



**HAL**  
open science

## **Brazilian Potato virus Y isolates identified as members of a new clade facilitate the reconstruction of evolutionary traits within this species**

Bérenger Janzac, A. Willemsen, J. M. Cuevas, Laurent Glais, Michel Tribodet, J. L. Verrier, S. F. Elena, Emmanuel Jacquot

### ► To cite this version:

Bérenger Janzac, A. Willemsen, J. M. Cuevas, Laurent Glais, Michel Tribodet, et al.. Brazilian Potato virus Y isolates identified as members of a new clade facilitate the reconstruction of evolutionary traits within this species. *Plant Pathology*, 2015, 64 (4), pp.799-807. 10.1111/ppa.12318 . hal-01208793

**HAL Id: hal-01208793**

**<https://hal.science/hal-01208793>**

Submitted on 27 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/267457255>

# Brazilian Potato virus Y isolates identified as members of a new clade facilitate the reconstruction of evolutionary traits within this species

ARTICLE *in* PLANT PATHOLOGY · OCTOBER 2014

Impact Factor: 2.97 · DOI: 10.1111/ppa.12318

DOWNLOADS

18

VIEWS

79

8 AUTHORS, INCLUDING:



**Anouk Willemsen**

Instituto de Biología Molecular y Celular de...

11 PUBLICATIONS 43 CITATIONS

SEE PROFILE



**José M Cuevas**

University of Valencia

47 PUBLICATIONS 784 CITATIONS

SEE PROFILE



**Santiago F Elena**

Spanish National Research Council

222 PUBLICATIONS 7,322 CITATIONS

SEE PROFILE



**Emmanuel Jacquot**

Agrocampus Ouest

51 PUBLICATIONS 512 CITATIONS

SEE PROFILE

Available from: Santiago F Elena  
Retrieved on: 19 August 2015

Comment citer ce document :

Janzac, B., Willemsen, A., Cuevas, J. M., Glais, L., Tribodet, M., Verrier, J. L., Elena, S. F., Jacquot, E. (2015). Brazilian Potato virus Y isolates identified as members of a new clade facilitate the reconstruction of evolutionary traits within this species. *Plant Pathology*, 64(4), 799-807. DOI : 10.1111/ppa.12318

## Brazilian *Potato virus Y* isolates identified as members of a new clade facilitate the reconstruction of evolutionary traits within this species

B. Janzac<sup>ab†</sup>, A. Willemsen<sup>c†</sup>, J. M. Cuevas<sup>c</sup>, L. Glais<sup>ad</sup>, M. Tribodet<sup>a</sup>, J.-L. Verrier<sup>e</sup>, S. F. Elena<sup>cf</sup> and E. Jacquot<sup>ab\*</sup>

<sup>a</sup>UMR 1349 IGEPP, INRA, Domaine de la Motte, F-35653 Le Rheu; <sup>b</sup>UMR 385 BGPI, INRA-Cirad-SupAgro Montpellier, Cirad TA A-54K, Campus international de Baillarguet, F-34398 Montpellier, France; <sup>c</sup>Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia, 46022 Valencia, Spain; <sup>d</sup>FN3PT/RD3PT, 43-45 rue de Naples, F-75008 Paris, France; <sup>e</sup>Imperial Tobacco Group, SEITA, Institut du Tabac, Domaine de la Tour, F-24100, Bergerac, France and <sup>f</sup>The Santa Fe Institute, Santa Fe, NM 87501, USA

*Potato virus Y* (PVY) is a plant virus distributed worldwide that causes damage to several species of the Solanaceae family. It was established long ago that groups of PVY isolates defined by phylogenetic analyses correlate strongly with those demarcated by differential biological properties. Consequently, life-history traits of this viral species can be inferred by phylogenetic analysis. In this study, characteristics of PVY isolates sampled in different tobacco fields in Brazil were analysed and most of the tested Brazilian PVY isolates were assigned to the recently described unconventional serogroup Y<sup>U</sup>. The analysis of molecular diversity of the coat protein (CP) cistron from some Y<sup>U</sup> isolates made it possible (i) to identify specific amino acid residues in the N-terminal of the CP protein and (ii) to assign some Y<sup>U</sup> isolates to a new PVY clade. The symptoms caused by isolates belonging to this new PVY 'Brazilian' clade and their ability to infect selected susceptible hosts led to the conclusion that neither veinal necrosis symptoms expressed on infected tobacco plants nor adaptation to potato or pepper hosts are ancestral characteristics of PVY. These observations suggest that PVY has gained a remarkable new biological property and broadened its host range over time.

**Keywords:** classification, host range, phylogeny, *Potyviridae*, serotype

### Introduction

*Potato virus Y* (PVY) is the type member of the genus *Potyvirus* (family *Potyviridae*). The PVY genome, a single-stranded positive-sense RNA molecule of *c.* 10 kb, encodes a polyprotein that is cleaved by three virus-encoded proteases into 10 products (Dougherty & Carrington, 1988). A short overlapping gene (PIPO), embedded within the previously described large ORF, was recently proposed for some potyviruses, including PVY (Chung *et al.*, 2008). PVY infects different solanaceous crops such as potato, tomato, tobacco and pepper. The diversity of biological, serological and molecular characteristics of PVY isolates has led to a complex classification of this viral species. The first level of classification groups isolates into strains according to the host from which they were collected (Kerlan & Moury, 2008).

PVY isolates collected from potatoes were originally subdivided into PVY<sup>O</sup>, PVY<sup>C</sup> and PVY<sup>N</sup> strains. These strains were defined according to their capacity to overcome potato resistance genes and their phenotype in infected tobacco plants. PVY<sup>O</sup> and PVY<sup>C</sup> isolates elicit

hypersensitive reactions (HR) on resistant potato cultivars carrying the *Nyibr* or the *Nc* resistance genes, respectively. PVY isolates that failed to elicit HR in the presence of the *Nyibr* or *Nc* genes in potato cultivars but were able to induce both mild mottle symptoms in most potato cultivars and veinal necrosis in tobacco leaves, were designated as members of the PVY<sup>N</sup> strain (Keller & Münster, 1961). This classification is also supported by serological properties of the isolates (Gugerli & Fries, 1983). Furthermore, it was established that the classification of PVY isolates in these three groups correlates with the phylogenetic trees resulting from molecular analyses (Moury, 2010). However, the observation of necrotic ringspot symptoms on potato tubers infected by some members of the PVY<sup>N</sup> group led to the identification of the PVY<sup>NTN</sup> subgroup (Le Romancer *et al.*, 1994). Subsequently, several subgroups constituted of either PVY<sup>O</sup>/PVY<sup>N</sup> recombinant isolates (Glais *et al.*, 2002) or isolates exhibiting new properties (e.g. virulence toward the potato *Nz* resistance gene) (Kerlan *et al.*, 1999) have also been proposed: e.g. PVY<sup>N-W</sup> (Chrzanoska, 1991), PVY<sup>ZE</sup> (Kerlan *et al.*, 1999) and PVY<sup>O5</sup> (Karasev *et al.*, 2011).

