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Serological analysis and coat protein sequence determination of potato virus Y (PVY) pepper pathotypes and differentiation from other PVY strains

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Abstract

The serological relationships of Potato Virus Y (PVY) isolates belonging to the pepper pathotypes 0, 1 and 1-2 were established by enzyme-linked immunosorbent assay (ELISA). PVY pepper pathotypes did not react with monoclonal antibodies which typically recognize non-pepper strains within the PVY group, leading to discrimination between these two groups of strains. No serological differences were found between the three PVY pepper pathotypes. The coat protein (CP) nucleotide and predicted amino acid sequences of the three different PVY pepper pathotypes were determined. The highest sequence similarity was found between pathotypes 0 and 1 (99.2%), while the lowest occurred between these two and pathotype 1–2 (98.1%). PVY strains from potato and tobacco appeared more distantly related. Phenetic analysis of the CP amino acid sequences showed that the PVY pepper pathotypes formed a tightly clustered group separate from other PVY strains.

Introduction

Potato Virus Y (PVY) is the type member of the genus *Potyvirus* (family Potyviridae), the largest group of RNA plant viruses (Murphy et al., 1995). This flexuous, rod-shaped virus is composed of a single-stranded, positive sense RNA encapsidated by about 2,000 copies of a single coat protein (CP). The 9.7 kb genomic RNA has a genome-linked viral protein (VPg) at the 5′ end and a 3′ poly (A) tail. The viral genome is expressed as a single polyprotein autoprocessed to generate the final gene products (Riechmann et al., 1992). PVY is non-persistently transmitted by aphids in nature with two viral proteins known to be involved in the transmission process: the viral CP and the non-structural viral protein, helper component (HC) (Pirone, 1991).

Potato Virus Y consists of a complex group of strains and pathotypes which have been traditionally identified and classified according to their biological properties. They cause serious losses in many important crops all over the world, including pepper (De Bokx and Huttinga, 1981). PVY isolates infecting pepper crops from mediterranean countries have been classified as pathotypes 0, 1 and 1–2, based on their differential interactions with the pepper (*Capsicum annuum* L.) resistance genes *pvr2*^{1} and *pvr2*^{2} at the *pvr2* locus (Gebre-Selassie et al., 1985). The *pvr2*^{1} allele confers complete resistance to PVY pathotype 0 and the *pvr2*^{2} allele to PVY pathotypes 0 and 1, whereas the PVY pathotype 1–2 is able to overcome both resistance genes (Palloix and Kyle, 1995). The same pathotype hierarchy was found and the same denomination used for local pepper PVY isolates in Spain (Luis-Arteaga, 1989; Pasko, 1993).

Most PVY strains or pathotypes are serologically closely related. Therefore, methods based on the antigenic properties have only occasionally resulted in successful discrimination between them (Gugerli and Fries, 1983; Jordan and Hammond, 1991). In fact, Spanish isolates of PVY belonging to different pepper

pathotypes have been reported to show similar antigenic properties (Soto et al., 1994), confirming that PVY serological relationships do not always correlate with biological properties (Shukla et al., 1992). On the other hand, the sequence identity of the CP gene is currently regarded as a useful criterion for resolving the taxonomy of the complex PVY group (Shukla and Ward, 1988; Ward et al., 1992; Van der Vlugt et al., 1993).

In this paper, we have analyzed the serological relationships of a collection of pepper PVY isolates belonging to the pathotypes 0, 1 and 1–2 using a large range of MAbs and PAbs. In addition, we have determined the nucleotide sequence of the CP gene of these pathotypes and have compared their predicted amino acid sequences with the published CP sequence of PVY strains from potato and tobacco. These results allowed us to differentiate between PVY pepper pathotypes and non-pepper PVY strains on the basis of both their antigenic behavior and their CP sequence homology. Although the CPs of some PVY isolates from pepper have already been sequenced (Chachulska et al., 1997), to our knowledge, this is the first report of CP sequences from each PVY pepper pathotype and therefore, this paper represents a contribution toward a clearer taxonomy of the PVY which support the current pepper pathotype classification.

