Potato virus Y group C isolates are a homogeneous pathotype but two different genetic strains

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Potato virus Y group C isolates (PVYC) have been characterized according to biological, molecular and genetic criteria. Two genetic strains, PVYC1 and PVYC2, were identified on the basis of genetic distances (among them and other PVY strains), host range (ability or inability to infect pepper), MAb response (ELISA recognition with MAb 10E3) and coat protein processing site. Some characteristics, such as aphid transmission and ELISA using other MAbs, did not correlate with classification into these two genetic strains. All isolates tested induced a hypersensitive response on potatoes bearing the Nc resistance gene, confirming the nature of PVYC isolates as a homogeneous pathotype.

Introduction

Potato virus Y (PVY) is an important pathogen in solanaceous crops. It belongs to the genus Potyvirus, of which it is the type species, in the plant virus family Potyviridae (Shukla et al., 1994). PVY has a single positive-sense genomic RNA about 10 kb long and forms flexuous virions. The genomic RNA contains a unique open reading frame encoding a polyprotein which is processed into functional viral proteins by virus-encoded proteases (P1, HC-Pro and NIa) (Riechmann et al., 1992). It is naturally transmitted by aphids in a non-persistent manner with great efficiency, causing epidemics in potato, tomato, pepper, tobacco and other solanaceous plants (De Bokx & Huttinga, 1981).

Historically, PVY has been classified into different strains according to host (De Bokx & Huttinga, 1981). The criteria used have included symptoms on test plants, serology and resistance responses. Three groups of PVY isolates have been identified: groups O, N and C (De Bokx & Huttinga, 1981).

PVYO group (common strain) causes mosaic symptoms when inoculated into tobacco plants, and severe symptoms such as crinkling, rugosity or leaf-drop streaks in potato. PVYN isolates (tobacco veinal necrosis strain) are defined by the necrotic symptoms they induce in tobacco, although they induce very mild symptoms in most potato cultivars. A MAb specific to PVYN isolates has been reported (Gugerli & Fries, 1983).

The PVYC isolates (sometimes referred to as PVC) elicit a hypersensitive response in potato cultivars bearing the Nc resistance gene (Cockerham, 1970). Symptoms in tobacco are reported to be indistinguishable from those induced by PVYO isolates, and they cause systemic mosaic or stipple streak symptoms in potato (De Bokx & Huttinga, 1981). No other definitive characteristic of PVYC isolates has been reported. The first isolate characterized as PVYC (the so-called PVC) was described as non-aphid-transmitted (Bawden & Kassanis, 1947), unlike other isolates of this group (De Bokx & Piron, 1978; De Bokx & Huttinga, 1981). Some isolates originally classified as PVYC were discovered to be a different potyvirus and renamed potato virus V (PVV) (Fribourg & Nakashima, 1984). These confusing findings, combined with the fact that PVYC isolates are less economically important, have led to the general assumption that PVYC is a strain, but clear proof of genetic strain status has been lacking.

The coat protein (CP) gene is the gene most frequently used for studies of genetic diversity in potyviruses (Shukla et al., 1994). Using genetic distances estimated from RFLP patterns of the CP gene obtained after immunocapture (IC)–
RT–PCR, a method was developed which allows the classification of PVY into true genetic strains (Blanco-Urgoiti et al., 1996). This is a rapid method for molecular typing of PVY, which is in good agreement with classical criteria and with genetic distances calculated from sequence comparisons. Three main clusters were obtained with isolates available at that time: PVY\(^{N}\) and PVY\(^{O}\) from potato, and non-potato isolates (PVY\(^{Np}\), including pepper, tomato and \textit{Datura} species isolates. The correlation coefficient between distances calculated from RFLP data and those obtained directly from sequence data was high enough (0.78) to presume no error when classifying PVY isolates into strains using RFLP data. No CP gene sequence information was available for any PVY when classifying PVY isolates into strains using RFLP data. No isolates from RFLP data and those obtained directly from isolates. The correlation coefficient between distances calculated from RFLP data and those obtained directly from sequence data was high enough (0.78) to presume no error when classifying PVY isolates into strains using RFLP data. No CP gene sequence information was available for any PVY isolate. Thus PVY\(^{C}\) isolates may be a useful tool to evaluate the suitability of the method for any PVY isolate with no prior sequence information.