The potato strain PVY classification is widely used in the literature even for PVY isolates not originally collected on potato plants. However, the ability of some

\*E-mail: emmanuel.jacquot@supagro.inra.fr

†These authors have contributed equally to this work.

isolates to infect other hosts has been taken into consideration in order to refine the PVY classification. Pepper was shown to be a non-host for some PVY isolates. Isolates serologically linked to the potato PVY<sup>O</sup> group and able to infect peppers have been assigned to the PVY<sup>C</sup> group. Biological differences between pepper-type and potato-type PVY<sup>C</sup> isolates, supported by differences in their genomic sequences (Moury *et al.*, 2002), led to the description of two distinct phylogenetic subgroups, PVY<sup>C1</sup> and PVY<sup>C2</sup>, for isolates able to infect specifically pepper or potato, respectively (Kerlan & Moury, 2008).

Despite the economical and agronomical impacts of PVY on tobacco production, studies of the tobacco/PVY pathosystem are scarce. Tobacco strain PVY isolates have been initially classified into three groups, M<sup>S</sup>M<sup>R</sup>, M<sup>S</sup>N<sup>R</sup> and N<sup>S</sup>N<sup>R</sup>, on the basis of mosaic (M) and necrotic (N) symptoms induced on tobacco hosts known to be susceptible (S) or resistant (R) to the root-knot nematode *Meloidogyne incognita* (Gooding & Tolin, 1973). Indeed, the necrotic response to PVY infection and the resistance to *M. incognita* were found to be pleiotropic effects of the *Rk* gene (Rufty *et al.*, 1983). However, this *Rk*-based indirect viral typing is inappropriate to describe the diversity of biological properties of PVY populations in the current agronomical context. Consequently, the tobacco strain PVY classification is rarely used to describe PVY isolates.

Based on the data described above, the nomenclature O, N, C, C1 and C2 used in the present work refers to phylogenetic PVY groups/subgroups having the specific biological properties described for isolates originally sampled on potatoes. The use of the nomenclature Y<sup>O/C</sup> and Y<sup>N</sup> refers to the description of serological characteristics of PVY isolates.

In this study, biological, serological and molecular characteristics of isolates originating from different tobacco growing areas in Brazil were analysed. This data helped to identify a possible new PVY clade and to reconstruct the phylogeny and evolutionary traits within the species.

## Materials and methods

### Plants and viruses

In 2007–2008 leaves were collected from different tobacco cultivars in Brazil (Fig. S1). PVY infection status of samples was confirmed by serological typing (see below) as described in Lacroix *et al.* (2010). Twenty-one PVY-infected samples were selected and analysed. Their main characteristics are reported in Table S1.

Isolates PVY<sup>N</sup>-605 (accession X97895) and PVY<sup>O</sup>-139 (accession U09509) were used in the experiments as reference isolates for N and O groups, respectively. The PVY<sup>C1</sup>-SON41 (accession AJ439544) and PVY<sup>C2</sup>-Adgen-C (accession AJ890348) isolates were included as two reference isolates for C1 and C2 subgroups, respectively.

The PVY-susceptible *Nicotiana tabacum* ‘Xanthi’ was used to produce inoculum for characterization experiments. Biological characterization of PVY isolates was performed using *N. tabacum*

‘Xanthi’, *Solanum tuberosum* ‘Bintje’ and *Capsicum annuum* ‘Yolo Wonder’ (provided by A. Palloix, INRA) as indicator hosts. Healthy and infected plants were maintained in separate thermo-regulated insect-proof greenhouses (18/25°C, night/day).

### Characterization of PVY isolates

The presence of PVY in the collected samples was assessed by DAS-ELISA using a polyclonal antiserum raised against PVY (INRA Rennes/FN3PT) as previously described (Lacroix *et al.*, 2011). To identify samples infected only by PVY, DAS-ELISAs were performed using other polyclonal antisera raised against *Potato virus A* (BIOREBA), *Potato virus X* (INRA Rennes/FNPPPT), *Cucumber mosaic virus* (INRA Avignon), *Tobacco mosaic virus* (CNRS, Strasbourg), *Tobacco vein mottling virus* (Sediag, France) and *Alfalfa mosaic virus* (LCA, Bordeaux).

For characterization experiments, PVY-infected tobacco leaves were used to inoculate Xanthi plants, as described by Lacroix *et al.* (2010). Then, crude sap obtained from infected Xanthi was used as viral source for both production of PVY sequences and inoculations of indicator hosts. Symptom expression and sanitary status of tests plants were monitored 40 days post-inoculation by visual observations and ELISA, respectively.

### RNA extraction and partial sequencing

Total RNAs were extracted from PVY-infected leaves using the SV Total RNA Isolation system kit (Promega). Eluted fractions were used in a two-step reverse transcription-polymerase chain reaction (RT-PCR) carried out with *Avian myeloblastosis virus* reverse transcriptase (Promega) and GoTaq Flexi DNA polymerase (Promega). The RT step was performed in the presence of the 3' UTR primer. Amplification of genomic regions was performed by PCR using degenerated primer pairs. The RT-PCR experimental conditions and sequences of the primers are described in Table S2. Regions of the PVY genome were amplified between primer pairs corresponding to the 3' end of protein P1 with the 5' end of the helper component-proteinase (HC-Pro) and/or the 3' end of the RNA polymerase (NIb)/coat protein (CP) with the 3' untranslated region (UTR). The amplified sequences were individually cloned between the T3 and T7 primer binding sites of the Strataclone vector using the Strataclone PCR Cloning kit (Agilent Technologies) according to the manufacturer's instructions. Viral sequences contained in recombinant plasmids were sequenced by GenoScreen (Lille, France) using T3 (5'-ATTAACCCTCACTAAAGGGA-3') and T7 (5'-TAATACGACTCACTATAGGG-3') universal primers.

