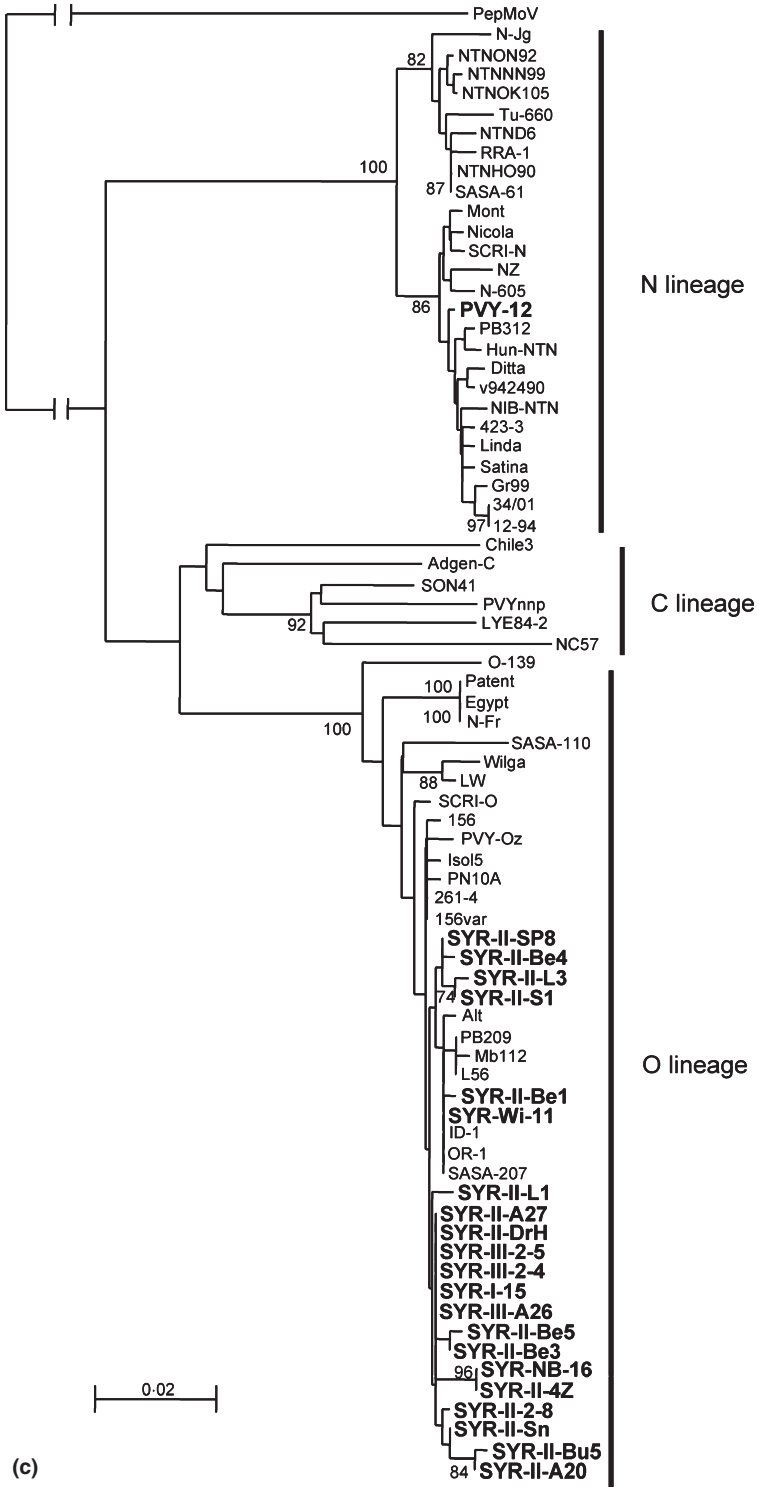


Figure 3 *Continued.*

isolate 156 from Germany (AJ889867; Schubert *et al*., 2007; Fig. 2). However, Ogawa *et al*. (2008) detected a further possible recombination point within the genome of isolate 156 at nucleotide position 2998. The same recombination point was detected in the present recombination analysis using BOOTSCAN ($P = 8{\cdot}447 \times 10^{-4}$), MAXCHI ($P = 9{\cdot}392 \times 10^{-5}$) and SISCAN ($P = 8{\cdot}447 \times 10^{-21}$). No such recombination was detected in the