In this paper, we present a genetic characterization of eight PVY\(^{c}\) isolates using the IC–RT–PCR–RFLP (restrictotype) method described above (Blanco-Urgoiti et al., 1996). The restrictotypes have allowed us to propose PVY\(^{C}\) as a PVY pathotype defined by the \(Nc\) gene, but not a homogenous genetic strain, unlike PVY\(^{O}\) and PVY\(^{N}\). The results were confirmed by sequence analysis of the CP gene, symptom analysis, MAb serotyping, host range and aphid transmission.

**Methods**

- **Virus isolates.** Freeze-dried leaf tissue from tobacco plants infected with PVY\(^{c}\) isolates 23, 26–30, 43 and 45 was part of a reference collection obtained from G. Adam (University of Hamburg, Germany). PVY\(^{O}\)32 and PVY\(^{N}\)134, used as controls in serotyping experiments, were field isolates from a variability study carried out in 1994 (Blanco-Urgoiti et al., 1994) and were also maintained in tobacco. PVY\(^{O}\)Palogan, used as a positive aphid transmission control, was part of the CIMA collection of PVY isolates. PVY-P21 from pepper, used as a positive control in the host range experiments, came from SIA (Zaragoza, Spain) (Soto et al., 1994).

- **Viral sequences.** CP gene sequences from the following virus isolates were used for analysis of genetic distances and the construction of phylogenetic trees: PVY-Chil, PVY-NsNr, PVY-MsNr, PVY-Pep, PVY-Is, PVY\(^{n}\)36 and PVY\(^{O}\)Can (Blanco-Urgoiti et al., 1996); PVY-P21 (Soto et al., 1994); PVY-PepN (GenBank accession number U10378; L. d’Aquino, T. Dalmay, J. Burgyan, A. Ragozzino & F. Scala, unpublished results). All these sequences, in addition to the following, were also used to calculate genetic distances between all pairs of sequences (Table 3): PVYN11, PVY-T, PVY-Jap, PVY-Nz, PVY-EurH, PVY-Hu, PVY-NTN, PVY-Go16, PVY-Chin, PVY-Fr, PVY-MsMr, PVYO2, PVYO4, PVY-PotUS, PVY-U, PVYO1 (Blanco-Urgoiti et al., 1996); PVY-Lb (GenBank accession number X92078); PVY-Chin2 (GenBank accession number U25672).

- **Plant material.** Potato plants \textit{Solanum tuberosum} cv. King Edward and cv. Félix were grown from tubers and maintained in an insect-proof glasshouse at 20–25 °C. \textit{Nicotiana tabacum} cv. Xanthi nc and \textit{Capsicum annuum} cv. Yolo Wonder were grown and maintained under similar conditions.

- **Hypersensitive response assay.** Three \(Nc\)–bearing potato plants (\textit{S. tuberosum} cv. King Edward) per isolate were rubbed on two of their leaves with virus extract in phosphate buffer (10 mM, pH 7), using carborundum as an abrasive. Local lesions were observed 7 days post-inoculation (p.i.). Inoculated and non-inoculated leaves of all plants were tested by Triple Antibody Sandwich (TAS)–ELISA (see below) at 15 days p.i.

- **Host range assay.** Phosphate buffer–DIECA virus extract, carborundum and active carbon (Marrou, 1967) were rubbed onto \textit{C. annuum} cv. Yolo Wonder to assay the host range of PVY\(^{c}\) isolates. Three to four pepper plants per isolate were inoculated with each of the following isolates: PVY\(^{N}\) 23, 26–30 and 43, and PVY-P21. Back-inoculation of infected pepper plants to potato (\textit{S. tuberosum} cv. Félix) and tobacco (\textit{N. tabacum} cv. Xanthi nc) plants was performed using the same method. Plants were tested by TAS–ELISA. Positive plants were also analysed by IC–RT–PCR–RFLP (Blanco-Urgoiti et al., 1996) to avoid the possibility of contamination with any pepper isolate.