Materials and methods

Viral pathotypes

A collection of PVY isolates belonging to pepper pathotypes 0, 1 and 1-2 were used. All the isolates were provided by M.P. Luis-Arteaga (SIA-DGA., Zaragoza, Spain), except isolate P-21-82 which was supplied by F. Ponz (CIT-INIA, Madrid, Spain) (Table 1). They were all isolated from Spanish field-grown pepper plants and maintained in a greenhouse by mechanical inoculation in tobacco (*Nicotiana tabacum* L. cv. Xanthi) and/or pepper plants (*C. annuum* L. cv. Yolo Wonder). They were identified as pepper pathotypes 0, 1 or 1–2 according to Gebre-Selassie et al. (1985). Isolates P-22-88 and P-27-95 were chosen as representatives of the pathotypes 1–2 and 1, respectively, and for simplicity, are designated as PVY 1-2 and PVY 1 throughout this paper. Since its initial collection, isolate P-21-82, a pathotype 0 isolate, was also kept by aphid inoculation in tobacco plants and termed PVY 0 AT to

Table 1. Reactivity in indirect-ELISA of PVY pepper isolates belonging to pathotypes 0, 1 and 1-2 with MAbs and PAbs to PVY

Pathotypes	Isolates	MAbs to PVY						Pab to PVY
		3E9	12C4	10E3	C9	PVY N	1E10	
0								
	P-27-81	+++[1]	+++	+++	−	−	−	+++
	PVY 0 AT[2]	++	+++	+++	−	−	−	+++
	PVY 0 NAT	++	+++	+++	−	−	−	+++
	P-82-90	++	++	+++	−	−	−	+++
	P-5-95	+++	+++	+++	−	−	−	+++
	P-20-95	++	++	+	−	−	−	+++
1								
	P-62-81	++	+++	+++	−	−	−	+++
	P-27-86	+++	+++	+++	−	−	−	+++
	P-28-95	+++	+++	+++	−	−	−	+++
	PVY 1[2]	++	+++	+++	−	−	−	+++
	P-8-96	+++	+++	+++	−	−	−	+++
1-2								
	PVY 1-2[2]	++	+++	+++	−	−	−	+++

[1]Serological reactivity, measured as the absorbance at 405 nm (A) after 10 min of substrate incubation: −, $A < 0.3$; +, $0.3 < A < 1$; ++, $1 < A < 2$; +++, $A > 2$. Mean values obtained from two independent experiments.

[2]PVY 0 AT, PVY 1 and PVY 1-2 refer to isolates P-21-82, P-27-95 and P-22-88, respectively.

differentiate it from a non-aphid transmissible variant of this isolate obtained in the laboratory by repeated mechanical inoculations in tobacco and named PVY 0 NAT (Canto et al., 1995).

Virus purification and RNA isolation

Virion preparations were obtained from leaves of systemically infected tobacco plants two to three weeks after inoculation (Murphy et al., 1990). Purified virus preparations were analyzed on sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE 12%) and virus concentrations were determined spectrophotometrically.

Viral RNA was isolated both from infected plants and purified virions (Logemann et al., 1987; Ullman et al., 1993). Briefly, two 10 mm discs from infected tobacco leaves were homogenized in guanidine buffer (8 M guanidine hydrochloride, pH 7, 20 mM MES, 20 mM ethylenediaminetetracetic acid (EDTA), 50 mM β-mercaptoethanol) and after phenol/chloroform extraction, the nucleic acids were ethanol precipitated and resuspended in 25 µl of sterile water. For RNA isolation from purified virions, viral preparations (2–10 µg) were diluted in a equal volume of 2 × extraction buffer (100 mM Tris-HCl, pH 8, 2 mM EDTA, 2% SDS), incubated with 0.5 mg/ml of Protease K (Boehringer Mannheim) at 65 °C for 10 min and after a phenol/chloroform extraction, the RNA was ethanol precipitated and finally resuspended in 10–20 µl of sterile water.

Monoclonal and polyclonal antibodies

A set of monoclonal antibodies (MAbs) prepared against the CP of different PVY strains was used for serological assays: MAbs 10E3, 12C4 and 3E9, which recognize a broad spectrum of viruses within the PVY group (Sanz et al., 1990), were supplied by INGENASA (Madrid, Spain); MAb 1E10, which recognizes potato PVY strains in the C group and some in the 0 group (Sanz et al., 1990; Blanco-Urgoiti et al., 1998a), was also purchased by INGENASA; MAbs C9 and PVY N, whose corresponding epitopes are present in all three potato PVY strain groups (0, N and C) and in the PVY N group, respectively (Gugerli and Fries, 1983), were provided by F.J. Legorburu (CIMA, Vitoria, Spain). A rabbit polyclonal antiserum (PAbs) against the whole particles of PVY 0 was produced in our laboratory by four intramuscular injections at 2-week intervals with approximately 0.5 mg (first injection) and 1 mg of purified virus emulsified with Freund's incomplete adjuvant (Sigma). Anti-PVY immunoglobulins (IgGs) were purified from rabbit antiserum using a Protein-A Sepharose CL 4B column (Pharmacia) according to the supplier's recommendations and precipitated with an equal volume of saturated ammonium sulfate solution. Additionally, purified IgGs were also labeled with alkaline phosphatase (AP) Type VII-T (Sigma) (Sambrook et al., 1989).

