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Characterization of typical pepper-isolates of PVY reveals multiple pathotypes within a single genetic strain

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Abstract

Potato virus Y (PVY) isolates originally coming from infected pepper plants, were biologically and genetically characterized, especially in comparison with PVY potato-isolates. Pepper PVY isolates could be differentiated from potato isolates in their host range, aphid transmission efficiencies, Mab serology, and genetic status. The genetic distances estimated for PVY pepper-isolates, based on their restrictotypes with five restriction enzymes and on their coat protein gene sequences, indicated that they form a single genetic strain with different pathotypic properties. This situation is essentially different to that of PVY potato-isolates.

Keywords: Potyvirus; Pepper; Genetic classification

1. Introduction

Potato virus Y (PVY) is an important pathogen, naturally transmitted by many aphid species in a non-persistent manner, that causes epidemics in potato, pepper, tomato, tobacco, and other solanaceous plants (De Bokx and Huttinga, 1981). It is the type species of the *Potyvirus* genus and it has a single positive sense genomic RNA about 10 kb long, inside flexuous virions. The genomic RNA is translated in a single polyprotein, which is processed into smaller proteins (Riechmann et al., 1992).

PVY isolates have been differentially classified depending on the host they were originally isolated from (De Bokx and Huttinga, 1981). Using criteria as symptomology, serology and resistance responses, potato isolates were classified into Y^O, Y^N and Y^C. A pathotypic classification has been proposed for pepper-PVY isolates based on their ability to overcome several resistance genes (Gebre Selassie et al., 1985). Thus, with respect to their responses against *pvr*2^1 and *pvr*2^2 resistance genes, pepper-PVY isolates were classified into three pathotypes: zero, which does not overcome any of the genes; 1, which overcomes *pvr*2^1 but not *pvr*2^2, and 1-2, which overcomes both resistance genes. Another pathotype (1-3) has recently been proposed (Luis-Arteaga et al., 1997). Potato- and pepper isolates have become differentiated to

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a degree such that potato isolates do not infect pepper mechanically nor viceversa (Gebre Selassie et al., 1985), although some potato isolates were found to infect pepper when inoculated with aphids (Fereres et al., 1993). Pepper isolates can also be differentiated from potato isolates serologically, because they do not react with monoclonal antibody Mab C9, which recognizes all potato isolates (Soto et al., 1994) and by transmissibility, because pepper isolates were more efficiently transmitted by aphids than potato isolates (Fereres et al., 1993). Tobacco isolates have been classified according to the symptoms induced in flue-cured tobacco cultivars resistant or susceptible to the root-knot nematode (Gooding, 1985; Gooding and Tolin, 1973), but a good strain classification is lacking for them. Tomato PVY-isolates have received less attention. Recently, in our laboratory, we have classified a collection of tomato-PVY isolates by genetic criteria (Blanco-Urgoiti et al., in preparation).

The use of different criteria has hampered a clear classification of PVY isolates into strains. From a genetic standpoint, it has been established that strains within a given potyvirus show a high level of sequence identity (94–99%), irrespective of the gene product considered (Shukla et al., 1994). It has, thus, become usual to approach the strain genetic variability of potyviruses through the analysis of the coat protein (CP) gene diversity, as a good accepted marker of the overall genome variability (Lehmann et al., 1997; Bousalem et al., 2000; Kreuze et al., 2000; Van Boxtel et al., 2000). We have developed a host-independent classification based on RFLP patterns of the coat protein gene, after immunocapture-RT-PCR, which allows the establishment of true genetic strains of PVY. We have proposed the term 'restrictotype' to define taxonomically each isolate (Blanco-Urgoiti et al., 1996). This rapid molecular typing approach distributed PVY isolates in three main clusters: potato PVY^{N}, potato PVY^{O} and non-potato isolates (PVY^{NP}), including two pepper, four tobacco and one *Datura* spp. isolates (Blanco-Urgoiti et al., 1996). These results suggested that pepper isolates formed a genetic strain, different from potato strains, as also suggested earlier by comparisons of coat protein gene and 3′UTR sequences (Van der Vlugt et al., 1993; Llave et al., 1999), and by Mab serology (Soto et al., 1994; Llave et al., 1999).

Recently, we used this method of typing to classify PVY^{C} (Blanco-Urgoiti et al., 1998). The PVY^{C} isolates cause a hypersensitive reaction in potato cultivars carrying the *Nc* resistance gene (Cockerham, 1970), so they can be considered as a pathotype. In this case, a collection of C isolates turned out to be formed by two different genetic strains, one classified as non-potato strain, C-isolates able to infect pepper (PVY^{C1}), and a new one (PVY^{C2}).