- **Analysis by TAS–ELISA.** PVY polyclonal antibody (Boehringer) was used to coat ELISA plates following supplier’s instructions. Four different ELISA analyses were carried out: two with plant extracts diluted 1:10 in Tris buffer (Nolasco et al., 1993) and two with extracts diluted 1:100. Mabs C9, 10E3, 1E10 and PVY(N) were used as serological indicators of variability (Soto et al., 1994). Alkaline phosphatase-conjugated antimouse MAb and 3-nitrophenyl phosphate substrate (Sigma) were used. ELISA readings were obtained as described in the COST-88 PVY Ringtest results (available from G. Adam on request).

- **Aphid transmission assay.** Aphid clones of \textit{Myzus persicae} \textit{Südzer} were kindly provided by A. Fereres (CSIC, Madrid) and maintained on tobacco plants. Twenty-five aphids, previously starved for 1 h, were placed on PVY source plant for 1 h. Groups of five aphids were then placed on each of five test plants. PVY\(^{c}\) and buffer-inoculated tobacco plants were used as positive and negative controls, respectively. The assay was performed twice. Test plants were analysed by ELISA and IC–RT–PCR–RFLP 15 days later.

- **CP gene sequencing.** IC–RT–PCR was performed for PVY\(^{c}\) isolates 27, 28, 30 and 45 using the methodology of Nolasco et al. (1993), with modifications as described by Blanco-Urgoiti et al. (1996). The oligonucleotide 5’ GATGTTGCCTTGAGATGATG, corresponding to nucleotides 8517–8536 of the published sequence of PVY-Hu (Thole et al., 1993), was used as the forward primer. The reverse primer was the same oligonucleotide used by Blanco-Urgoiti et al. (1996). A 935 nucleotide fragment was obtained, which included the last 54 nucleotides of the Nib, the full CP cistron and the first 75 nucleotides of the 3’ UTR. These fragments were cloned into the plasmid vector pUC13. Clones were used to determine the nucleotide sequence of the CP cistron of each isolate. Nucleotide sequences were obtained by the dideoxynucleotide chain termination method (Sanger et al., 1977) using the Sequenase 2.0 DNA sequencing kit (Amersham). All sequences were determined on both strands in one or several clones.

- **Restrictotyping.** Restrictotyping of PVY\(^{c}\) isolates was performed as described by Blanco-Urgoiti et al. (1996).

- **Phylogenetic analysis.** Genetic distances were determined and phylogenetic trees for both RFLP and sequence analyses were constructed as described by Blanco-Urgoiti et al. (1996). PVY\(^{N}\)36 and PVY\(^{O}\)Can were used as outgroup members to root the tree.

**Results**

**Biological characterization**

All PVY\(^{c}\) isolates elicited a hypersensitive response on inoculated leaves of the \(Nc\) gene carrier potato cv. King...
Table 1. Mechanical infectivity of PVY\textsuperscript{c} isolates to pepper (Capsicum annuum cv. Yolo Wonder)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Infected plants/inoculated plants</th>
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<tbody>
<tr>
<td>PVY\textsuperscript{c}23</td>
<td>0/4</td>
</tr>
<tr>
<td>PVY\textsuperscript{c}26</td>
<td>2/3</td>
</tr>
<tr>
<td>PVY\textsuperscript{c}27</td>
<td>0/4</td>
</tr>
<tr>
<td>PVY\textsuperscript{c}28</td>
<td>2/4</td>
</tr>
<tr>
<td>PVY\textsuperscript{c}29</td>
<td>2/3</td>
</tr>
<tr>
<td>PVY\textsuperscript{c}30</td>
<td>0/4</td>
</tr>
<tr>
<td>PVY\textsuperscript{c}43</td>
<td>0/4</td>
</tr>
<tr>
<td>PVY-P21</td>
<td>2/2</td>
</tr>
</tbody>
</table>

Edward, causing local lesions 7 days p.i. Non-inoculated leaves were negative when tested by ELISA 15 days p.i., confirming virus confinement in the inoculated leaves. The control PVY\textsuperscript{O} isolate infected the plants systemically, with no local lesions on inoculated leaves (results not shown). Tobacco plants infected with PVY\textsuperscript{C} isolates showed a milder mosaic than those infected with PVY\textsuperscript{O}.

