Host Range and Sequence Analysis of an Isolate of Potato Virus Y Inducing Veinal Necrosis in Pepper

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ABSTRACT

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An isolate of potato virus Y (PVY), obtained from pepper (*Capsicum annuum* L.) showing veinal necrosis and named PVY-nnp, was compared with other PVY isolates for host range. In addition, the 3′ noncoding region (3′-NCR) and coat protein gene (CP-gene) of PVY-nnp were cloned, sequenced, and aligned with the same regions of other PVY isolates. Biological characterization showed that PVY-nnp differs from all other PVY strains including isolates commonly infecting pepper. The nucleotide sequence comparison of PVY-nnp with other PVY isolates exhibited a homology ranging from 85.5 to 99.7% for the 3′-NCR, and from 87.5 to 94% for the CP-gene. The deduced amino acid sequence of the CP had a similarity ranging from 94.7 to 97.3%. These results indicate that PVY-nnp is a PVY isolate in spite of its peculiar biological properties.

Additional keywords: PVY coat protein sequence, PVY 3′ noncoding region sequence, PVY taxonomy

Potato virus Y (PVY) is the type member of the potyvirus group, which is the largest viral group and contains many economically important viruses (17). The viral RNA is encapsidated by about 2,000 subunits of coat protein (CP) with an M_r of 33,000–34,000 to form a flexuous particle. The genome consists of one single-stranded, positive-sense RNA molecule of approximately 9.7 Kb, with a 3′ poly(A) tail and a 5′ covalently linked protein. The genome codes for a polyprotein processed by several viral proteases (7,23,28). Sequence data for the coat protein gene (CP-gene) and for the 3′ noncoding region (3′-NCR) of a large number of potyviruses and their strains are now available. PVY occurs worldwide and is responsible for diseases in potato, tobacco, tomato, and pepper. Many different strains of the virus have been isolated that cause symptoms ranging from mild mosaic to severe necrosis (31).

Strains of PVY are separated into tobacco veinal necrosis (PVY^N), common (PVY^O), and potato stipple streak (PVY^C) groups according to the symptoms induced on indicator plants and aphid transmissibility (4). Gooding and Tolin (11) proposed an alternative scheme in which three distinct PVY strains (PVY M^SM^r, PVY M^SN^r, and PVY N^SN^r) are identified on the basis of host-specific reactions induced in flue-cured tobacco cultivars resistant or susceptible to the root knot nematode. However, it seems unlikely that these strain groups are similar to the classical PVY^N, PVY^O and PVY^C strains (33). PVY isolates infecting pepper are less studied and are classified as PVY-0, PVY-1, and PVY-1-2 in accordance with their ability to overcome resistance genes. Within these groups, isolates are further defined as "common" or "necrotic" types (19). Common isolates induce vein-banding symptoms in pepper, while necrotic ones also cause veinal necrosis (22). Necrotic isolates are less widespread but induce more severe symptoms and cause greater yield losses than the common isolates. Biological and serological comparisons of PVY from pepper and PVY from potato or tobacco suggest that PVY from pepper belongs to a different potyviral species (21,32).

The taxonomy of potyviruses based on biological and serological properties is unsatisfactory (8,17) and several authors (1,14,17) hypothesized that within the potyvirus group there is a continuum of variants or strains that cannot be distinguished into strains and species. Recently, nucleotide sequence of the genome and amino acid sequence of the CP seem to provide suitable information for identifying strains and species in this group (28–30,38,39). In this study, we compared a necrotic PVY isolate from *Capsicum annuum* L., designated PVY-nnp, with other PVY isolates, mainly from tobacco and potato, using biological properties, 3′-NCR and CP-gene nucleotide sequences, and CP amino acid sequence. The results indicate that PVY-nnp has peculiar biological properties, but clearly is a strain of PVY.