### Data set, alignment and recombination analysis

The CP-coding regions of the 21 PVY isolates originating from Brazil were sequenced (Table S1). Sequences of the CP cistron from reference PVY isolates (PVY<sup>N</sup>-605, PVY<sup>O</sup>-139, PVY<sup>C1</sup>-SON41 and PVY<sup>C2</sup>-Adgen-C) were retrieved from GenBank (Table S3). The isolate Chile3, which has been defined as a sister group of groups N, O and C (Moury, 2010), was included as an additional reference isolate. CP sequences from PVY isolates sampled in different French tobacco growing areas in 2007 (Lacroix *et al.*, 2010) were also included in the sequence data set (see Table S1 for accession numbers). The final assembled data set consisted of a total of 120 CP sequences, including the 21 Brazilian isolates and sequences downloaded from GenBank (Tables S1 & S3).

The nucleotide sequences were aligned at the amino acid level using MUSCLE as implemented in MEGA5 (Edgar, 2004) then back translated to nucleotides with PAL2NAL v. 14 (Suyama *et al.*, 2006) and identical sequences were removed with RAXML v. 8.0 (Stamatakis, 2014). Recombination analysis was performed using RDP3 (Martin *et al.*, 2010) employing several algorithms for the detection of putative recombinant sequences. Only the breakpoints detected by more than two out of the eight methods implemented were considered and recombinant sequences were excluded from the data set used in subsequent analyses. The recombination analysis was also done separately for the different PVY groups to remove any within-group recombinants before performing selection analyses. *Pepper severe mosaic virus* (Pep-SMV, accession AM181350; Ahn *et al.*, 2006) was introduced in the phylogenetic analyses as an out-group.

### Phylogenetic analysis

The nucleotide substitution model that best fitted the data was estimated using jMODELTEST 2 v. 2.1.4 (Darriba *et al.*, 2012). Maximum likelihood (ML) phylogenetic analysis was conducted using RAXML PTHREADS-BASED v. 8.0 (Stamatakis, 2014), under the GTR +  $\Gamma_4$  substitution model. Bayesian phylogenetic analysis was performed using BEAST v. 1.8.0 (Drummond & Rambaut, 2007) with the same substitution model. Due to absence of information on sampling dates for some of the GenBank collected sequences, sampling times were not specified. Estimation was done using the relaxed uncorrelated exponential clock model with a log-normal distribution set as the mean prior. This combination of the clock model and the mean prior distribution was tested to be the most plausible model calculating Bayes factor  $K = P(D|M_1)/P(D|M_2)$  where  $P(D|M_x)$  is the posterior probability of a model  $x$  given the data  $D$ . Two independent Markov chain Monte Carlo (MCMC) algorithms were completed with a chain length of  $10^8$  sampling every 1000 trees to establish convergence of all parameters. The trees were summarized onto a single target tree using TREEANNOTATOR v. 1.8.0 implemented in the BEAST package, discarding the first 10% of sampled trees as burn-in.

## Results

### Serological characterization of PVY isolates

Leaves showing typical symptoms of PVY infection were sampled from different tobacco growing areas in Paraná, Rio Grande do Sul and Santa Catarina states located in the south of Brazil (Table S1). Serological typing targeting the most prevalent viral species in tobacco was carried out to identify single (i.e. PVY only) or mixed infections in tested samples. To assign detected PVY isolates to their appropriate serogroup, all PVY-infected samples were tested by ELISA with monoclonal antibodies raised against  $Y^N$  (INRA Rennes / FN3PT) and  $Y^{O/C}$  (Neogen). Only a few Brazilian PVY isolates could be assigned to the  $Y^O$  or  $Y^N$  serogroups by this method (Table S1; Fig. 1). Indeed, 15 out of the 21 Brazilian samples determined as PVY-positive by ELISA using PVY-polyclonal antisera, gave negative PVY detection results (i.e. considered as healthy samples) when serological assays were carried out with the  $Y^N$ - or  $Y^{O/C}$ -specific monoclonal antisera used in this work. For this reason,

these 15 PVY isolates were assigned into the ‘unconventional’ serogroup ‘ $Y^U$ ’ recently described by Lacroix *et al.* (2011).

### Recombination analysis

A set of 120 CP sequences, including Brazilian isolates assigned into the  $Y^U$  serogroup (see above), were used to locate these isolates within the global PVY phylogenetic tree and to test the hypotheses of life-history traits ancestry. After removing identical and recombinant sequences, 117 CP sequences were analysed (Fig. 1). Recombination analysis was performed using the whole data set and also separately for the different PVY phylogenetic groups. Recombinant isolates found in this study are  $PVY^{O-}$ SASA-110 (accession AJ585195),  $PVY^O$ -tamarillo (accession FM244834) and the French isolate  $PVY^C$ -278 (accession KJ741178).

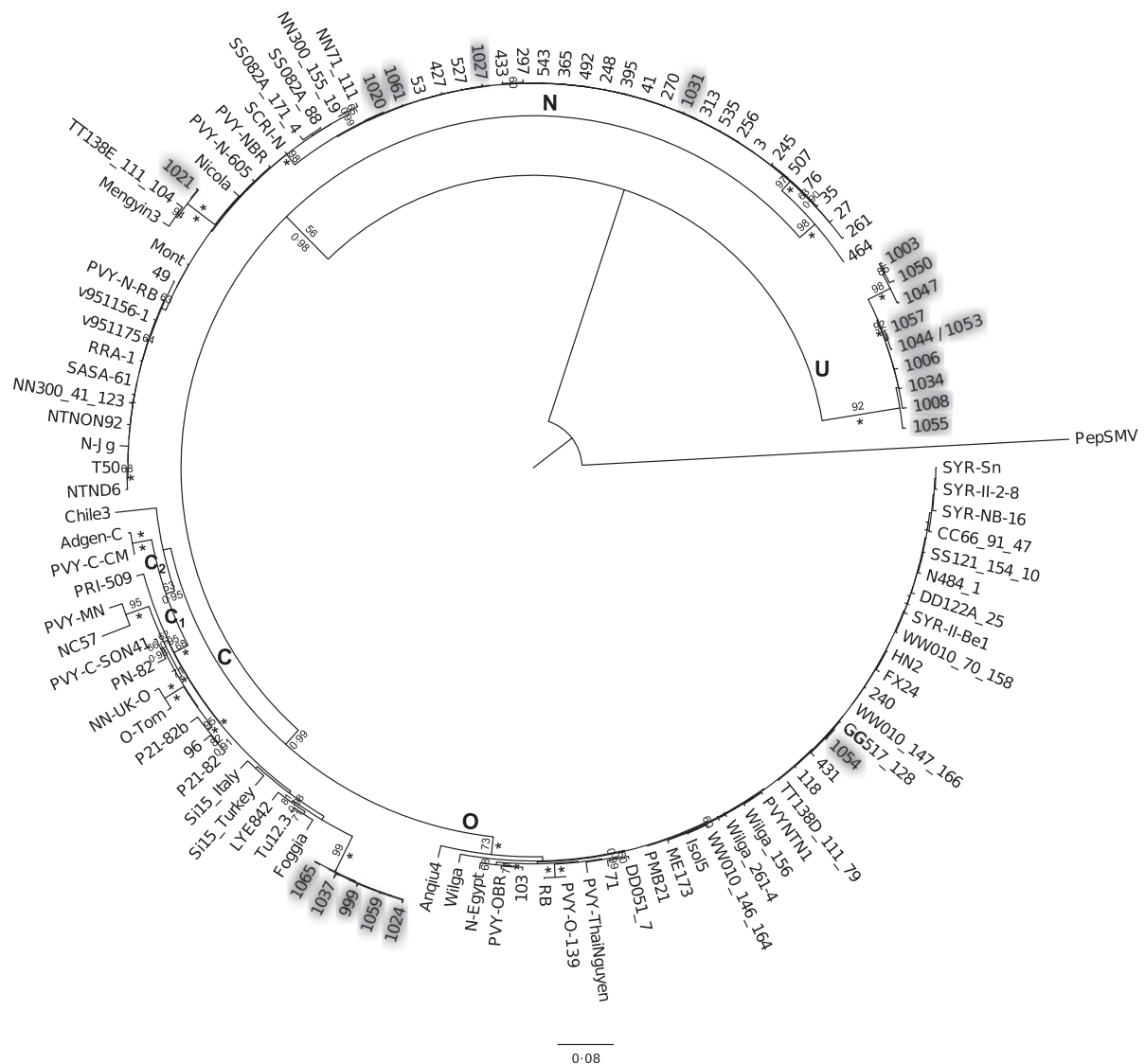
### Some Brazilian PVY isolates form a new distant phylogenetic group