In this paper, we have undertaken a multidisciplinary approach for further characterization of typical pepper PVY-isolates of different pathotypes. The combination of present and earlier biological characterization such as host range and serology depict a picture of a compact and quite homogeneous group of isolates. The genetic analysis of the coat protein gene, both by RFLP-based and sequence phylogeny, confirm that typical pepper isolates of PVY form a single genetic strain, although showing a degree of genetic variation that is sufficient to form different pathotypes.

2. Materials and methods

2.1. Plant material

Nicotiana tabacum cv. Xanthi nc, *N. benthamiana*, *Chenopodium amaranticolor*, *Capsicum annuum* cv. Yolo Wonder and *Solanum tuberosum* cv. Bintje were grown and maintained in a glasshouse at 20–25°C.

2.2. Virus isolates

P21-82 (Soto et al., 1994), P27-81, P62-81, P27-86, P22-88PRW, P8-96, P34-95, P27-95, and PN-82 isolates were provided by M. Luis from SIA (Zaragoza, Spain) and Son-41-VR-2, To-72 (Gebre Selassie et al., 1985), K16.94, Si 15 and Tu 12.3 were provided by G. Marchoux from INRA (Monfavet, France). All isolates had been obtained from pepper plants, except Son-41-VR-2,

which was obtained by successive infections on pepper var. Florida VR-2 of the isolate Son-41, originally isolated from *Solanum nigrum*, and To-72, isolated from *lycopersicum Sculentum*. All of them were able to infect pepper mechanically.

2.3. Host range assay

Phosphate buffer-DIECA virus extract, carborundum and active carbon (Marrou, 1967) were rubbed onto the different plant species described above to assay host range of PVY isolates. Isolates used belonging to strain PVY^O were 32 and Palogán, isolate belonging to strain PVY^N was A134 (Blanco-Urgoiti et al., 1998) and isolates coming from pepper were P62-81, P21-82 and P27-81 (see above). Plants were tested by DAS-ELISA as described (Soto et al., 1994).

For aphid assays, they were subjected to 1 h of preacquisition starving, 5 min of acquisition access time, and 24 h of inoculation time. Twenty aphids per test plant were used in all assays.

2.4. Coat protein gene sequencing

Immunocapture-RT-PCR was performed for isolates P21-82, Si15, K16.94, Tu12.3, PN-82 and P62-81 as described by Blanco-Urgoiti et al. (1998). The 935 nucleotide fragments obtained were cloned and sequenced.

2.5. Restrictotyping

Restrictotyping of PVY pepper-isolates was performed after IC-RT-PCR as described (Blanco-Urgoiti et al., 1996).

2.6. Phylogenetic analysis

Genetic distances were determined and the phylogenetic trees derived from both RFLP and sequence analyses were constructed as described (Blanco-Urgoiti et al., 1996). Coat protein gene sequences from the following virus isolates were used for analysis of genetic distances based on RFLP and sequences to construct the phylogenetic trees: PVY-EurH, PVY-Hu, PVY-O4, PVY-PotUs, PVY-NsNr, PVY-MsNr, PVY-Is, PVY-52, PVY-C27, PVY-C30, PVY-C28 and PVY-C45 (described in Blanco-Urgoiti et al., 1996). Potato isolates used in the host range experiment were not included here because their coat protein gene sequences are not available.

The EMBL accession numbers of the sequences reported in this paper are AJ005639 (P62-81), AJ303093 (Si 15), AJ303094 (K16.94), AJ303095 (Tu 12.3), AJ303096 (PN-82), AJ303097 (P21-82).

3. Results

3.1. Biological characterization

Results of the comparative studies on host range and symptomology of potato and pepper isolates of PVY mechanically inoculated are shown in Table 1. In *Chenopodium amaranticolor*, local lesions on inoculated leaves were observed with all the isolates tested (potato and pepper isolates), and in *Nicotiana benthamiana*, all isolates produced similar mild mosaic and stunting. *Nicotiana tabacum* Xanthi nc infected with the potato isolates belonging to the strain PVY^O ex-

Table 1
Symptoms on test plants mechanically inoculated with potato or pepper isolates of PVY

Host/PVY isolate	Potato isolates		Pepper isolates
	O	N	
Chenopodium amaranticolor	Local lesions	Local lesions	Local lesions
Nicotiana benthamiana	Mild mosaic, stunting	Mild mosaic, stunting	Mild mosaic, stunting
Nicotiana tabacum Xanthi nc	Severe mosaic	Veinal necrosis	Mild mosaic
Capsicum annuum Yolo Wonder	No infection	No infection	Severe mosaic
Solanum tuberosum Bintje	Stunting	Stunting	No infection