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MATERIALS AND METHODS

Virus isolates. PVY-nnp was isolated in southern Italy from pepper showing veinal necrosis of leaves. The isolate was grown in *Nicotiana tabacum* L. Burley 64 and *C. annuum* Bahamian Hot Chile. PVY-mp, a common pepper isolate, was from the same geographic area as PVY-nnp. The potato isolates PVY^N CH 605 (Gugerli) 603, PVY^N Gineke 605, PVY^O Paul Kruger 706, and PVY^C Zeeuwse Blauwe 509 were supplied by the Instituut voor Planteziektenkundig Onderzoeg (I.P.O.) collection, Wageningen, The Netherlands. All isolates were maintained in Burley 64.

Hosts. Plants used in this study included standard virus indicators, some weeds commonly occurring in horticultural crops of southern Italy, commercial pepper lines ,and other *Capsicum* species and varieties. *Solanum tuberosum* L. Duke of York was supplied by R. Wustman, Research Station for Arable Farming and Field Production of Vegetables, Lelystad AK 8200, The Netherlands. Bahamian Hot Chile, *C. frutescens* L. Agronomico 8 and Tabasco, and *C. baccatum* L. var. *pendulum* were supplied by K. E. Conway, Oklahoma State University (Stillwater). *Capsicum annuum* Anaheim F6 was supplied by G. Marchoux, Station de Pathologie Vegetale, 84143 Monfavet, France. *Capsicum baccatum* var. Anaheim was supplied by J. L. Sherwood, Oklahoma State University (Stillwater). Commercial pepper included Drago, F1 P374, F1 P894, F1 P5210, F1 P9011, Rino F1, Stratos, Zarco, Heldor, Predi F1, Sidor, Sonar, Jolly Rosso, Pacific, Duplo Hy, Friariello di Napoli, and Antares F1. The other indicator plants were from local sources.

Biological assays. Infected tobacco-leaf tissues were ground in 0.01 M sodium citrate buffer pH 7.4 and the inoculum was applied to leaves of healthy plants previously dusted with carborundum (600 mesh). The presence of the virus in symptomless plants was checked by back inoculation, using sap from both inoculated and apical leaves, on *Datura metel* L., a host susceptible to all known isolates of PVY.

Virus and viral RNA purification.

PVY-nnp was purified and the genomic RNA extracted as described by Thole et al (34). The viral RNA was further fractionated by linear-log sucrose gradient (2) and the fractions analyzed by electrophoresis in 1% agarose gels (26). RNA fractions were precipitated with 2.5 volumes of cold ethanol, 1/20 volume of 4 M sodium acetate.

cDNA cloning. RNA fractions containing full-length genomic RNA of PVY-nnp were selected to prepare the cDNA. Oligo (dT) was used to initiate first-strand cDNA synthesis and the second strand was synthesized in the presence of ribonuclease H and DNA polymerase I, as described by Gubler and Hoffman (13), using a cDNA synthesis kit (Amersham, Buckinghamshire, UK). The double-stranded cDNA was blunt-ended and ligated into a SmaI-digested, dephosphorylated pUC-18 plasmid. Recombinant plasmids were transformed into *Escherichia coli* DH5α competent cells.

cDNA sequencing and computer analysis. The nucleotide sequences of cDNA inserts were established by the dideoxynucleotide chain-termination method (27) utilizing Sequenase Version 2.0 DNA Sequencing Kit (US Biochemical Co., Cleveland, OH). The nucleotide and deduced amino acid sequences were compared with those of other PVY isolates available in GenBank, pepper mottle virus (PepMoV), and tobacco vein mottling virus (TVMV), using the GCG analysis software package Version 7.1 (5).