The maximum likelihood tree, presented in Figure 1, aimed to locate members of the  $Y^U$  serogroup in the phylogenetic tree; the three main groups, C, N and O, were clearly identified, plus an extra phylogenetically distant group, U, formed exclusively by Brazilian  $Y^U$  isolates (Table S1). However, it is important to note that not all Brazilian  $Y^U$  isolates were assigned to the U group; these isolates rather cluster in the C group (Fig. 1; isolates 1065, 1037, 999, 1059 and 1024). This split could be geography-driven because all U isolates were collected from close locations in the states of Paraná and Santa Catarina (Fig. S1; Table S1), whereas none of the isolates collected in the most southern state of Brazil (Rio Grande do Sul) cluster in the U phylogenetic group. However, the hypothesis for a geography-driven structure within the Brazilian CP sequences cannot be confirmed with certainty due to the small amount of Brazilian samples.

### Characterization of biological and molecular properties of Brazilian PVY isolates

To better characterize the diversity among the Brazilian PVY isolates, biological and molecular properties of a few  $Y^U$  isolates were studied and compared to  $Y^N$  and  $Y^{O/C}$  isolates sampled in Brazil. Thus, among the available samples, the biological properties of the four  $Y^U$  (1034, 1037, 1044 and 1059), one  $Y^N$  (1031) and one  $Y^{O/C}$  (1054) isolates were analysed on tobacco, potato and pepper. These hosts had PVY-susceptible genotypes (i.e. without resistance against PVY) and are commonly used as indicator hosts for PVY description. Symptoms observed on infected plants were compared with those induced by the  $PVY^O$ -139 (Singh & Singh, 1996),  $PVY^N$ -605 (Jakab *et al.*, 1997) and  $PVY^{C1}$ -SON41 (Gebre Selassie *et al.*, 1985) reference isolates. Collected data are reported in Table 1. As expected, isolate 1031,





**Figure 1** Maximum likelihood (ML) tree of 117 PVY isolates as inferred from the nucleotide sequences of the coat protein. The different PVY groups (C: C1 and C2, O, N and U) are indicated. Sequences from Brazilian isolates are highlighted in grey and the slash separator indicates those with identical sequences. Numbers on branches indicate ML bootstrap support values (above) and Bayesian probabilities (below). Asterisks indicate maximal support values and maximal probabilities. ML bootstrap support values and Bayesian probabilities under 50 and 0.90, respectively, are not shown.

a member of the N group according to serological ( $Y^N$ ) and phylogenetic analyses (see Fig. 1), is able to infect tobacco and potato and induces vein necrosis on tobacco plants. Isolate 1054, assigned to the  $Y^{O/C}$  group by serological assays, induces vein necrosis on tobacco and systemic mosaic symptoms on potato but is not able to infect pepper. However, PVY isolates belonging to the O group, including isolate 1054, are expected to induce mosaic symptoms on infected *N. tabacum* 'Xanthi'. Thus, the serological type-O and the biological type-N properties observed for isolate 1054 suggest that this isolate either has a PVY<sup>N</sup>/PVY<sup>O</sup> recombinant genome (with a type-O CP and a type-N tobacco necrotic property) as

described for PVY<sup>N</sup>-W isolates (Glais *et al.*, 2002) or presents mutation(s) of the necrotic determinant(s) (Faurez *et al.*, 2012). The four Brazilian  $Y^U$  isolates were able to induce mosaic symptoms on non-inoculated leaves of infected *N. tabacum* plants, but were not infectious on potato. However, these isolates had heterogeneous behaviours on pepper plants, as only isolate 1059 was able to infect pepper while isolates 1034 and 1044 were not.

In addition to biological properties of these Brazilian tobacco isolates, the genetic diversity of the 3' P1/5' HC-Pro and 3' N1b/CP/3' UTR regions of their genome was investigated (Fig. 2). Analysis of the trees obtained with these genomic data highlights that Brazilian isolate 1031

Table 1 Biological and serological properties of the Brazilian and reference *Potato virus Y* (PVY) isolates

Isolate <sup>a</sup>	Area <sup>b</sup>	Serotype <sup>c</sup>	Group <sup>d</sup>	Host range <sup>e</sup>			
				<i>Nicotiana tabacum</i> 'Xanthi'		<i>Solanum tuberosum</i> 'Bintje'	<i>Capsicum annuum</i> 'Yolo Wonder'
				Symptoms <sup>f</sup>	ELISA <sup>g</sup>		
N605	Switzerland	Y <sup>N</sup>	N	Nec	+	+	–
O139	Canada	Y <sup>O/C</sup>	O	Mo	+	+ <sup>h</sup>	+ <sup>h</sup>
SON41	France	Y <sup>O/C</sup>	C1	Mo	+	–	+
1031	PS, Brazil	Y <sup>N</sup>	N	Nec	+	+	nd
1034	PS, Brazil	Y <sup>U</sup>	U	No	+	–	–
1037	PS, Brazil	Y <sup>U</sup>	C1	Mo	+	–	nd
1044	SCS, Brazil	Y <sup>U</sup>	U	Mo	+	–	–
1054	PS, Brazil	Y <sup>O/C</sup>	O	Nec	+	+	–
1059	RGS, Brazil	Y <sup>U</sup>	C1	Mo	+	–	+

nd: not determined.