Indirect-ELISA

PVY pepper pathotypes were tested for their serological relationships by indirect enzyme-linked immunosorbent assay (ELISA) (Converse and Martin, 1991) using the above mentioned MAbs and PAb. An anti-mouse and anti-rabbit AP-conjugated PAbs (Sigma) were used as second antibodies. Experiments were carried out with crude extracts of virus-infected tobacco plants ground 1/10 (w/v) in phosphate-buffered saline (PBS) (0.15 M NaCl, 0.015 M sodium phosphate, pH 7.4), clarified for 30 s in a microfuge and finally diluted to 1/50 in sodium carbonate buffer, pH 9.6. Color reactions were developed using *p*-nitrophenyl phosphate as a substrate and absorbances were recorded at 405 nm. ELISA absorbances were regarded as positive if they were three times higher than those of the healthy plants.

Cloning and sequencing of the CP genes

Standard molecular procedures were as described (Sambrook et al., 1989). A single strand complementary DNA (cDNA) to the CP gene of the isolates PVY 0 AT, PVY 0 NAT, PVY 1 and PVY 1-2 was synthesized by reverse transcription of viral RNA preparations using the CP1 oligonucleotide downstream primer (5′ ATATCGGATCCGGAGAGACAC 3′), and the avian myeloblastosis virus reverse transcriptase (Promega). cDNA was subsequently amplified by polymerase chain reaction (PCR) using the CP1 and the CP2 upstream primer (5′ CTAAGAAGCTTCACTGAAATG 3′). Both primers were selected from highly conserved regions among different PVY isolates. The CP1 primer is located 26 nucleotides downstream from the 3′ end of the CP gene within the 3′ untranslated region (UTR), and the CP2 primer lies 75 nucleotides upstream from the 5′ end of the CP gene of PVY N (Robaglia et al., 1989). A *Hind* III and *Bam* HI recognition sequences (underlined)

were created in both primers, respectively, to facilitate further cDNA cloning. PCRs were carried out with the Ampli-Taq DNA polymerase (Perkin Elmer) using the following amplification procedure: initial denaturation at 94 °C for 5 min, followed by 25 cycles of 30 s at 94 °C, 1 min at 58 °C, 1 min 30 s at 72 °C and final 5 min elongation at 72 °C.

After digestion with *Bam* HI and *Hind* III, cDNA fragments were ligated to *Bam* HI-*Hind* III-digested pBluescript SK II (−) (Stratagene) using T4 DNA ligase (Promega). Recombinant plasmids were used to transform competent *Escherichia coli* DH5α. Positive clones were also tested by restriction enzyme analysis of small-scale preparations of plasmid DNA. At least, two independent clones were obtained for each isolate.

Nucleotide sequence analysis of the CP genes was conducted with purified plasmids as templates by mean of the ABI PRISM sequencer (Applied Biosystems). Each nucleotide determination was done on cDNA clones in both directions and directly on cDNA obtained by RT-PCR from viral RNA.

Sequence comparison and computer analysis

The nucleotide sequence and the predicted amino acid sequences of the CP of the isolates PVY 0 AT, PVY 0 NAT, PVY 1 and PVY 1-2 were compared and aligned with available sequences in the GenBank database. Multiple sequence alignments and phenetic relationships were performed using CLUSTAL W (Thompson et al., 1994), version 1.6 and the University of Wisconsin GCG program package. The CP N-terminus was identified by amino acid sequence similarity with the cleavage site established for other PVY strains and potyviruses (Riechmann et al., 1992).

The CP sequences of the following PVY strains were included in the comparisons: PVY Ch, MsNr, NsNr (Sudarsono et al., 1993), LB (Revers et al., unpubl. GeneBank accession no. X92078), N (Robaglia et al., 1989), NZ (Hay et al., 1989), 0 (Griffin et al., unpubl. GeneBank accession no. M91435), 0 Am (Bravo-Almonacid and Mentaberry, 1989), D, 18 (Shukla et al., 1988b), NT (Hidaka et al., 1992).

Results

Electrophoretic analysis of the PVY CP

Virion purification yields were similar for the four isolates PVY 0 AT, PVY 0 NAT, PVY 1 and PVY 1-2. SDS-PAGE analysis of dissociated-virus preparations showed an unique major band with an apparent molecular weight of about 35 kDa corresponding to the viral CP (Figure 1). Electrophoretic mobility for the rest of the PVY pepper isolates tested in this study were also similar as judged by Western blot analysis (not showed).

Serological relationships of the PVY pepper pathotypes

Different PVY isolates belonging to pepper pathotypes 0, 1 and 1–2 were tested for their serological relationships by indirect-ELISA. They all reacted with MAbs 3E9, 12C4, 10E3 and also with PAb to PVY (Table 1). None of the isolates reacted with MAbs C9 and PVY N, nor with MAb 1E10 (Table 1).