RESULTS

Host reactions. Reactions of indicator plants to inoculation with PVY-nnp are reported in Table 1. Mosaic and vein banding induced by PVY-nnp on *N. glutinosa* and *D. metel are* typical symptoms for PVY infection. The absence of infection in *D. stramonium* L. is a common feature for all PVY strains, while vein-banding and mottling symptoms in *N. tabacum* are similar to those described for PVY^O and PVY^C infections. PVY-nnp failed to infect Duke of York and *Physalis floridiana* Rybd., which are indicator plants commonly used for identifying PVY strains. In addition, PVY-nnp never induced local lesions in *Chenopodium amaranticolor* Coste & Reyn. and *C. quinoa* Willd. The lack of reaction in these plants was also observed for the PVY^N and PVY^C isolates, while the PVY^O isolate usually induced local lesions in *Chenopodium* (data not shown). The species, varieties, and lines of *Capsicum* reacted differently to the PVY isolates (Table 2). Depending on the season in which they were inoculated, Rino F1 and Pacific showed only mosaic or veinal necrosis, while in Anaheim F6 and *C. baccatum* var. Anaheim both symptoms were observed. Bahamian Hot Chile, P 5210, and Predi F1 showed only veinal necrosis symptoms (Fig. 1). Duplo Hy, F1 P894 F1 P9011, Stratos, and Zarco were not infected by any of the tested isolates. PVY-mp infected only Friariello, Heldor, Rino F1, Sidor, Sonar, and Anaheim, but it never induced necrotic symptoms. All potato PVY isolates failed to infect the tested *Capsicum* plants, with the exceptions of PVY^O Paul Kruger 706, which induced vein-banding symptoms on Friariello, and PVY^C Zeeuwse Blauwe 509, which caused latent infection only in the inoculated leaves of Anaheim.

Table 1. Reaction of indicator plants to potato virus Y isolate PVY-nnp inoculation[a]

Indicator plants	Reaction
Nicotiana tabacum L. 'White Burley'	M, VB, Mo
N. tabacum L. 'Burley 64'	M, VB, Mo
N. tabacum L. 'Samsun NN'	M, VB, Mo
N. tabacum L. 'Xanthi nc'	M, VB, Mo
N. glutinosa L.	Nb, M
N. silvestris Speg. & Comes	M
N. occidentalis Wheeler	M
N. rustica L.	–
N. bigelovii S. Wats.	Cns
N. debneyi Domin	Cns
N. glauca R. Grah.	–
Datura stramonium L.	–
D. metel L.	M, VB
Lycopersicon esculentum Mill. 'Rutgers'	Cns, M
L. esculentum Mill 'Supermarmande'	–
Solanum tuberosum L. 'Duke of York'	–
S. marginatum L.	–
Chenopodium amaranticolor Coste & Reyn.	–
C. quinoa Willd.	–
C. album L.	–
C. foetidum Lam.	–
C. murale L.	–
Physalis floridiana Rybd.	–
Ph. peruviana L.	–
Ph. oxycarpa Brot.	M
Sonchus oleraceus L.	–
Cucurbita pepo L.	–
Cucumis sativus L.	–
Gomphrena globosa L.	–

[a] M: mosaic; VB: vein banding; Mo: mottling; Nb: necrosis of inoculated leaves; –: not infected; Cns: systemic veinal chlorosis.

Table 2. Reaction of *Capsicum* species, varieties, and lines to the inoculation of the potato virus Y (PVY) isolates[a]

	PVY-nnp	PVY-mp	PVY^N CH 605	PVY^N Gineke	PVY^O PK706	PVY^C ZB509
Capsicum annuum L.						
Anaheim F 6	N, M, VB	–	–	–	–	–
Antares F1	–	–	–	–	–	–
Bahamian Hot Chile	N	–	–	–	–	–
Drago	–	–	–	–	–	–
Duplo Hy	–	–	–	–	–	–
F1 P374	M, VB	–	–	–	–	–
F1 P894	–	–	–	–	–	–
F1 P5210	N	–	–	–	–	–
F1 P9011	–	–	–	–	–	–
Friariello di Napoli	M, VB	M, VB	–	–	VB	–
Heldor	–	M, VB	–	–	–	–
Jolly Rosso	M	–	–	–	–	–
Pacific	N, M	–	–	–	–	–
Predi F1	N	–	–	–	–	–
Rino F1	N, VB, M	M, VB	–	–	–	–
Sidor	M, VB	M, VB	–	–	–	–
Sonar	M, VB	M, VB	–	–	–	–
Stratos	–	–	–	–	–	–
Zarco	–	–	–	–	–	–
C. frutescens L.						
Agronomico 8	–	–	–	–	–	–
Tabasco	–	–	–	–	–	–
C. baccatum L.						
var. Anaheim	N, M	M	–	–	–	lb
var. *pendulum*	–	–	–	–	–	–

[a] N: veinal necrosis; M: mosaic; VB: vein banding; –: not infected; lb: latent infection in the basal leaves.