<sup>a</sup>Isolates N605 (Jakab *et al.*, 1997), O139 (Singh & Singh, 1996) and SON41 (Gebre Selassie *et al.*, 1985) are reference isolates for PVY groups N, O and C1, respectively. Other listed isolates were collected in 2007–2008 from *N. tabacum* in Brazil.

<sup>b</sup>Isolates from Brazil were collected from PS: Paraná state; RGS: Rio Grande do Sul state; SCS: Santa Catarina state.

<sup>c</sup>Serotype according to monoclonal antibodies anti-PVYN (YN, INRA Rennes/FN3PT) or anti-PVYO/C (YO/C, Neogen). Y<sup>U</sup> was used for unconventional PVY isolates that were not detected with monoclonal antisera listed.

<sup>d</sup>PVY phylogenetic groups according to analyses performed in this work. U group corresponds to the proposed new lineage in PVY species described in this study.

<sup>e</sup>For each isolate, a total of four *N. tabacum* 'Xanthi', six *S. tuberosum* 'Bintje' and six *C. annuum* 'Yolo Wonder' were manually inoculated at 5–6 weeks after sowing and maintained under greenhouse conditions as described in Lacroix *et al.* (2010).

<sup>f</sup>Symptoms recorded at 40 days post-inoculation. Mo and Nec: mosaic and necrotic symptoms in non-inoculated upper leaves, respectively.

<sup>g</sup>Virus detection in non-inoculated leaves was performed by DAS-ELISA with the PVY polyclonal antiserum (INRA/FN3PT) as described in Lacroix *et al.* (2010). Plants were considered infected (+) when optical density value at 405 nm was at least more than three times the mean value of the negative controls (healthy controls), otherwise plants were considered non-infected (–).

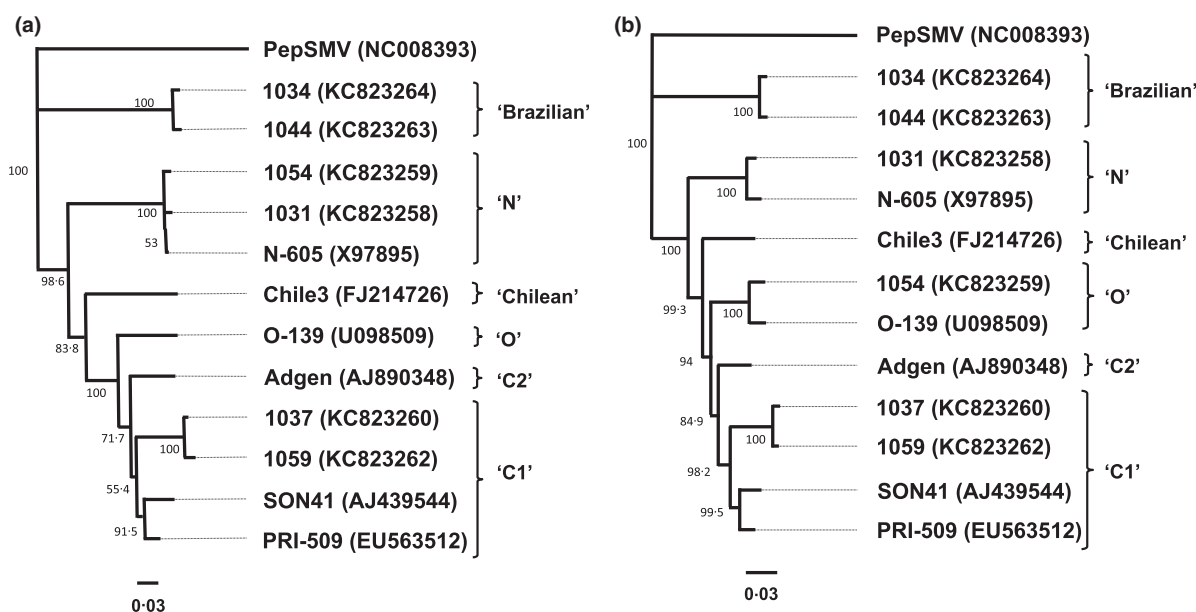
<sup>h</sup>Not tested in this study, results according to Moury (2010).

is closely related to the reference PVY<sup>N</sup>-605 for the two genomic regions (99% nucleotide identity). Isolate 1054 is closely related to PVY<sup>N</sup>-605 and PVY<sup>O</sup>-139 for the genomic regions 3' P1/5' HC-Pro and 3' N1b/CP/3' UTR, respectively. This confirms the N/O recombinant status of this isolate, as previously suggested by the comparative analysis of its serological and biological properties. When compared with PVY sequences retrieved from GenBank, the four Y<sup>U</sup> isolates (1034, 1037, 1044 and 1059) shared from 78 to 90% and from 82 to 93% nucleotide identities for 3' P1/5' HC-Pro and 3' N1b/CP/3' UTR regions, respectively. Among these isolates, 1034 and 1044, both assigned to the U group for both genomic regions (Figs 1 & 2a,b), were associated with the lowest percentage of nucleotide identity obtained in the analysis. This low percentage identity between these two U isolates and conventional N and O isolates is supported by the presence of U-specific sequence patterns mainly observed in the 5' end of the CP gene. After translation of the available CP sequences and amino acid alignment, the signature analysis carried out with VESPA (Korber & Myers, 1992) highlighted (i) a deletion of the codon at position 22 for U isolates, (ii) a few Y<sup>U</sup>-specific amino acid residues in the N-terminal of the CP protein (Fig. 3), and (iii) the absence of recombination sites. Comparing a signature pattern analysis of U relative to all other groups and relative to only the Brazilian sequences present in other groups (data not shown), the