genome of any $PVY^{SYR}$ isolates. In addition, the recombination point at HC-Pro/P3 was not at an identical position for isolate 156 (nt 2392) and SYR-II isolates (nt 2414). The phylogenetic analysis of PVY isolates (Ogawa *et al*., 2008; Fig. 3a,b) showed that isolate 156 was closely related to isolates of $PVY^{NTN}$, such as 34/01 (Poland; AJ890342) and 12-94 (Poland; AJ889866), and $PVY^{N}W$ (Germany; 156var; AJ889868), that share a close geographic origin. SYR-II isolates, however, were closely related to isolates of $PVY^{NTN}$ and $PVY^{N}W$ from Syria, which may suggest convergent evolution. Moreover, unlike isolates of SYR-II, there is no report on the ability of isolate 156 to induce PTNRD, so it was classified as an isolate of strain $PVY^{N}W$ (Schubert *et al*., 2007).

In conclusion, SYR-I and SYR-II isolates of $PVY^{SYR}$ are to be classified as a new strain within the $PVY^{N}$ strain group. The name $PVY^{NTN\text{-}NW}$ is proposed for this novel strain because of its shared properties with $PVY^{NTN}$ and $PVY^{N}W$.

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## References

Beczner L, Horvath H, Romhanyi L, Foster H, 1984. Etiology of tuber ringspot disease in potato. *Potato Research* **27**, 339–52.

Blanco-Urgoiti B, Sanchez F, de San Roman CP, Dopazo J, Ponz F, 1998. *Potato virus Y* group C isolates are a homogeneous pathotype but two different genetic strains. *Journal of General Virology* **79**, 2037–42.

Boonham N, Walsh K, Hims M, Preston S, North J, Barker I, 2002. Biological and sequence comparisons of *Potato virus Y* isolates associated with potato tuber necrotic ringspot disease. *Plant Pathology* **51**, 117–26.

Chikh Ali M, Maoka T, Natsuaki KT, 2007. The occurrence and characterization of new recombinant isolates of PVY displaying shared properties of $PVY^{N}W$ and $PVY^{NTN}$. *Journal of Phytopathology* **155**, 409–15.

Chikh Ali M, Maoka T, Natsuaki KT, 2008a. The emergence and prevalence of a heterogeneous recombinant subpopulation of *Potato virus Y* in Syria. In: *Proceedings of the 14th International Congress of Virology, August 10-15, 2008, Istanbul, Turkey*. 383.

Chikh Ali M, Maoka T, Natsuaki KT, 2008b. Whole genome sequence and characterization of a novel isolate of PVY inducing tuber necrotic ringspot in potato and leaf mosaic in tobacco. *Journal of Phytopathology* **156**, 413–8.

Chrzanowska M, 1991. New isolates of the necrotic strain of *Potato virus Y* ($PVY^{N}$) found recently in Poland. *Potato Research* **34**, 179–82.

Chrzanowska M, Doroszewska T, 1997. Comparison between PVY isolates obtained from potato and tobacco plants grown in Poland. *Phytopathologia Polonica* **13**, 63–71.

Crosslin JM, Hamm PB, Hane DC *et al*., 2006. The occurrence of $PVY^{O}$, $PVY^{N}$, and $PVY^{N:O}$ strains of *Potato virus Y* in certified potato seed lot trials in Washington and Oregon. *Plant Disease* **90**, 1102–5.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, 2005. *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*. Amsterdam, Netherlands: Elsevier Academic Press.

Gibbs MJ, Armstrong JS, Gibbs AJ, 2000. Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics* **16**, 573–82.

Glais L, Tribodet M, Kerlan C, 2002. Genomic variability in *Potato potyvirus Y* (PVY): evidence that $PVY^{N}W$ and $PVY^{NTN}$ variants are single or multiple recombinants between $PVY^{O}$ and $PVY^{N}$ isolates. *Archives of Virology* **147**, 363–78.

Glais L, Tribodet M, Kerlan C, 2005. Specific detection of the $PVY^{N}$-W variant of *Potato virus Y*. *Journal of Virological Methods* **125**, 131–6.

Gray S, Karasev A, Lorenzen J, Whitworth J, Nolte P, Perry K, 2008. Emerging diversity in *Potato virus Y* poses new challenges for the US potato industry. *Phytopathology* **98**(Suppl. 6), 61.

Jones RAC, 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *Annals of Applied Biology* **117**, 93–105.

Kerlan C, Tribodet M, Glais L, Guillet M, 1999. Variability of *Potato virus Y* in potato crops in France. *Journal of Phytopathology* **147**, 643–51.

Le Romancer M, Kerlan C, Nedellec M, 1994. Biological characterization of various geographical isolates of *Potato virus Y* including superficial necrosis on potato tubers. *Plant Pathology* **43**, 138–44.