Sequence analysis. Four recombinant clones, nnp 27/28-5, 158, 27/29-7, and 62, containing 432, 981, 1,015, and 1,716 nucleotides, excluding the poly(A) tail, respectively, were used to determine the nucleotide sequence of the 3′-NCR and the CP-gene, and to deduce the amino acid sequence of the CP of PVY-nnp (Fig. 2). The genomic RNA of PVY-nnp has a 332 nucleotide long 3′-NCR and a poly(A) tail at the 3′ terminus. The CP cistron is 801 nucleotides encoding a protein of 267 amino acids, beginning with a GCA (alanine) and ending with TGA stop codon. The nucleotide homology percentages of PVY-nnp 3′-NCR and CP-gene and the amino acid similarity and identity percentages of CP are compared with the other available sequences of PVY and with those of PepMoV and TVMV (Table 3). PVY-nnp 3′-NCR shares homology percentages from 85.5 to 99.7% (average 91.8%) with the available PVY 3′-NCR sequences, and 52.1 and 41.3% with the sequences of PepMoV and TVMV, respectively. The PVY-nnp CP-gene shows homology percentages from 87.5 to 94% (average 90.2%) with the other PVY sequences, corresponding, for the deduced amino acid sequence, to similarity percentages from 94.7 to 97.3% (average 95.6%) and identity percentages from 92.1 to 94.3% (average 92.9%). The PVY CP-gene homology percentages are 68.3% with PepMoV and 57.1% with TVMV, which correspond, for the CP, to similarity and identity percentages of 81.2 and 72.6% for PepMoV and 69.4 and 53.9% for TVMV.

Fig. 1. Bahamian Hot Chile pepper infected with potato virus Y isolate PVY-nnp, showing veinal necrosis on apical leaves.

DISCUSSION

Biological tests showed that PVY-nnp does not belong to any of the PVY^N, PVY^O, or PVY^C groups because it did not cause veinal necrosis in the tested *Nicotiana* species and it failed to infect Duke of York and *P. floridiana* (4,12). Reactions of *Capsicum* species and varieties were helpful for the differentiation of the PVY isolates. PVY-nnp induced severe symptoms on many pepper lines and varieties, while the "normal" pepper isolate, PVY-mp, infected fewer lines and varieties without inducing necrotic symptoms. Traditional potato isolates used in this study, with some exceptions, were unable to infect pepper, at least when plants were mechanically inoculated, as also reported by other authors (10,20). Furthermore, no local lesion hosts for PVY-nnp were found among 52 plant species, varieties, and lines of different genera and families. Since potato PVY isolates appear to be unable to infect pepper, and pepper PVY isolates seem to be nonpathogenic on *S. tuberosum* (10), the role of potato as source of PVY for pepper crops should be reconsidered. More relevant sources could be weeds and other crops such as tobacco or tomato. Moreover, since necrotic symptoms on pepper sometimes disappear or are substituted for by mosaic symptoms (22), it is possible that necrotic pepper PVY isolates occur frequently in crops but may be undetected, or confused with common isolates. The results of biological assays support the previously reported hypothesis that pepper PVY isolates are distinct from other PVY isolates (21,32).

PVY strains are reported to be closely related serologically (4,11). In our study, immunoblot analysis showed that an antiserum to a necrotic pepper isolate reacted with the CPs of all tested isolates, indicating that PVY-nnp is related to the different PVY strains used (data not shown). However, McDonald and Kristjansson (21) recently reported that a pepper PVY isolate did not react with antibodies to PVY^N and PVY^O.