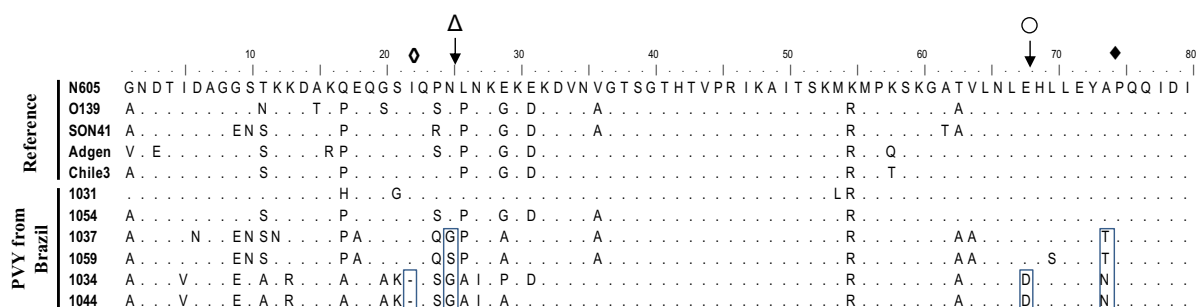
results are very similar, suggesting that the deletion at position 22 must have driven the formation of this phylogenetically distant group of PVY. Lastly, the analysis of the CP amino acid sequence alignment revealed that the N25 conserved in O-139, N-605, C1-SON41, Adgen and Chile3 is replaced in Brazilian Y<sup>U</sup> isolates by G (isolates 1037, 1034 and 1044) or S (isolate 1059).

## Discussion

This is the first description of properties of a newly discovered clade U in the PVY phylogeny, consisting of sequences of isolates coming from Brazil. This group distinguishes itself by a 3-nucleotide deletion and the presence of a set of atypical amino acids, which are mainly located in the N-terminus of the CP protein. PVY group-specific antibodies are known to target epitopes located in the variable N-terminal region of the CP protein (Ounouna *et al.*, 2002). Thus, modifications of amino acid sequence in this region could impact the efficiency of interactions between CP and monoclonal antibodies and consequently induce a lack of serological detection of isolates with previously described PVY-specific monoclonal antisera. The N-terminus is exposed to the exterior of the particle, thus making it a potential target of selection. Moury & Simon (2011) have shown that a nonpolar amino acid at position 25 in the CP of PVY confers a fitness advantage on isolates when infecting tobacco



**Figure 2** Phylogenetic trees obtained from alignments of the 3' P1/5' HC-Pro (a) and 3' N1b/CP/3' UTR (b) PVY sequences. *Pepper severe mosaic virus* (PepSMV) was used as an out-group to root the trees. Phylogenetic trees were constructed using the maximum likelihood method implemented into MEGA5 (Tamura *et al.*, 2011) software with the Tamura and Nei nucleotide substitution model (Tamura & Nei, 1993). Bootstrap percentages obtained with 1000 bootstrap samples are reported on branches. The scale bar represents the relative genetic distance (number of substitutions per nucleotide). Accession numbers (GenBank) for sequences of the PVY isolates are listed.



**Figure 3** Amino acid (AA) alignment of the N-terminal sequence of the viral CP from reference and Brazilian PVY isolates. Amino acids identical to the N605 sequence are represented by dots. Specific patterns in the CP sequence of U group members correspond to the deletion of the AA at position 22 (◊) and to specific AA residues at positions 25 (Δ), 68 (○) and 74 (★); these specific AAs are highlighted by boxes. Arrows indicate AA positions known for their involvement in PVY fitness on potato host (Moury & Simon, 2011).

compared to potato. As the biological assays performed with Y<sup>U</sup> isolates highlight the non-infectious status of these isolates on potato, it is likely that the nonpolar amino acid at position 25 present in isolates 1037, 1034 and 1044 had been positively selected for fitness gain in the tobacco host. Moreover, these authors also reported that the replacement E68K in CP increased aphid transmission efficiency but drastically decreased virus competitiveness in potato plants. In the Brazilian isolates 1034 and 1044, the residue E68 is replaced by D. This E68D mutation, even if it maintains an acidic residue, could impact PVY fitness in tobacco plants, as previously described for E400D in the HC-Pro protein sequence of PVY (Rolland *et al.*, 2009). In addition to these variations, amino acid 74 of the CP allows the discrimination

of both Y<sup>U</sup> and U isolates from reference isolates. This residue should also be considered as a candidate in future work on the specific characteristics of unconventional PVY<sup>U</sup> isolates.

The reconstruction of ancestral states in a given species presents numerous challenges because of the limitations of available data, the missing of ancient historical records and the frequent lack of correlation between phylogenetic trees and biological traits. However, here, because (i) some PVY biological traits are unique in major clades, (ii) correlation exists between phylogeny and biological traits in clades, and (iii) a new basal clade sharing original biological traits was found, the reconstruction of the ancestral traits and the evolutionary history in PVY species can be properly addressed.



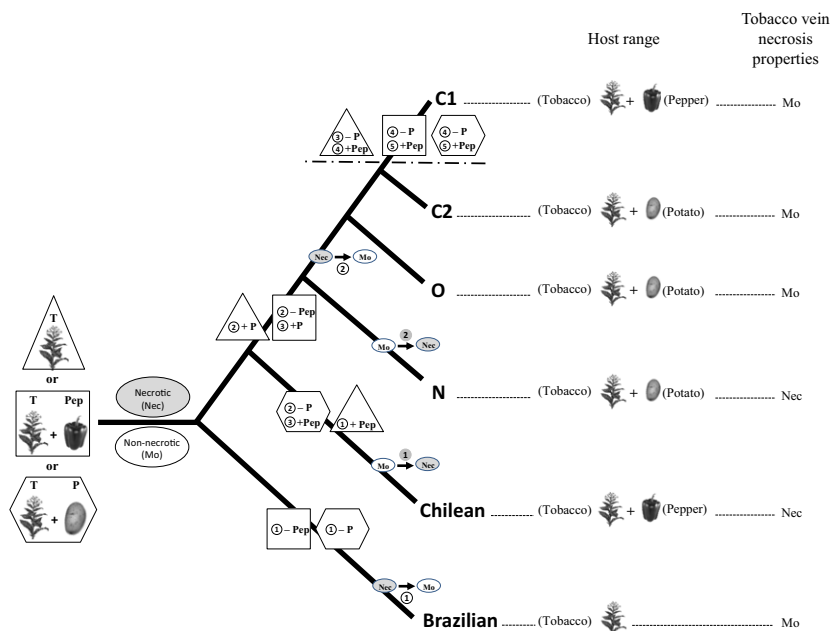
The phylogenetic tree resulting from the analysis of CP sequences revealed that the Y<sup>U</sup> isolates could be assigned into different PVY branches of the phylogenetic trees (Fig. 1). However, isolates 1003, 1006, 1008, 1034, 1044, 1047, 1050, 1053, 1055 and 1057 do not cluster within an existing PVY clade and appear to be the first described members of a new lineage in the PVY species, referred to in this study as the Brazilian U clade. The same result was obtained by phylogenetic analysis with the 3' P1/5' HC-Pro region of the viral genome.