Table 2
Transmission by aphids of different PVY isolates from pepper source plants to potato test plants

Assay	Source plant	Virus isolate	Vector	Test plant	Transmission %[a]
1	Pepper	P21-82	*M. persicae*	Potato	0 (0/5)
	Pepper	P21-82	*A. gossypii*	Potato	0 (0/5)
	Pepper	A134	*M. persicae*	Potato	100 (5/5)
	Pepper	P21-82	*M. persicae*	Pepper	100 (5/5)
2	Pepper	P21-82	*M. persicae*	Potato	0 (0/5)
	Pepper	P21-82	*M. persicae*	Pepper	100 (5/5)

[a] Percent infected plants. Number of plants infected/total number of plants tested in parentheses.

hibited a strong mosaic, while PVY^N isolate produced vein necrosis when inoculated in this species. Pepper isolates, belonging to different pathotypes, induced the same type of mild mosaic. In this sense, tobacco can be considered as a differential host based on the symptoms developed by potato- or pepper-isolates. As described earlier (Gebre Selassie et al., 1985), potato isolates were not mechanically transmissible to pepper and viceversa. Earlier results (Fereres et al., 1993) proposed *Nicotiana glutinosa* as a differential host between one pepper- and one potato-isolate using aphids, because the potato isolate was able to infect and the pepper isolate was not. We have now tested more pepper isolates, the behavior is not homogeneous, some isolates infecting this host and some not (not shown).

We have earlier observed that potato isolates were transmitted by aphids from potato or tobacco to pepper, although in a lower and more unstable way than the pepper isolates (Fereres et al., 1993). Table 2 (assay 1) shows that a pepper-PVY isolate was unable to infect potato plants (cv. Bintje) when *M. persicae* or *A. gossypii* were used as vectors. In the same assay, isolate A134 (belonging to PVY^N strain), inoculated in pepper by aphids, was efficiently transmitted to potato test plants using *M. persicae* as vector. The pepper PVY isolate was also transmitted readily to pepper test plants. The large number of aphids used to inoculate each test plant assured, when possible, a 100% transmission rate. Assay 2 showed again that *M. persicae* failed to transmit the pepper PVY isolate P21-82 to potato test plants.

3.2. Genetic classification of pepper PVY-isolates

The results described above and earlier findings showed that typical pepper-PVY isolates were easily distinguished from potato isolates by host range and Mab serotyping (Soto et al., 1994). Next, we tried to find out if a collection of pepper PVY isolates, coming from different Mediterranean countries, corresponded to a different and homogeneous genetic strain with respect to potato isolates, as suggested by Van der Vlugt et al. (1993) and by our own earlier work (Blanco-Urgoiti et al., 1996). Most of the 12 isolates were classified by the donors into pathotypes with respect to the resistance genes $pvr2^1$ and $pvr2^2$ (Gebre Selassie et al., 1985) and re-confirmed by us (Table 3). Pathotype 1-3 was described by Luis-Arteaga et al. (1997). Isolates Son-41-VR-2 and To-72 were described as belonging to pathotypes 1-2 and zero, respectively, when infected on pepper (Gebre Selassie et al., 1985). The differential response of potato- and pepper-isolates to a Mab (Soto et al., 1994) directed to an epitope originally described to be present in all potato PVY isolates (Gugerli and Fries, 1983) suggested that coat protein gene should be a useful genome fragment for genetic classification. Immunocapture followed by RT-PCR and RFLP of the CP gene was performed to determine the restrictotypes of the twelve pepper PVY-isolates (Table 3). As an illustration of this process, some selected RFLP results of pepper and potato isolates and the conversion into restrictotypes are shown in Fig. 1. The overall pattern similarity found for pepper isolates and the clear differences with potato isolate patterns are exemplified in this

figure. The genetic distances between all isolates were calculated based on RFLP data and a dendrogram was constructed (Fig. 2a), including several potato PVY^N, Y^O and Y^C-isolates and non-potato isolates (Blanco-Urgoiti et al., 1996). The use of restrictotypes for genetic clustering of PVY isolates with no prior sequence information was validated earlier (Blanco-Urgoiti et al., 1996). The phylogenetic tree shows the clustering of all pepper isolates in a single genetic strain, into the PVY^{NP} group.