In the potyvirus group, the similarity percentage of CP amino acid sequence ranges from 38 to 71% among different species, and from 90 to 99% among strains of the same species. Variations are mainly located in the N-terminal region, whose length and sequence vary considerably among different members, but not among strains (29). The size of PVY-nnp CP is similar to that of the other PVY isolates and its sequence shared similarity and identity percentages (averages 95.6 and 92.9%, respectively) as high as those shown for strains of the same species. Frenkel et al (9) observed that the homology percentage of the 3′-NCR of potyviruses ranges from 39 to 53% among different members and from 83 to 99% among strains of a species. The 3′-NCR of PVY-nnp is as long as those of the other PVY isolates and showed an identity percentage (average 91.8%) similar to that of strains of the same species. In this paper, sequences of 3′-NCR, CP-gene and CP of a necrosis-inducing pepper strain of PVY are reported for the first time. Homology between these sequences and those of other PVY isolates, mainly obtained from potato and tobacco, indicates that PVY-nnp does not belong to a different viral species, as biological tests may suggest. In addition, the low homology percentage between PVY-nnp

Table 3. Comparison of the 3′ noncoding region (3′-NCR) and coat protein (CP) gene nucleotide sequences and CP-deduced amino acid sequence of potato virus Y (PVY) isolate PVY-nnp with those of other PVY isolates, PepMoV and TVMV[a]

Virus isolates (reference)	A	B	C	D
PVY^N-Japan (EMBL D01242, D12570)	86.1	88.6	95.1	92.1
PVY^N-The Netherlands (37)	85.8	88.5	95.1	92.1
PVY^N-New Zealand (15)	85.8	89.0	95.8	93.1
PVY-Germany (40)	85.5	87.5	95.5	92.1
PVY^N-France (24)	88.6	91.1	96.2	94.3
PVY^H-Hungary (34)	99.7	88.8	95.1	92.5
PVY^O-U.S.A. (EMBL M81435)	99.7	91.1	95.5	93.6
PVY^O-Japan (16)	99.0	91.1	95.8	93.2
PVY^O-Argentina (3)	97.2	90.1	94.7	92.5
PVY-Israel (25)	90.8	94.0	96.2	94.3
PVY-China (41)	...	90.8	95.1	92.5
PVY^N-Chile (33)	...	90.8	95.5	92.8
PVY^N-Hungary (33)	...	88.8	95.1	92.1
PVY M^SN^R-U.S.A. (33)	...	92.2	96.6	92.5
PVY N^SN^R-U.S.A. (33)	...	92.2	97.3	93.6
PVY-U.S.A. (33)	...	89.8	95.5	93.6
PVY^O-U.S.A. (18)	...	90.6	95.5	93.2
PepMoV (36)	52.1	68.3	81.2	72.6
TVMV (6)	41.3	57.1	69.4	53.9

[a]In A and B homology percentages of the 3′ NCR and CP-gene sequences are reported, respectively. In C and D similarity and identity percentages of CP sequence are reported, respectively.

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1     AGG GCA GTG GAT GAG GAG GAG CTA CGA ATC TTC ACT GAA ATG ATT GTT GCA CTG GAT GAT

61    GAA TTT GAG TGT GTT CCT TAT GAA GTA CAC CAC CAG GCA AAC GAC ACA ATT GAT GCT GGA
                                                    ala asn asp thr ile asp ala gly
                                                     *
121   GGG AGC AGT AAG AAA GAT GCG AAG CCA GAA CAG GAT AGC ATC CAA CCA AGT TCT AAC AAG
      gly ser ser lys lys asp ala lys pro glu gln asp ser ile gln pro ser ser asn lys

181   GGA AAG GAT AAG GAC GTG AAT GCT GGT ACA TCT GGG ACA CAT ACT GTA CCA AGA ATA AAG
      gly lys asp lys asp val asn ala gly thr ser gly thr his thr val pro arg ile lys

241   GCT ATA ACG TCA AAA ATG AGA ATG CCT AAA AGC AAA GGA GCA GCC GCG CTG AAC TTA GAA
      ala ile thr ser lys met arg met pro lys ser lys gly ala ala ala leu asn leu glu

301   CAC TTA CTC GAG TAT GCT CCA CAA CAG ATA GAC ATC TCA AAT ACT CGG GCA ACT CAA TCA
      his leu leu glu tyr ala pro gln gln ile asp ile ser asn thr arg ala thr gln ser