Aphid transmission assays revealed that PVY\textsuperscript{C} isolates 23, 26, 27 and 29 were transmitted, while PVY\textsuperscript{C} isolates 28, 30 and 43 were not. PVY\textsuperscript{C}45 had lost infectivity by the time this assay was carried out, so we were unable to test its transmissibility. The transmission assay was performed twice, and while the PVY\textsuperscript{O} Palogen isolate used as a positive control was transmitted in both cases (80% transmission), PVY\textsuperscript{C} isolates were less efficiently transmitted.

PVY\textsuperscript{N} and PVY\textsuperscript{O} isolates from potato do not infect pepper mechanically (Gebre Selassie et al., 1985) but this assay has not previously been reported for PVY\textsuperscript{C} isolates. Results obtained in the host range assay are presented in Table 1: PVY\textsuperscript{C} isolates 26, 28 and 29 infected pepper as efficiently as did PVY-P21. Two groups of PVY\textsuperscript{C} isolates were established: PVY\textsuperscript{C}23, isolates 26, 28 and 29, were infective in pepper; PVY\textsuperscript{C}2, isolates 23, 27, 30 and 43, were non-infective in pepper. PVY\textsuperscript{C}29 was back-inoculated from pepper to potato and tobacco. Passage through pepper of this PVY\textsuperscript{C} isolate did not alter its host range since it remained infective in potato and tobacco. It was also observed that pepper acts as a filter plant: when inoculated with a mixture of PVY\textsuperscript{C} isolates 27 and 28, only PVY\textsuperscript{C}28 was detected in infected plants 15 days p.i. (data not shown).

Serological characterization

Serological characterization was performed using commercially available MAbs for PVY (Table 2). The assays were performed by TAS-ELISA as described in Methods.

ELISA tests were positive with MAbs C9 and 1E10 for all PVY\textsuperscript{C} isolates, as expected. PVY\textsuperscript{O}32 was also positive for 1E10, and both PVY\textsuperscript{O}32 and PVY\textsuperscript{N}134 were C9-positive. ELISA readings for these two MAbs were similar for all the isolates tested.

The results with MAb 10E3 differentiated the PVY\textsuperscript{C} isolates into the same two groups: PVY\textsuperscript{C} isolates 26, 28 and 29 (PVY\textsuperscript{C2}) were positive to this MAb, in common with most PVY isolates (Pérez de San Román et al., 1987; Sanz et al., 1990), while PVY\textsuperscript{C} isolates 23, 27, 30 and 43 (PVY\textsuperscript{C3}) were not reactive with this MAb.

MAb PVY(N) recognized all PVY\textsuperscript{C} isolates in the ELISA, despite the fact that this MAb has been reported to be positive only against PVY\textsuperscript{N} isolates (Gugerli & Fries, 1983). PVY\textsuperscript{O}32 readings were similar to those of the negative control, while PVY\textsuperscript{N}134 (the positive control for this MAb) gave values similar to the PVY\textsuperscript{C} isolates.

A mixture of MAbs 10E3 + 1E10 was used as a positive control, since this has been reported as the only combination of MAbs able to recognize any PVY isolate (Final Report, PVY-Ringtest 1991 in COST-88, Braunsweig). This mixture recognized all PVY\textsuperscript{C}, PVY\textsuperscript{O} and PVY\textsuperscript{N} isolates in the ELISA test, although readings for PVY\textsuperscript{C} 23, 27, 30 and 43 were lower than for the remaining isolates.

Table 2. Results of serotyping PVY isolates with four MAbs

The difference between minimum and maximum values of the ELISA readings (taken at 405 nm), divided by four, was used to determine the range corresponding to one `+` (see COST-88 PVY Ringtest results, available from G. Adam upon request). Values range to a maximum of four plus signs.