To further confirm the results obtained with RFLPs data, the sequence of the coat protein gene of six pepper isolates was determined (P21-82, Si 15, K16.94, Tu 12.3, PN-82 and P62-81). Genetic distances were estimated from these nucleotide sequences (Fig. 2b), using the same algorithm as for the RFLP-derived distances. The phylogenetic tree obtained from sequence data resulted in the same clustering of the pepper isolates in the PVY^{NP} group.

Table 3
Restrictotypes obtained for a collection of twelve pepper-PVY isolates

Name	Origin	Pathotype	Restrictotype[a]
P21-82	Spain	zero	D30 E1 H15 R127 T190
P62-81	Spain	1	D30 E2 H15 R127 T190
K16.94	Tunisie	1	D32 E2 H15 R127 T190
Si 15	Sicile	1	D32 E1 H15 R127 T158
Tu 12.3	Turkey	1	D32 E2 H15 R192 T190
P27-81	Spain	zero	D30 E2 H15 R512 T158
PN-82	Spain	1	D30 E2 H15 R512 T158
P8-96	Spain	n.d.	D30 E2 H15 R127 T190
P34-95	Spain	n.d.	D30 E2 H15 R127 T190
P27-95	Spain	1	D30 E2 H15 R127 T190
P22-88PRW	Spain	1-3	D30 E2 H15 R192 T190
P27-86	Spain	1	D30 E1 H15 R127 T190

[a] Capital letters in the restrictotype are the initials of the five restriction enzymes used (*Dde*I, *Eco*RV, *Hinf*I, *Rsa*I, *Taq*I). The numbers after the letters are the particular array of bands, expressed as 0s and 1s, of the collection of patterns kept in our laboratory. n.d., not determined.

4. Discussion

In this paper, we present a biological and molecular characterization of typical pepper isolates of PVY, which has allowed us to propose that they form a genetic cluster different from potato isolates. As to their host range, pepper and potato were differential hosts, especially potato, because pepper isolates were not able to infect this plant, not even using aphids. We have earlier shown that potato isolates can be aphid-transmitted to pepper, although with a low efficiency (Fereres et al., 1993). The infection obtained was unstable. *Nicotiana tabacum* Xanthi nc can also be considered as differential based on symptomology. In a complementary approach, the results obtained in genetic clustering of the coat protein gene using RFLPs and sequences indicated that pepper and pepper-infecting isolates (To-72 and Son-41-VR-2) could be classified within the PVY^{NP} group. We have recently reported that the PVY^C isolates that infect pepper also belong to the PVY^{NP} strain (Blanco-Urgoiti et al., 1998). All these results lead us to propose that pepper-infecting PVY isolates form a true genetic strain, different from isolates non-infecting pepper, confirming and expanding earlier suggestions made by us and others (Van der Vlugt et al., 1993; Soto et al., 1994; Blanco-Urgoiti et al., 1996, 1998; Llave et al., 1999).

Our results show that the genetic variability of pepper-infecting PVY isolates is much smaller than the one found within PVY isolates from potato. Thus, all pepper-infecting isolates analyzed in this paper belong to the same genetic strain (PVY^{NP}), even the PVY^{C1} group, a potato-isolate group able to infect pepper, whereas potato isolates fall into at least four different strains, PVY^N, PVY^O, PVY^{C1} (in the PVY^{NP} strain), and PVY^{C2} (Blanco-Urgoiti et al., 1998). These results indicate that the specificity of the different isolates of PVY with respect to their host