CP sequences of the PVY pepper pathotypes

The CP-coding region of PVY 0 AT, PVY 0 NAT, PVY 1 and PVY 1-2 were amplified by RT-PCR and cloned. An unique cDNA fragment of 902 bp, including the complete CP gene, flanking regions of the 3′ NIb, and the beginning of the 3′ UTR was obtained.

As expected, nucleotide and deduced amino acid sequence analysis of the entire CP of all these pathotypes revealed an uninterrupted reading frame with no

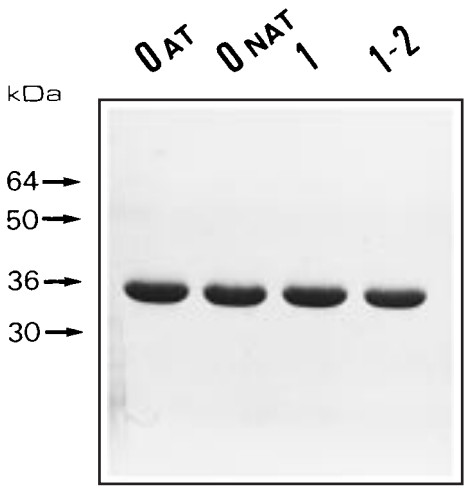

Figure 1. Sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE 12%) of virus preparations obtained from PVY 0 AT, PVY 0 NAT, PVY 1 and PVY 1-2 infected tobacco plants. Molecular weight markers are indicated on the left. Gel stained with Coomasie blue.

methionine initiation codon for the CP gene (Figure 2). Three putative cleavage sites by the NIa between the NIb and CP genes (Vance et al., 1992) were found at position 1 (Q/A), 20 (Q/G) and 88 (Q/S) on the polyprotein sequence (Figure 3). We assumed that the Q/A dipeptide sequence present at position 1 is the proteolytic cleavage site at the N-terminus of the CP. This site contains a Val residue at position −4 which is conserved among several potyvirus CP cleavage sites. Therefore, the first amino acid residue of the CP of pepper pathotypes is Ala, as reported for most PVY strains (Van der Vlugt et al., 1993). Immediately following the first Q/A putative cleavage site, the DAG motif, required for aphid transmission (Pirone, 1991), can be found as well (Figure 3). Therefore, the CP genes of all these pepper pathotypes are 801 nucleotides long, encoding a 267 residues protein with a calculated Mr of 29.8 kDa. This value is significantly lower that the apparent Mr of 35 kDa, as estimated by SDS-PAGE of purified preparations of each PVY pepper pathotype (Figure 1).

Nucleotide and amino acid comparison

A multiple sequence alignment was conducted among the CP nucleotide and deduced amino acid sequences of PVY 0 AT, PVY 0 NAT, PVY 1, PVY 1-2 (Figures 2 and 3). As expected, no nucleotide differences were seen between the CP of the isolates PVY 0 AT and PVY 0 NAT, since they are variants of the same isolate with different aphid transmission properties. Twelve nucleotide replacements were found in PVY 1 with respect to the consensus sequence (Figure 2), of which ten were silent mutations and two involved amino acid changes: $A_{32}G$ (where the first nucleotide is the one from the consensus sequence at the position indicated and the second one the variant) led to an Asn (consensus sequence) to Ser (PVY 1) change at amino acid position 11 ($N_{11}S$), and $A_{89}G$ changed a Lys to Arg at position 30 ($K_{30}R$) (Figure 3). Thirteen nucleotide substitutions were consistently found in PVY 1-2 (Figure 2) of which nine were silent mutations and four induced amino acid changes: $A_{32}G$ led to an Asn to Ser at amino acid position 11 ($N_{11}S$), $A_{173}G$ led to a Lys to Arg at position 58 ($K_{58}R$) and $G_{295}A$-$T_{296}C$ at the same codon led to a Val to Thr at position 99 ($V_{99}T$) (Figure 3). The $N_{11}S$ change in both PVY 1 and PVY 1-2 and the $K_{30}R$ in PVY 1-2 were located in the N-terminal half of the protein which is known to be the most variable region of the potyvirus CP (Shukla et al., 1988a). It should be noted that the substitutions closer to the N-terminus ($N_{11}S$, $K_{30}R$ and $K_{58}R$) did not lead to charge and/or hydrophobicity changes at these positions. The $V_{99}T$ replacement, however, involved a hydrophobicity change.

Within each isolate, a few nucleotide heterogeneities (two for PVY 0 AT, six for PVY 0 NAT, two for PVY 1, one for PVY 1-2) between independent cDNA clones were also detected with each appearing in only one of the sequenced clones (Figures 2 and 3). Some of these replacements led to amino acid exchanges: One for PVY 0 AT ($C_{404}T : Met_{135}Thr$), four for PVY 0 NAT ($T_{217}C : Tyr_{73}His$, $G_{348}A : Met_{116}Ile$, $A_{385}G : Asn_{129}Asp$, $T_{422}C : Val_{141}Ala$) and two for PVY 1 ($A_{104}T : Asn_{35}Ile$, $G_{715}A : Val_{239}Ile$) (Figures 2 and 3). However, neither of these nucleotide changes were found when CP genes were sequenced directly from PCR amplification products.