<table>
<thead>
<tr>
<th>MAb</th>
<th>C23</th>
<th>C26</th>
<th>C27</th>
<th>C28</th>
<th>C29</th>
<th>C30</th>
<th>C43</th>
<th>O32</th>
<th>N134</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td></td>
</tr>
<tr>
<td>1E10</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td></td>
</tr>
<tr>
<td>10E3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PVY(N)</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td></td>
</tr>
<tr>
<td>10E3 + 1E10</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
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<td>++++</td>
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</tr>
</tbody>
</table>
Molecular analysis

IC–RT–PCR–RFLP (Blanco-Urgoiti et al., 1996) was performed to determine the restrictotypes of PVYC isolates (Fig. 1). Four different restrictotypes were found: D32 E1 H15 R119 T190 for PVYC 23 and 30; D32 E2 H15 R119 T190 for PVYC 27 and 43; D30 E2 H16 R127 T190 for PVYC 26, 28 and 29; and D30 E1 H15 R127 T174 for PVYC 45. As a background for the phylogenetic tree, restrictotypes were computer-predicted for the PVYNP isolates whose CP sequences were available (PVY-Chil, PVY-NsNr, PVY-MsNr, PVY-P21, PVY-Pep, PVY-PepN and PVY-Is), as well as for PVYO36 and PVYNCan, both from potato. Genetic distances were calculated from these data and the corresponding phylogenetic tree constructed (Fig. 2 a). PVYC isolates clustered into the same two groups as mentioned previously: PVYC 26, 28, 29 and 45 (PVYC1) and PVYC 23, 27, 30 and 43 (PVYC2). The PVYC1 group was included within the NP cluster (Blanco-Urgoiti et al., 1996); the PVYC2 group differed significantly (P > 99%) from the rest of the PVY isolates. PVYO36 and PVYNCan were used as outgroup members, because preliminary results suggested that PVYC isolates were genetically closer to the NP group than to any potato PVY group (data not shown).

Since no sequence information for the CP gene of any PVYC isolate was available at the time the restriction enzymes for restrictotype analysis were chosen (Blanco-Urgoiti et al., 1996), and to confirm the results obtained for the previous dendrogram, the CP cistron of one isolate from each restrictotype was sequenced (PVYC 27, 28, 30 and 45). A new processing site for PVYNla protease was found in PVYC2 isolates 27 and 30 (Fig. 3); position p’ 1 of the CP cleavage site was a valine residue, as derived from the nucleotide sequence.

This amino acid residue has not been previously reported for PVY, and it is also rare for Potyviridae, having been found only once before in one isolate of lettuce mosaic virus (Dinant et al., 1991). The CP sequences of these isolates were compared with the CP sequence of a PVV isolate (GenBank accession number X61279; M. L. Moore, E. Maiss & P. R. Mills, unpublished results). The percentage of identity calculated for the core was 76%, whereas for isolates of a single species it has been reported as over 90% (Ward et al., 1995).

Genetic distances were estimated from these nucleotide sequences (Fig. 2 b), using the same background as for the RFLP-derived distances. The phylogenetic tree obtained from sequence data resulted in the same two groups of PVYC isolates: PVYC2 28 and 45 (PVYC1) within the NP cluster (P > 95%) and PVYC2 27 and 30 (PVYC2) in a branch significantly
Thus, according to commonly used terminology, isolates of different (P > 99%) from the other PVY isolates. The position of the PVYC isolates within the previous PVYN strain was slightly different when the two trees (RFLP- and sequence-derived) were compared. Overall, the PVYN branch was more compact in the sequence-derived tree. The correlation coefficient between distances obtained from RFLP data and those obtained from sequence comparison was 0.70.

To establish similarities between different strains of PVY isolates, genetic distances between the published CP sequences of all PVY isolates were calculated as described (Blanco-Urgoiti et al., 1996). The average genetic distance between PVYO and PVYN isolates was calculated and expressed in terms of percentage of similarity (Table 3), avoiding PVY-Ru (Blanco-Urgoiti et al., 1996), referred to as a recombinant (Revers et al., 1996), and PVY-VN (GenBank accession number U06789), because its genetic distance with PVY-Ru is only 0.0309. The remaining PVY isolates were grouped to calculate average similarity according to the clusters obtained from the phylogenetic tree (Fig. 2b). PVY-Chil was not included because it does not clearly belong to the PVYN branch. Four PVY clusters were compared: PVYO, PVYN, PVY(C1) (isolates 28 and 45) + PVYN and PVY(C2) (isolates 27 and 30). As shown in Table 3, the average similarity within groups was over 94%, while, in most cases, similarity between groups was below 91%. The similarity between PVY(C2) (27 and 30) and the branch formed by the NP cluster was comparable to its similarity with PVYO.