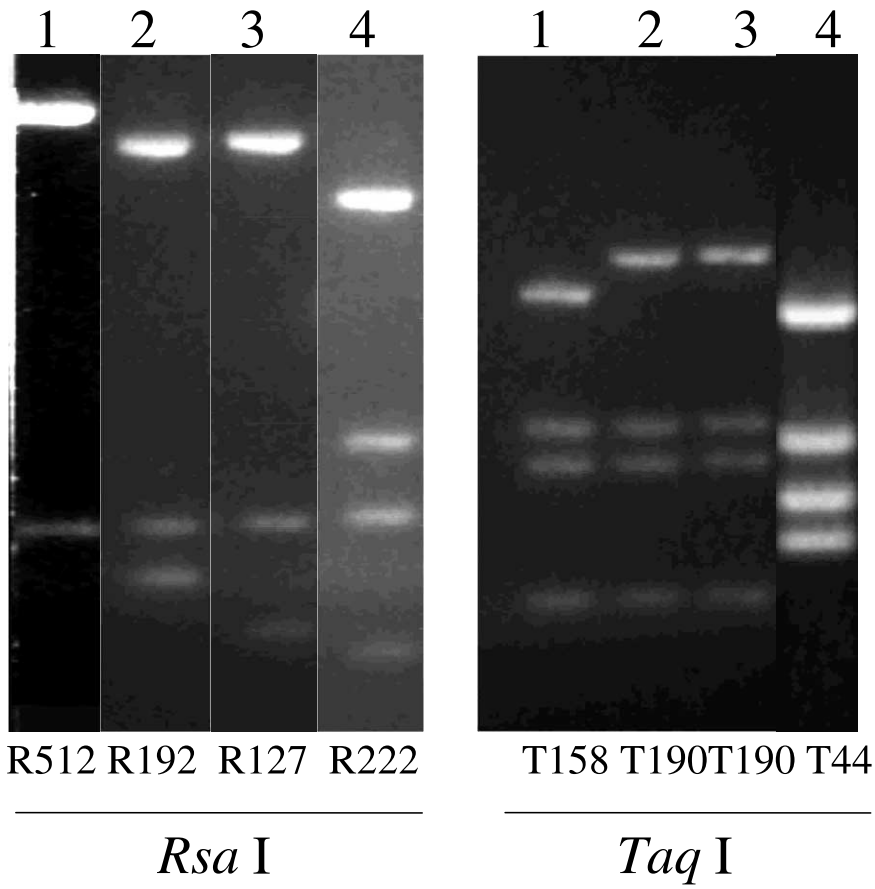

Fig. 1. Example illustrating some *Rsa*I and *Taq*I restriction patterns obtained from three pepper isolates: P27-81 (lane 1), P22-88PRW (lane 2), and P27-86 (lane 3), and one potato isolate, A134 (lane 4). Numbers and letters at the bottom of the panel represent the name of the restrictotype (see Table 3).

is more strict in the case of pepper isolates, since they do not infect potato, neither mechanically nor by aphids. In another experimental work using immunocytochemistry, we have observed that pepper isolates were not able to accumulate in protoplasts purified from inoculated potato leaves (unpublished results). It appears that pepper infection requires more specialization than other hosts, like potato or tomato, whose infecting isolates distribute into several strains, or tobacco, which is infected by both potato- and pepper-isolates. The fact that potato plants are tuber-multiplied may have led to a strain differentiation that we do not find in pepper isolates. We can assert that host specificity is an important criterion for specialization and strain differentiation in PVY. In other words, the host seems to be an important factor in PVY evolution and probably the strain specialization in the virus is an effect of coevolution with the plant. It is still not possible to analyze the case of PVY isolated from other cultivated solanaceous plants, because enough data are not available yet. Preliminary results with PVY tomato-isolates indicate that this case is different from pepper isolates and that they form several strains (Blanco-Urgoiti et al., in preparation).

We have argued in the past the need for a single, genetic criterion for strain classification (Blanco-Urgoiti et al., 1996). A good example of

the confusion that can be induced when using a non-genetic criterion is illustrated by our recent finding that the classically termed PVY^{C} strain of potato-PVY isolates is in fact a pathotype formed by two very different strains (Blanco-Urgoiti et al., 1998). In the present work we have questioned if this was also the case for pepper-PVY isolates, for which four pathotypes have been proposed (Gebre Selassie et al., 1985; Luis-Arteaga et al., 1997). Earlier work suggested that pepper-PVY was indeed a different case (Llave et al., 1999). Our results confirm this suggestion and show that pepper-PVY isolates are a single genetic strain, containing several pathotypes, exemplifying a situation opposite to PVY^{C} isolates. We found several groups of isolates with an RFLP-derived