361   CAG TTT GAT ACG TGG TAT GAA GCA GTG CGG ATG GCA TAC GAC ATA GGG GAA ACA GAA ATG
      gln phe asp thr trp tyr glu ala val arg met ala tyr asp ile gly glu thr glu met

421   CCA ACT GTG ATG AAT GGG CTT ATG GTT TGG TGC ATT GAA AAT GGA ACC TCG CCA AAT GTC
      pro thr val met asn gly leu met val trp cys ile glu asn gly thr ser pro asn val

481   AAC GGA GTT TGG GTT ATG ATG GAT GGA AGT GAA CAA GTT GAA TAT CCG TTG AAA CCA ATC
      asn gly val trp val met met asp gly ser glu gln val glu tyr pro leu lys pro ile

541   GTT GAG AAT GCA AAA CCG ACC CTT AGG CAA ATC ATG GCA CAT TTC TCA GAT GTT GCA GAA
      val glu asn ala lys pro thr leu arg gln ile met ala his phe ser asp val ala glu

601   GCG TAT ATA GAA ATG CGC AAC AAA AAG GAA CCA TAC ATG CCA CGA TAT GGT TTA GTT CGA
      ala tyr ile glu met arg asn lys lys glu pro tyr met pro arg tyr gly leu val arg

661   AAC TTG CGG GAT GGA AGT TTA GCG CGC TAT GCC TTT GAC TTT TAT GAA GTT ACA TCA CGA
      asn leu arg asp gly ser leu ala arg tyr ala phe asp phe tyr glu val thr ser arg

721   ACA CCA GTG AGG GCC AGA GAA GCG CAA ATA CAG ATG AAG GCC GCA GAA TTA AAA TCA GCT
      thr pro val arg ala arg glu ala gln ile gln met lys ala ala glu leu lys ser ala

781   AAA CCT CGA CTT TTC GGG TTG GAT GGT GGC ATC AGT ACA CAA GAG GAG AAC ACA GAG AGG
      lys pro arg leu phe gly leu asp gly gly ile ser thr gln glu glu asn thr glu arg

841   CAC ACC ACC GAG GAT GTA TCT CCA AGT ATC CAT ACT CTA CTT GGA GTT AAG AAC ATG TGA
      his thr thr glu asp val ser pro ser ile his thr leu leu gly val lys asn met OPA

901   TTG TAG TGT CTC TCC GGA CGA TAT ATA AGT ATT TAC ATA TGC AGT AAG TAT TTT GGC TTT

961   TCC TGT ACT ACT TTT ATC ATA ATT AAT AAT CAG TTT GAA TAT TAC TAA TAG ATA GAG GTG

1021  GCA GGG TGA TTT CGT CAT TGT GGT GAC TCT ATC TGT TAA TTT CGC ATT ATT AAG TCT TAG

1081  ATA AAA GTG CCG GGT TGT CGT TGT TGT GGA TGA TTC ATC GAT TAG GTG ATG TTG CGA TTC

1141  TGT CGT AGC AGT GAC TAT GTC TGG ATC TAT CTG CTT GGG TGG TGT TGT GAT TTC GTC ATA

1201  ACA GTG ACT GTA AAC TTC AAT CAG GAG ACA(An)
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Fig. 2. Nucleotide sequence of the 3′ noncoding region and coat protein gene of potato virus Y isolate PVY-nnp, and amino acid sequence of the predicted coat protein (GenBank accession n. U10378). The asterisk marks the first amino acid of the sequence; "OPA" and "A_n" indicate the stop codon and the poly(A) tail, respectively.

and PepMoV 3′-NCRs indicates that these viruses belong to different species, although their CPs share a high level of similarity (81.2%). This result agrees with the observation of Vance et al (35).

In conclusion, we suggest that the observed differences in host range between pepper and potato PVY isolates indicate a specialization within the viral species. Pepper isolates may constitute a new strain group in which necrotic isolates (PVY-nnp type) and common isolates (PVY-mp type) are distinguishable from each other based on symptoms induced on pepper plants. This hypothesis agrees with the suggestion of Van der Vlugt et al (38) of a host adaptation between PVY and *Capsicum*.

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