**Discussion**

In this paper we present a molecular and biological characterization of PVYC. We propose that these isolates, historically considered as a single virus strain, differ sufficiently in their characteristics to be considered as two genetic strains. Isolates of both strains share the ability to induce a hypersensitive response in potato cultivars bearing the Nc gene. Thus, according to commonly used terminology, isolates of these two strains belong to the same virus pathotype.

Isolates under the common name of PVYC are not genetically homogeneous, as demonstrated using two phylogenetic trees, one derived from genetic distances calculated from RFLP data and the other derived from sequence data. Two clusters were established: PVY(C1) and PVY(C2). PVY(C1) isolates were included in the NP cluster (Blanco-Urgoiti et al., 1996), while PVY(C2) isolates were independent of the other PVY isolates described so far. The correlation coefficient between the two types of genetic distance was comparable to that obtained by Blanco-Urgoiti et al. (1996) using 22 PVY isolates (none of them a PVYC), validating the use of restrictotypes for genetic clustering of PVY isolates with no prior sequence information. Sequences of four PVYC isolates were obtained to verify the genetic status of the two PVYC groups. The presence of a valine as the first amino acid in the CP of both PVYC isolates sequenced (PVYC27 and PVYC30) supports the proposition of two different genetic strains within PVYC.

PVYC has traditionally been considered as non-aphid-transmissible. Assays performed in this study indicate that some PVYC isolates are aphid-transmissible, and that this feature is independent of genetic strain. Other biological and serological characteristics correlated with the strain of PVYC. Thus, the ability or inability to infect pepper turned out to be a PVYC strain-specific property. Interestingly, the PVYC isolates of the PVYN cluster (PVYC1) were able to infect pepper while other potato isolates are not (Gebre Selassie et al., 1985). This could imply some type of coevolution within PVY and its plant hosts, as suggested for the Potyviridae in general (Ward et al., 1995). Serological tests also differentiated the same two groups based on their recognition by MAb 10E3. This was the most clear-cut distinguishing feature obtained from the complex serological profile of PVYC isolates. PVYC isolates were recognized by MAb PVYN, which has been reported to be PVYN-specific (Gugerli & Freis, 1983). A further anomaly with this MAb (and with three further PVYN-specific MAbs) is that two Canadian isolates of PVYN react negatively with MAb PVYN despite causing vein necrosis in tobacco (MacDonald & Singh, 1996). All PVYC isolates were positive to MAb 1E10. Thus, the mixture 10E3 + 1E10 (commercially available) has proved to be the only combination able to detect any PVY, since MAb C9 is unable to detect pepper PVY (Soto et al., 1994).

As described above, the two genetic strains of PVYC belong to the same pathotype. There are other resistance genes against PVY that define pathotypes. For example, pepper PVYs are classified in pathotypes (Gebre Selassie et al., 1985). In this case, genetic distances estimated from their restrictotypes show that isolates from different pathotypes fall into the same genetic strain (F. Ponz, unpublished results). Another resistance gene (Ny) that differentiates two types of PVYO isolate has been reported (Jones, 1990). Analysis of restrictotype genetic distances showed that both types of PVYO belong to the same genetic strain, with no correlation.
between the resistance response and genetic distances (B. Blanco-Urgoiti, M. Tribodet, S. Leclere, F. Ponz, C. Pérez de San Román, F. J. Legorburu & C. Kerlan, unpublished results). This result reinforces the view that the concept of pathotype is different to the ‘strain’ concept, which should always refer to a genetic characteristic of at least a particular region of the virus.

In order to establish similarities between strains of PVY, and using 30 sequences of the CP gene, we calculated the average of sequence similarities inter- and intra-clusters (Table 3). We found that the two PVY\textsuperscript{C} groups can be considered as different genetic strains because the similarity between them is comparable to the similarity between PVY\textsuperscript{O} and PVY\textsuperscript{EN}. PVY\textsuperscript{O} and PVY\textsuperscript{N} clusters are more homogeneous than the CP cluster, which presents the most variable genetic background. Unfortunately, it is not possible to establish a criterion of similarity to determine the strain of a hypothetical new isolate. This depends on the strain and the virus species, as reported for other potyviruses, such as yam mosaic virus (Aleman-Verdaguer et al., 1997).

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