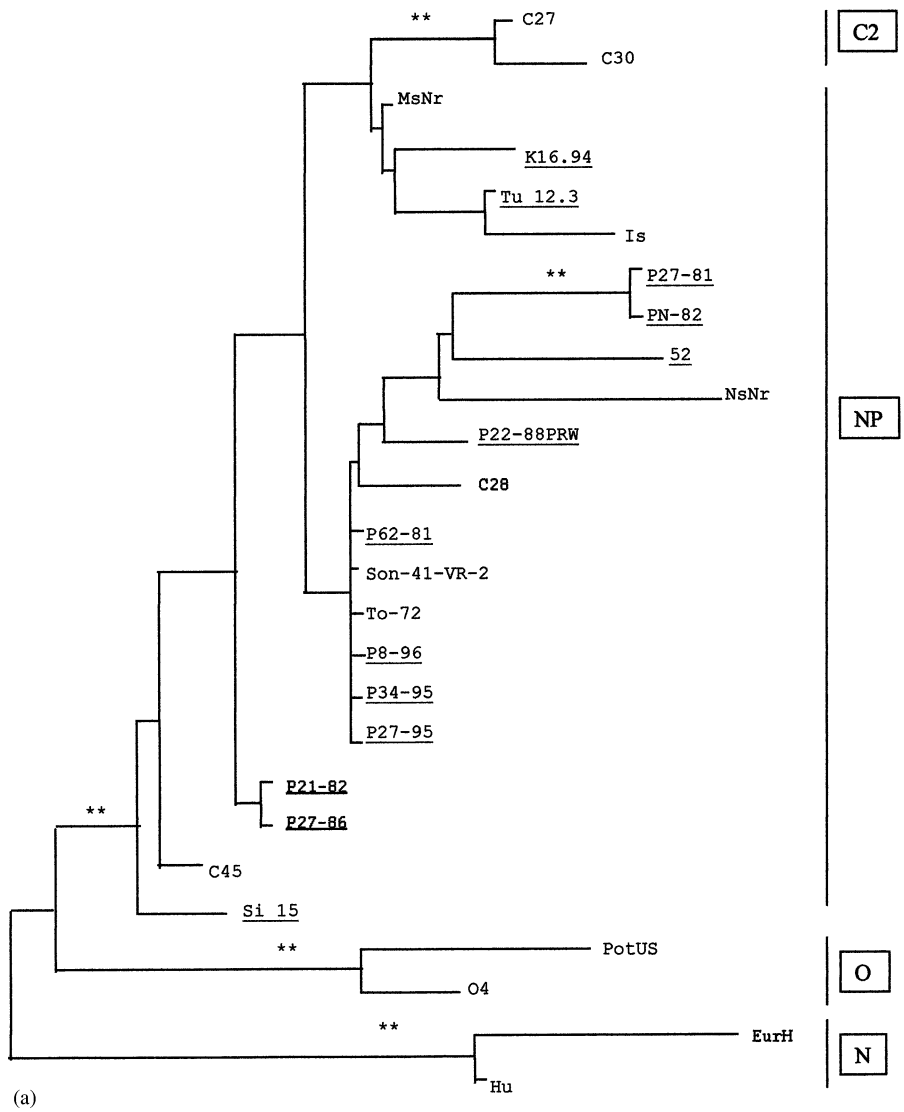

Fig. 2. Phylogenetic trees derived from genetic distances calculated from RFLP data (a) and from CP sequence data (b). '*' statistically significant branch ($\geq 95\%$); '**' highly statistically significant branch ($\geq 99\%$). Isolates originally coming from pepper are underlined.

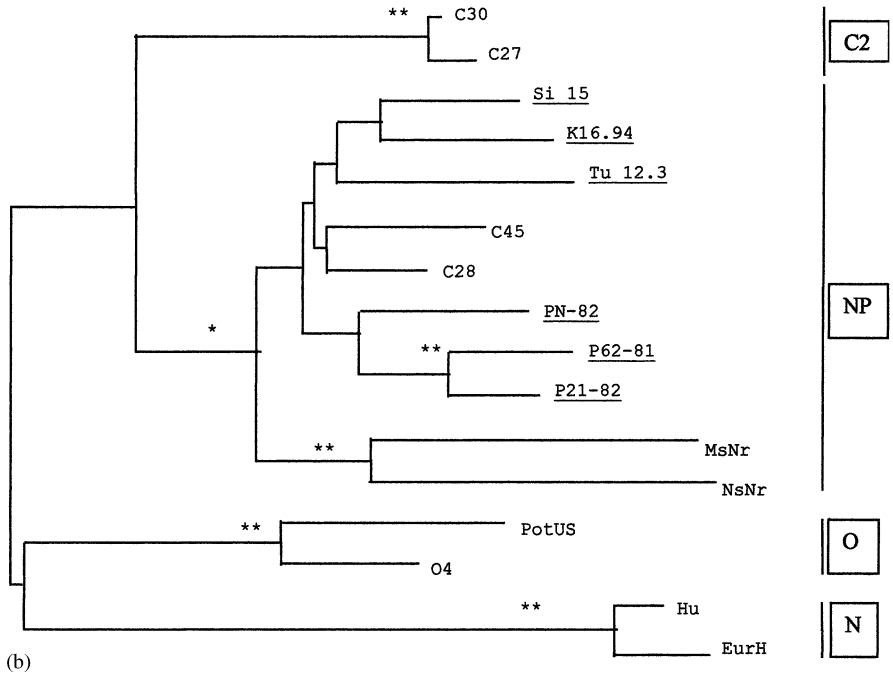

Fig. 2. (*Continued*)

genetic distance of zero, which belonged to different pathotypes: P62-81 (pathotype 1), Son-41-VR-2 (1-2) and To-72 (zero); P27-81 (zero) and PN-82 (1); P21-82 (zero) and P27-86 (1). Even if the CP gene is not the avirulence determinant for the resistance gene, such results stress again that an intrinsic genetic classification such as the strain one, should not be tried to correlate with a biological one like pathotypes, because they are based on different unrelated criteria.