The comparison of the CP sequences of these PVY pepper pathotypes with those of PVY strains from potato and tobacco shows a higher degree of amino acid sequence similarity among the isolates belonging to pathotypes 0, 1 and 1-2, while the CPs of non-pepper PVY strains appear more distantly related (Figure 3, Table 2). Within PVY pepper pathotypes, the highest sequence similarity was found between PVY 0 AT and PVY 1 (99.2%), while the lowest occurred between these two and PVY 1-2 (98.1%) (Table 2). As expected, the similarities found between the pepper pathotypes and other published PVY sequences were greater than 90%, while CP similarities between PVY and other potyviruses were 50–75% (not shown). Even considering the non-consistent amino acid changes found between independent clones for each PVY pepper isolate, all pepper pathotypes showed CP sequence similarities ranging from 97.2% to 99.2% (not shown). Phenetic analysis based on the complete CP amino acid sequences of these PVY pepper pathotypes and other PVY isolates showed that PVY pepper pathotypes form a sub-cluster clearly separate from the non-pepper PVY strains (Figure 4).

Discussion

Previous studies have showed that no serological differences could be established between PVY pepper pathotypes 0 and 1 with four of the six MAbs used in our study (Soto et al., 1994). We have now confirmed these results and have extended them to the PVY 1-2 pathotype. Therefore, it appears that a serological distinction between the three PVY pepper pathotypes