It was established that isolates belonging to N and Chilean groups (Moury, 2010) are able to induce necrotic symptoms on tobacco whereas isolates belonging to the O, C and U groups do not share this biological property. Moury (2010) has suggested that the ancestral state of PVY was more likely to be necrotic, based on (i) the most parsimonious evolutionary scenarios of evolution of the necrotic ability trait and (ii) the fitness cost due to amino acid substitutions that confer the tobacco necrotic vein property to an isolate (Rolland *et al.*, 2009). However, the position of the non-necrotic Brazilian U lineage, which radiate earlier than other PVY lineages, does not support this theory (Fig. 4). The tobacco veinal necrosis trait requires the amino acid triplet N<sub>339</sub>K<sub>400</sub>E<sub>419</sub> in the HC-Pro protein (Faurez *et al.*, 2012). Analysis of the HC-Pro residues encoded by the necrotic isolates 1031 and 1054 confirmed the presence of this amino acid triplet (data not shown). The isolates 1034, 1037, 1044 and 1059, non-necrotic on tobacco, code for triplets containing X<sub>339</sub>R<sub>400</sub>D<sub>419</sub> residues known to impair the necrotic capacity of PVY isolates (data not shown). Considering the evolutionary steps involved in the tobacco vein necrotic trait, based on substitutions at amino acid positions, it cannot be discriminated whether the ancestral PVY isolate was necrotic or non-necrotic. However, the amino acid

substitutions that confer necrotic properties in tobacco impact the fitness of the virus (Rolland *et al.*, 2009), suggesting a non-necrotic ancestral state for PVY isolates.

The evolution of PVY infectivity on solanaceous hosts may be inferred by analysis of host range data together with the phylogenetic position of the different PVY clades (Fig. 4). Correlations between the PVY phylogeny and host range data have established that none of the potato isolates belong to the C1 group (Moury, 2010), except for potato isolate PRI-509, which was assigned in the C1 group based on CP analysis but excluded from this group when the full length PRI-509 sequence was analysed (Dullemans *et al.*, 2011). As pepper PVY isolates do not belong to the N, O or C2 groups, it has been suggested that a host barrier exists between these distinct phylogenetic groups (d'Aquino *et al.*, 1995). This theory is also supported by the fact that most recombination events in PVY occurred between N and O group members (Glais *et al.*, 2002) whereas very few recombination events involved sequences from isolates belonging to the C1 group. This proposed host barrier could explain the fact that pepper PVY isolates belonging to C1 or Chilean groups are not infectious on potato cultivars (Table 1; Moury, 2010). Thus, it is possible, using a limited number of pathotype changes, to reconstruct the evolutionary history of the PVY host range. At least four pathotype changes are required to fit with the described phylogeny because (i) isolates able to infect potato or pepper are not assigned to monophyletic groups and (ii) isolates in the Brazilian U clade, which had radiated earlier, are neither infectious on potato nor on pepper, (Fig. 4). The most parsimonious hypothesis assumes the ancestral state of PVY to be adapted only to tobacco, so requiring four changes of PVY host adaptation during the evolutionary process; alternative scenarios require five evolutionary steps.

**Figure 4** Evolutionary scenarios of potato- and pepper-infectivity, and of tobacco vein necrotic properties of PVY. Based on whole PVY genome alignments (Moury, 2010), the Chilean clade has been considered ancestral to the N clade. For PVY host adaptations, the different possible ancestral states and their associated evolutionary scenario are illustrated by triangles, rectangles or hexagons; a circled number inside the appropriate symbol indicates each evolutionary step and loss or gain of adaptation to a host is indicated by a – or + sign, respectively. Individual host species involved in the different scenarios are represented by plant icons and/or by a one-to-three-letter code corresponding to T (tobacco), Pep (pepper) and P (potato). Ancestral phenotypes of PVY isolates on tobacco, i.e. non-necrotic or necrotic, are indicated with white and grey ellipses, respectively.



To conclude, identification and characterization of members of the Brazilian U lineage give important new insights into the evolutionary history of the biological traits in the PVY species. Gibbs *et al.* (2008) suggested that potyviruses radiated with the advent of agriculture. In the same way, it is not inconsistent to think that the domestication of the potato in Peru, initiated c. 8000 years ago ([www.potato2008.org](http://www.potato2008.org)), and later its worldwide cultivation, promoted species jumps and the emergence of new isolates of PVY previously adapted to the tobacco host. Nevertheless, the present description of unconventional PVY isolates illustrates that the current perception of diversity of this species should not be considered exhaustive. Even if the improvement of knowledge and the possibility to correlate data from phylogenetic trees with those from biological traits makes evolutionary history reconstructions easier, the lack of fossils or accurate historical records associated to this viral species prevents the absolute validation of proposed theories.

### Acknowledgements

The authors wish to thank Claudir Lorencetti (Alliance One from Brazil) who kindly supplied tobacco PVY isolates. Transfer of materials from Brazil to France was carried out using an official letter of authorization 'LOA' delivered to L. Glais (INRA Rennes/FNPPPT Paris) by plant protection organizations. The authors gratefully acknowledge Dr Alain Palloix (INRA, Avignon) who kindly provided pepper seeds for the host range study and Maryse Guillet (FN3PT, Le Rheu) who kindly provided serum for PVY DAS-ELISA. A. W. and S. F. E. were supported by grants BFU2012-30805 from Spanish MINECO and 22371 from the John Templeton Foundation. The opinions expressed in this publication are those of the authors and do not necessarily reflect the views of the John Templeton Foundation. J. M. C. was supported by the JAE-Doc program from CSIC. This study was supported by The Imperial Tobaccos Group and the Association for Research on Nicotianae (ARN).