The close genetic distance between isolates belonging to the four pepper-PVY pathotypes suggest that few changes are required in the genome of pepper-PVY isolates to overcome the corresponding pepper resistance genes. This seems actually to be the general case for potyviruses. Thus, all cases of new pathotypes reported to date involve very minor changes in the avirulence gene (Nicolas et al., 1997; Schaad et al., 1997; Keller et al., 1998; Masuta et al., 1999; Rajamäki and Valkonen, 1999; Mestre et al., 2000; Jenner et al., 2000; Hämäläinen et al., 2000). This implies that significant biological changes such as pathotyping can be obtained with minimal genetic changes without changing the genetic strain status. It can be inferred that obtaining new pathotypes should not be difficult in PVY infections of pepper in the field. This notion has some circumstancial experimental support, based on the reported obtention of pathotype 1-3 during greenhouse manipulations (Luis-Arteaga et al., 1997). The resistances related to these pathotypes are most likely a recessive allelic series of a single genetic locus (Palloix and Kyle, 1995). The mechanism of one of them, *pvr*2^1, has been shown to be impairment in virus cell-to-cell movement (Arroyo et al., 1996). Another unrelated PVY-resistance gene, *Pvr*4, has not been overcome so far (Dogimont et al., 1996). This is a dominant resistance that seems to be mediated by a different mechanism (Caranta et al., in preparation). It will be interesting to analyze if eventual *Pvr*4-overcoming isolates will also belong to the same genetic strain, as in the case studied in this paper.

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References

Arroyo, R., Soto, M.J., Martínez Zapater, J.M., Ponz, F., 1996. Impaired cell-to-cell movement of potato virus Y in pepper plants carrying the y(a)(*pr*2(1)) resistance gene. Mol. Plant Microbe Interac. 9, 314–318.

Blanco-Urgoiti, B., Sánchez, F., Dopazo, J., Ponz, F., 1996. A strain-type clustering of potato virus Y based on the genetic distance between isolates calculated by RFLP analysis of the amplified coat protein gene. Arch. Virol. 141, 2425–2442.

Blanco-Urgoiti, B., Sánchez, F., Pérez de san Román, C., Dopazo, J., Ponz, F., 1998. PVYC isolates are a homogeneous pathotype but two different genetic strains. J. Gen. Virol. 79, 2037–2042.

Bousalem, M., Dallot, S., Guyader, S., 2000. The use of phylogenetic data to develop molecular tools for the detection and genotyping of Yam mosaic virus. Potential application in molecular epidemiology. J. Virol. Methods 90, 25–36.

Cockerham, A., 1970. Genetical studies on resistance to potato viruses X and Y. Heredity 25, 309–348.

De Bokx JA, Huttinga H, 1981. Potato virus Y. CMI/AAB Descriptions of Plants Viruses No. 242.

Dogimont, C., Palloix, A., Daubze, A.M., Marchoux, G., Selassie, K.G., Pochard, E., 1996. Genetic analysis of broad spectrum resistance to potyviruses using doubled haploid lines of pepper (*Capsicum annuum* L.). Euphytica 88, 231–239.

Fereres, A., Pérez, P., Gemeno, C., Ponz, F., 1993. Transmission of spanish pepper- and potato-PVY isolates by aphid (homoptera: *Aphididae*) vectors: epidemiological implications. Commun. Ecosystem Ecol. 22, 1260–1265.

Gebre Selassie, K., Marchoux, G., Delcolle, 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, 621–630.

Gooding, G.V., 1985. Relationship between strains of potato virus Y and breeding for resistance, cross protection, and interference. Tobacco Sci. 29, 99–104.

Gooding, G.V., Tolin, S.A., 1973. Strains of potato virus Y affecting flue-cured tobacco in the South eastern United States. Plant Dis. Rep. 57, 200–204.

Gugerli, P., Fries, P., 1983. Characterization of monoclonal antibodies to potato virus Y and their use for virus detection. J. Gen. Virol. 64, 2471–2477.