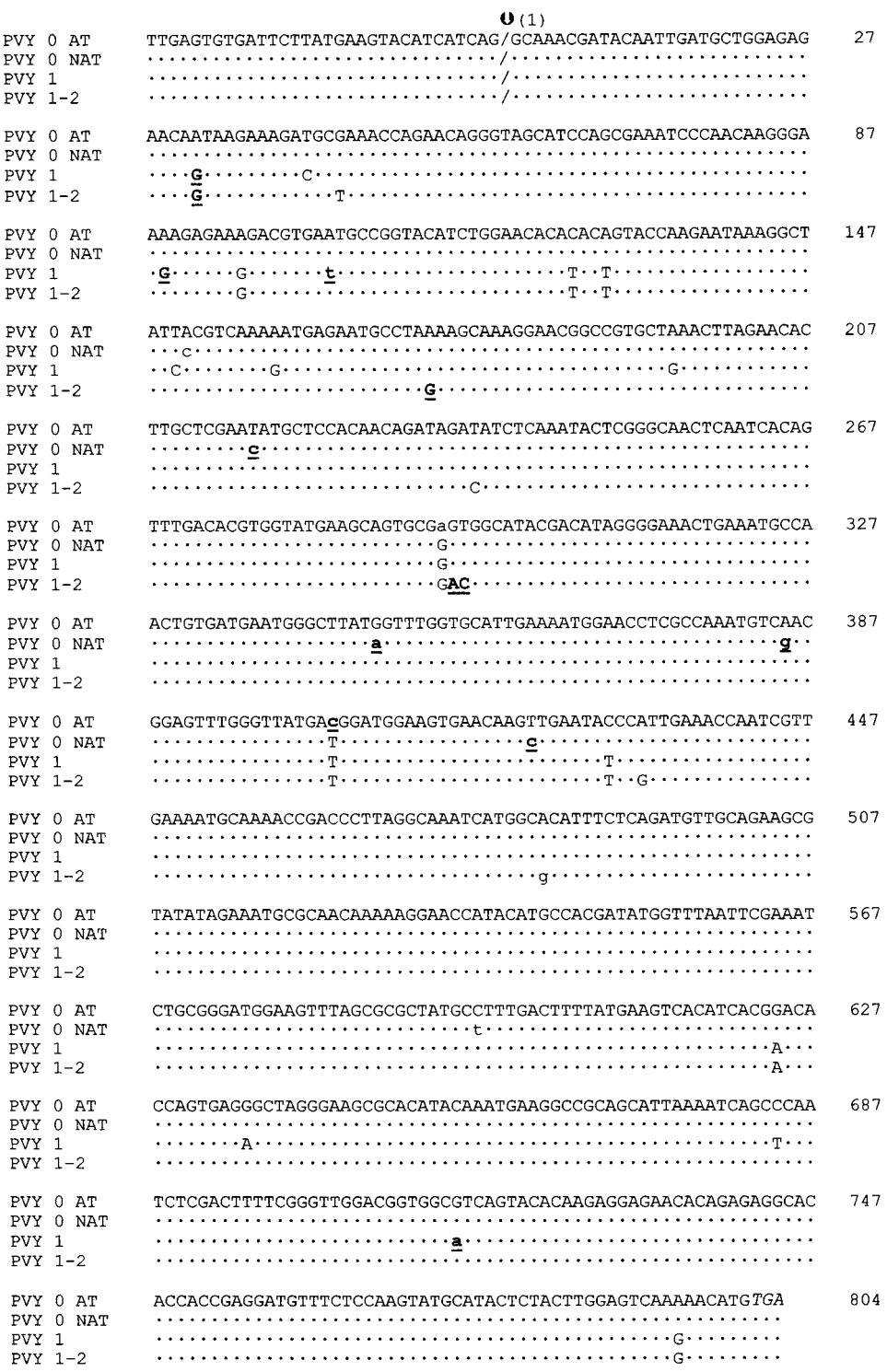

Figure 2. Alignment of the nucleotide sequences of the CP genes of pepper pathotypes PVY 0 AT, PVY 0 NAT, PVY 1 and PVY 1-2. The putative 5′ end of the CP gene is indicated by a vertical arrow. Base differences are indicated below the PVY 0 AT sequence. "." indicates that the same base is present. Nucleotide changes present in a single clone are showed in small lettering. Nucleotide changes representing amino acid substitution are underlined. Numbers to the right of the sequences show the position from the N-terminus of the CP.