### References

- Ahn HI, Yoon JY, Hong JS *et al.*, 2006. The complete genome sequence of *Pepper severe mosaic virus* and comparison with other potyviruses. *Archives of Virology* **151**, 2037–45.
- d'Aquino L, Dalmay T, Burgyan J, Ragozzino A, Scala F, 1995. Host range and sequence analysis of an isolate of *Potato virus Y* inducing vein necrosis in pepper. *Plant Disease* **79**, 1046–50.
- Chrzanowska M, 1991. New isolates of the necrotic strain of *Potato virus Y* (PVY-N) found recently in Poland. *Potato Research* **34**, 179–82.
- Chung BYW, Miller WA, Atkins JF, Firth AE, 2008. An overlapping essential gene in the *Potyviridae*. *Proceedings of the National Academy of Sciences, USA* **105**, 5897–902.
- Darriba D, Taboada GL, Doallo R, Posada D, 2012. jMODELTEST 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.
- Dougherty WG, Carrington JC, 1988. Expression and function of potyviral gene products. *Annual Review of Phytopathology* **26**, 123–43.
- Drummond AJ, Rambaut A, 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.
- Dullemans AM, Cuperus C, Verbeek M, van der Vlugt RAA, 2011. Complete nucleotide sequence of a potato isolate of strain group C of *Potato virus Y* from 1938. *Archives of Virology* **156**, 473–7.
- Edgar RC, 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–7.
- Faurez F, Baldwin T, Tribodet M, Jacquot E, 2012. Identification of new *Potato virus Y* (PVY) molecular determinants for the induction of vein necrosis in tobacco. *Molecular Plant Pathology* **13**, 948–59.
- Gebre Selassie K, Marchoux G, Delecote B, Pochard E, 1985. Variabilité naturelle des souches du virus Y de la pomme de terre dans les cultures de piment du sud-est de la France. Caractérisation et classification en pathotypes. *Agronomie* **5**, 621–30.
- Gibbs AJ, Ohshima K, Phillips MJ, Gibbs MJ, 2008. The prehistory of potyviruses: their initial radiation was during the dawn of agriculture. *PLoS ONE* **3**, e2523.
- Glais L, Tribodet M, Kerlan C, 2002. Genomic variability in *Potato potyvirus Y* (PVY): evidence that PVY<sup>NW</sup> and PVY<sup>NTN</sup> variants are single to multiple recombinants between PVY<sup>O</sup> and PVY<sup>N</sup> isolates. *Archives of Virology* **147**, 363–78.
- Gooding GV, Tolin SA, 1973. Strains of *Potato virus Y* affecting flue-cured tobacco in southeastern United States. *Plant Disease Reporter* **57**, 200–4.
- Gugerli P, Fries P, 1983. Characterization of monoclonal-antibodies to *Potato virus Y* and their use for virus detection. *Journal of General Virology* **64**, 2471–7.
- Jakab G, Droz E, Brigneti G, Baulcombe D, Malnoe P, 1997. Infectious *in vivo* and *in vitro* transcripts from a full-length cDNA clone of PVY-N605, a Swiss necrotic isolate of *Potato virus Y*. *Journal of General Virology* **78**, 3141–5.
- Karasev AV, Hu XJ, Brown CJ *et al.*, 2011. Genetic diversity of the ordinary strain of *Potato virus Y* (PVY) and origin of recombinant PVY strains. *Phytopathology* **101**, 778–85.
- Keller ER, Münster J, 1961. Betrachtungen über den Y-Virusbefall der nach der Schweiz gelieferten Saatkartoffeln aus europäischen Ländern und die ergriffenen Gegenmassnahmen. *European Potato Journal* **4**, 341–53.
- Kerlan C, Moury B, 2008. Potato virus Y. In: Mahy BWJ, Van Regenmortel MHV, eds. *Encyclopedia of Virology*. Oxford, UK: Elsevier, 287–96.
- 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.
- Korber B, Myers G, 1992. Signature pattern analysis: a method for assessing viral sequence relatedness. *AIDS Research Human Retroviruses* **8**, 1549–60.
- Lacroix C, Glais L, Kerlan C, Verrier JL, Jacquot E, 2010. Biological characterization of French *Potato virus Y* (PVY) isolates collected from PVY-susceptible or -resistant tobacco plants possessing the recessive resistance gene *va*. *Plant Pathology* **59**, 1133–43.
- Lacroix C, Glais L, Verrier JL, Charlier C, Lorencetti C, Jacquot E, 2011. Impact of tobacco recessive resistance gene *va* on biological properties of Brazilian *Potato virus Y* (PVY) isolates. *Plant Pathology* **60**, 1048–54.
- Le Romancer M, Kerlan C, Nedellec M, 1994. Biological characterization of various geographical isolates of *Potato virus Y* inducing superficial necrosis on potato tubers. *Plant Pathology* **43**, 138–44.
- Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuve P, 2010. rDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* **26**, 2462–3.
- Moury B, 2010. A new lineage sheds light on the evolutionary history of *Potato virus Y*. *Molecular Plant Pathology* **11**, 161–8.
- Moury B, Simon V, 2011. dN/dS-based methods detect positive selection linked to trade-offs between different fitness traits in the coat protein of *Potato virus Y*. *Molecular Biology and Evolution* **28**, 2707–17.

- Moury B, Morel C, Johansen E, Jacquemond M, 2002. Evidence for diversifying selection in *Potato virus Y* and in the coat protein of other potyviruses. *Journal of General Virology* **83**, 2563–73.
- Ounouna H, Kerlan C, Lafaye P, Loukili MJ, El Gaaied A, 2002. Production of monoclonal antibodies against synthetic peptides of the N-terminal region of *Potato virus Y* coat protein and their use in PVY strain differentiation. *Plant Pathology* **51**, 487–94.
- Rolland M, Kerlan C, Jacquot E, 2009. The acquisition of molecular determinants involved in *Potato virus Y* necrosis capacity leads to fitness reduction in tobacco plants. *Journal of General Virology* **90**, 244–52.
- Rufty RC, Powell NT, Gooding GV, 1983. Relationship between resistance to *Meloidogyne incognita* and a necrotic response to infection by a strain of *Potato virus Y* in tobacco. *Phytopathology* **73**, 1418–23.
- Singh M, Singh P, 1996. Nucleotide sequence and genome organization of a Canadian isolate of the common strain of potato virus Y (PVY<sup>O</sup>). *Canadian Journal of Plant Pathology* **18**, 209–24.
- Stamatakis A, 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–3.
- Suyama M, Torrents D, Bork P, 2006. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic Acids Research* **34**, W609–12.
- Tamura K, Nei M, 1993. Estimation of the number of nucleotide substitutions in the control of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512–26.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–9.

## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** Geographic regions from where Brazilian isolates were collected.

**Table S1.** Biological data of collected PVY isolates.

**Table S2.** Primers used for reverse transcription and PCR to amplify genomic sequences of PVY.

**Table S3.** List of sequences retrieved from GenBank.