Lorenzen JH, Meacham T, Berger PH *et al*., 2006a. Whole genome characterization of *Potato virus Y* isolates collected in the western USA and their comparison to isolates from Europe and Canada. *Archives of Virology* **151**, 1055–74.

Lorenzen JH, Piche L, Gudmestad NC, Meacham T, Shiel P, 2006b. A multiplex PCR assay to characterize *Potato virus Y* isolates and identify strain mixtures. *Plant Disease* **90**, 935–40.

McDonald JG, Singh RP, 1996a. Host range, symptomatology and serology of isolates of *Potato virus Y* (PVY) that shared properties with both the $PVY^{N}$ and $PVY^{O}$ strain groups. *American Potato Journal* **73**, 309–15.

McDonald JG, Singh RP, 1996b. Response of potato cultivars to North American isolates of $PVY^{NTN}$. *American Potato Journal* **73**, 317–23.

Martin D, Rybicki E, 2000. RDP: detection of recombination amongst aligned sequences. *Bioinformatics* **16**, 562–3.

Martin D, Williamson C, Posada D, 2005. RDP2: recombination detection and analysis from sequence alignment. *Bioinformatics* **21**, 260–2.

Maynard-Smith J, 1992. Analyzing the mosaic structure of genes. *Journal of Molecular Evolution* **34**, 126–9.

Nagy PD, 2008. Recombination in plant RNA viruses. In: Roossinck MJ, ed. *Plant Virus Evolution*. Heidlberg, Germany: Springer-Verlag, 133–56.

Nie X, Singh RP, 2002. A new approach for the simultaneous differentiation of biological and geographical strains of *Potato virus Y* by uniplex and multiplex RT-PCR. *Journal of Virological Methods* **104**, 41–54.

Nie X, Singh RP, 2003a. Evolution of North American $PVY^{NTN}$ strain Tu 660 from local $PVY^{N}$ by mutation rather than recombination. *Virus Genes* **26**, 39–47.

Nie X, Singh RP, 2003b. Specific differentiation of recombinant $PVY^{N:O}$ and $PVY^{NTN}$ isolates by multiplex RT-PCR. *Journal of Virological Methods* **113**, 69–77.

Ogawa T, Tomitaka Y, Nakagawa A, Ohshima K, 2008. Genetic structure of a population of *Potato virus Y* inducing potato tuber necrotic ringspot disease in Japan; comparison with North American and European populations. *Virus Research* **131**, 199–212.

Ohshima K, Sako K, Hiraishi C *et al.*, Nakagawa A, Matsuo K, Ogawa T, Shikata E, Sako N, 2000. Potato tuber necrotic ringspot disease occurring in Japan: its association with Potato virus Y necrotic strain. *Plant Disease* **84**, 1109–15.

Page RDM, 1996. TreeView: an application to display phylogenetic trees on personal computer. *Computer Applications in the Biosciences* **12**, 357–8.

Piche LM, Singh RP, Nie X, Gudmestad NC, 2004. Diversity among *Potato virus Y* isolates obtained from potatoes grown in the United States. *Phytopathology* **94**, 1368–75.

Posada D, Crandall KA, 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. *Proceedings of the National Academy of Sciences, USA* **98**, 13757–62.

Salminen MO, Carr JK, Burke DS, McCutchan FE, 1995. Identification of breakpoints in intergenotypic recombinants of HIV type 1 by bootscanning. *AIDS Research and Human Retroviruses* **11**, 1423–5.

Sawyer SA, 1999. *GENECONV: a Computer Package for the Statistical Detection of Gene Conversion*. St Louis, MO, USA: Washington University, Department of Mathematics. http://www.math.wustl.edu/~sawyer.

Schubert J, Fomitcheva V, Sztangret-Wisniewski J, 2007. Differentiation of *Potato virus Y* using improved sets of diagnostic PCR-primers. *Journal of Virological Methods* **140**, 66–74.

Singh RP, Valkonen JPT, Gray SM *et al.*, 2008. The naming of *Potato virus Y* strains infecting potato. *Archives of Virology* **153**, 1–13.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The CLUSTAL-X windows interface: flexible strategies for multiple-sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **22**, 4673–80.

Van den Heuvel JFJM, Van der Vlugt RAA, Verbeek M, De Haan PT, Huttinga H, 1994. Characteristics of a resistance-breaking isolate of potato virus Y causing potato tuber necrotic ringspot disease. *European Journal of Plant Pathology* **100**, 347–56.