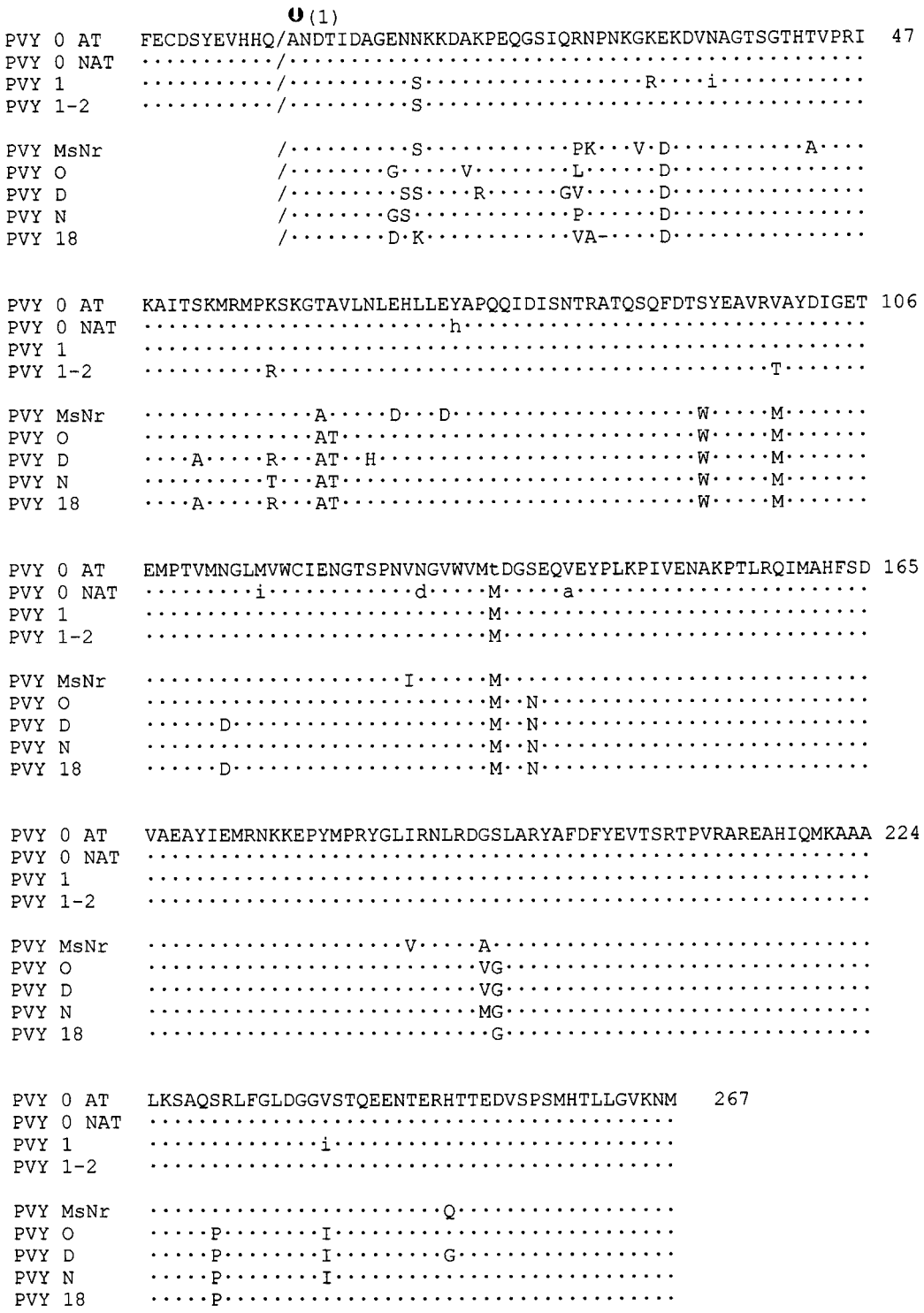

Figure 3. Alignment of the predicted amino acid sequences of the CP genes of different PVY isolates. Amino acid differences are indicated below the PVY 0 AT sequence. "." indicates the same amino acid present. Non-consistent amino acid changes deduced from a single clone are showed in small lettering.

Table 2. Percentage sequence similarity between the CP sequences of different PVY isolates calculated using Distances (GCG) on sequences aligned using Pile Up (GCG package, Wisconsin University)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 PVY 0 AT	—	99.2	98.1	94.7	94.7	95.1	92.8	93.2	91.7	92.5	94.7	92.8	93.2	93.9
2 PVY 1		—	98.1	94.5	94.5	94.1	91.6	92.9	90.8	92.5	93.7	92	92.5	93.3
3 PVY 1-2			—	93.6	93.3	93.3	92.1	92.5	90.6	92.8	93.3	91.7	92.1	93.9
4 PVY MsMr				—	95.5	92.8	91.0	92.8	91.3	91.3	92.8	92.1	92.5	92.1
5 PVY NsNr					—	92.8	91.3	92.8	92.1	91.7	92.8	92.8	93.2	92.4
6 PVY 0						—	94.3	95.5	92.5	95.5	98.1	92.8	92.8	95.4
7 PVY 0 Am							—	94.3	91.3	91.7	94.3	91.7	92.5	93.2
8 PVY Ch								—	93.2	93.6	96.6	93.6	93.6	92.8
9 PVY Lb									—	90.2	93.2	98.1	97.0	90.9
10 PVY D										—	95.8	90.6	90.6	95.4
11 PVY N											—	93.6	93.6	95.4
12 PVY NT												—	98.1	90.9
13 PVY NZ													—	91.3
14 PVY 18														—

and thus correlation between their antigenic behavior and their differential pathogenicity is not yet possible. So far, serology seems to be a misleading approach for tracing relationships not only within PVY strains from potato but within PVY pepper pathotypes as well. Indeed, Blanco-Urgoiti et al. (1998b) were unable to establish an absolute differentiation between PVY isolates from potato, based on their biological, serological and molecular properties. However, our data confirm the fact that PVY pepper pathotypes react negatively with MAbs C9, PVY N and 1E10, whereas typical non-pepper PVY strains react positively (Sanz et al., 1990; Gugerli and Fries, 1983) and therefore, although the use of these MAbs did not distinguish between PVY pepper pathotypes, but they allowed us to discriminate pepper strains from non-pepper strains and they are thus useful for diagnosis of PVY from pepper.

As a complement to the conventional methods based on host range, symptomatology and serological relationships, CP sequence similarities appear to be an increasingly useful criteria for sorting out the taxonomy of the potyvirus group (Shukla and Ward, 1988). We determined the CP gene sequence of the different PVY pepper pathotypes and found that their 267 amino acid CPs showed only minor changes, explaining their identical antigenic behavior. Only one of the amino acid changes found in PVY 1 and PVY 1-2 (both at position 11) occurred in the surface amino terminal domain, which is the most immunodominant region of the entire CP (Shukla et al., 1988a), while the remaining three (PVY 1, at position 30; PVY 1-2, at positions 58 and 99) occurred in the trypsin resistant core domain of the protein. This similarity of CP sequence agrees with the results from Blanco-Urgoiti et al. (1998a) who have recently showed that PVY isolates from pepper pathotypes 0 and 1 fall into the same genetic strains by using genetic distances estimated from CP restrictotypes. This indicates that the concept of pathotype, which refers to a host resistance response is different from the genetic strain concept, which refers to a genetic characteristic of at least a particular region of the virus (Blanco-Urgoiti et al., 1998a).

On the other hand, multiple sequence alignments of the CP of PVY pepper pathotypes and other PVY strains led us to group the pepper pathotypes separately from the non-pepper strains. However, no discrimination within these pathotypes on the basis of their CP amino acid sequences was possible, and therefore, biological properties seem to be the only reliable criteria for classification of PVY pepper pathotypes.