Hämäläinen, J.H., Kekarainen, T., Gebhardt, C., Watanabe, K.N., Valkonen, J.P.T., 2000. Recessive and dominant genes interfere with the vascular transport of Potato virus A in diploid potatoes. Mol. Plant Microbe Interact. 13, 402–412.

Jenner, C.E., Sánchez, F., Nettleship, S.B., Foster, G.D., Ponz, F., Walsh, J.A., 2000. The cylindrical inclusion gene of Turnip mosaic virus encodes a pathogenic determinant to the Brassica resistance gene TuRB01. Mol. Plant Microbe Interact. 13, 1102–1108.

Keller, K.E., Johansen, I.E., Martin, R.R., Hampton, R.O., 1998. Potyvirus genome-linked protein (VPg) determines pea seed-borne mosaic virus pathotype-specific virulence in *Pisum sativum*. Mol. Plant Microbe Interac. 11, 124–130.

Kreuze, J.F., Karyeija, R.F., Gibson, R.W., Valkonen, J.P.T., 2000. Comparisons of coat protein gene sequences show that East African isolates of Sweet potato feathery mottle virus form a genetically distinct group. Arch. Virol. 145, 567–574.

Lehmann, P., Petrzik, K., Jenner, C., Greenland, A., Spak, J., Kozubek, E., Walsh, J.A., 1997. Nucleotide and amino acid variation in the coat protein coding region of turnip mosaic virus isolates and possible involvement in the interaction with the brassica resistance gene TuRB01. Physiol. Mol. Plant Pathol. 51, 195–208.

Llave, C., Martínez, B., Díaz-Ruíz, J.R., López-Abella, D., 1999. Serological analysis and coat protein sequence determination of potato virus Y (PVY) pepper pathotypes and differentiation from other PVY strains. Eur. J. Plant Pathol. 105, 847–857.

Luis-Arteaga, M., Arnedo Andrés, M., Gil Ortega, R., 1997. New potato virus Y pathotype in pepper. Capsicum Eggplant Newslett. 16, 85–86.

Marrou, J., 1967. Amèlioration des mèthodes de transmission mècenique des virus par absortion des inhibiteurs d'infection sur le charbon vègètal. Comtes Rendus de l'Académie d'Agriculture Français 53, 972–981.

Masuta, C., Nishimura, M., Morishita, H., Hataya, T., 1999. A single amino acid change in viral genome-associated protein of potato virus Y correlates with resistance breaking in 'Virgin A Mutant' tobacco. Phytopathology 89, 118–123.

Mestre, P., Brigneti, G., Baulcombe, D.C., 2000. An Ry-mediated resistance response in potato requires the intact active site of the NIa proteinase from potato virus Y. Plant J. 23, 653–661.

Nicolas, O., Dunnington, S.W., Gotow, L.F., Pirone, T.P., Hellmann, G.M., 1997. Variations in the VPg protein allow a potyvirus to overcome va gene resistance in tobacco. Virology 237, 452–459.

Palloix, A., Kyle, M., 1995. Proposal revision of gene nomenclature for potyvirus resistance genes in Capsicum sp. Capsicum Eggplant Newslett. 14, 26–29.

Rajamäki, M.L., Valkonen, J.P.T., 1999. The 6K2 protein and the VPg of potato virus A are determinants of systemic infection in *Nicandra physaloides*. Mol. Plant Microbe Interact. 12, 1074–1081.

Riechmann, J.L., Lain, S., García, J.A., 1992. Highlights and prospects of potyvirus molecular biology. J. Gen. Virol. 73, 1–16.

Schaad, M.C., Lellis, A.D., Carrington, J.C., 1997. VPg of tobacco etch potyvirus is a host genotype-specific determinant for long-distance movement. J. Virol. 71, 8624–8631.

Shukla, D.D., Ward, C.W., Brunt, A.A., 1994. Genome structure, variation and function. In: The Potyvidae. CAB International, Wallingford, pp. 74–112.

Soto, M.J., Luis Arteaga, M., Fereres, A., Ponz, F., 1994. Limited degree of serological variability in pepper strains of potato virus Y as revealed by analysis with monoclonal antibodies. Ann. Appl. Biol. 124, 37–43.

Van Boxtel, J., Thomas, C.L., Maule, A.J., 2000. Phylogenetic analysis of two potyvirus pathogens of commercial cowpea lines: implications for obtaining pathogen-derived resistance. Virus Genes 20, 71–77.

Van der Vlugt, R., Leunissen, J., Goldbach, R., 1993. Taxonomic relationships between distinct potato virus-Y isolates based on detailed comparisons of the viral coat proteins and 3′-nontranslated regions. Arch. Virol. 131, 361–3751.