The non-consistent nucleotide changes found among the independent clones obtained for each particular isolate may be either true heterogeneities in the sequence or errors caused by the Taq-DNA polymerase. Interestingly, PVY 0 NAT, which was maintained in the laboratory through successive mechanical passages and lost the ability to be aphid-transmitted, had the greatest number of heterogeneities. On the other hand, PVY 0 AT was aphid-transmitted and had the lowest number of heterogeneities. This suggests a bottleneck-like process created by vector transmission for which a few sequence variations would be allowed in the viral population and consequently reduction of polymorphism would occur. In fact, it is assumed that the quasispecies

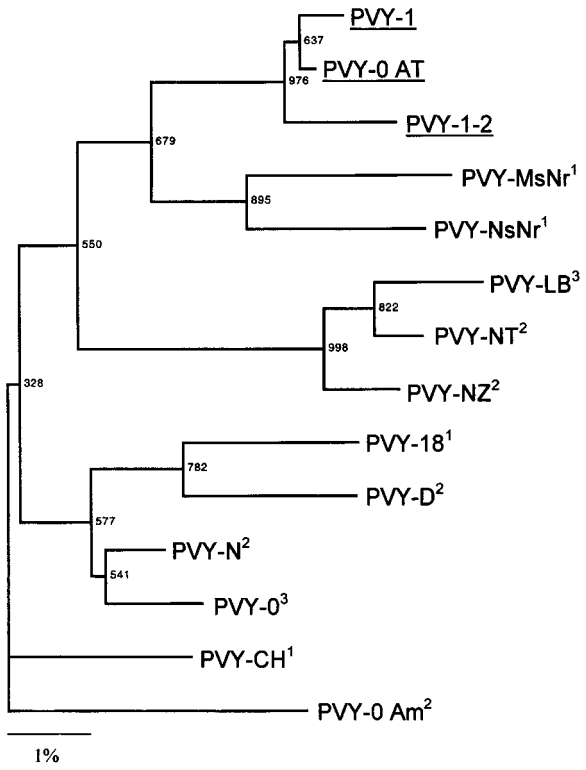

Figure 4. Cluster dendrograms showing the relationships among PVY pepper pathotypes (underlined) and other PVY strains as deduced from comparisons of the predicted viral CP sequences. Phenetic analysis was done with the CLUSTAL W Vers. 1.6 with bootstrapping (1000 replicates) using the neighbor joining option in CLUSTAL W. All horizontal distances are proportional to sequence differences indicated by inset scale, but vertical distances are arbitrary. Number adjacent to nodes are bootstrap scores (out of 1000 replicates). [1]Tobacco isolates. [2]Potato isolates. [3]Data not available.

of non-circulative plant viruses are frequently forced through bottlenecks as narrow as a few infectious genomes during vector transmission (Pirone and Blanc, 1996). A similar phenomenon, in this case created by host resistance to infection might occur in the case of PVY 1 and PVY 1-2, which are the only pathotypes able to overcome the *pvr2*[1] and *pvr2*[1]-*pvr2*[2] resistance genes in pepper. From the above, it can be inferred that PVY 0 NAT, which bore the highest number of nucleotides heterogeneities, would not be under such selection, and therefore it could stochastically incorporate non-deleterious mutations, owing to the high rate of mutation during RNA genome replication (Drake, 1993) and resulting in a higher range of genome variation. The fact that such mutations had been incorporated into the PVY 0 NAT genome and not into the PVY 0 AT would implicate a negative effect of such mutations on aphid transmission.

It is well known that one or a few amino acid substitutions in the CP of certain viruses is sufficient to affect macromolecular interactions between the virus and the host, leading to a dramatic impact on the biological properties of the virus (McKern et al., 1994). For example, the CP has been implicated in the elicitation of the *Capsicum* spp. L^2 and L^3 genes-mediated resistance against pepper tobamoviruses (Berzal-Herranz et al., 1995; De la Cruz et al., 1997; Gilardi et al., 1998). In potyviruses, several amino acids in the surface-exposed N-terminal region of the CP have been involved in host range and virulence behavior of sugarcane mosaic virus (SCMV) and Johnsongrass mosaic virus (JGMV) (Xiao et al., 1993; Suranto et al., 1998). Valkonen et al. (1995) have showed that the CP of potato virus A isolates may play an important role in the elicitation of a hypersensitive resistance response in potato plants. On the other hand, the resistance of pepper plants carrying the *pvr2*[1] gene to PVY 0 infection has been showed to be due to the impaired intercellular movement of the virus (Arroyo et al., 1996), in which the potyvirus CP seems to be directly involved (Dolja et al., 1994, 1995). In our case, any of the amino acid differences observed between the CP sequences of the three pepper pathotypes could be somehow involved in the ability of PVY 1 and PVY 1-2 to break the resistance conferred by the *pvr2*[1] and *pvr2*[2] genes. However, since only minor changes were found among these pathotypes, we cannot rule out that another unidentified gene might be involved in the ability to overcome these pepper resistance genes.

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