BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF A RECOMBINANT ISOLATE OF *POTATO VIRUS Y* ASSOCIATED WITH A TOMATO NECROTIC DISEASE OCCURRING IN ITALY

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SUMMARY

The biological and molecular characterization of a *Potato virus Y* (PVY) isolate, denoted PVY^{C}-to, associated with a necrotic phenotype of tomato plants grown in the province of Foggia (Apulia, southern Italy) is reported. The fully sequenced PVY^{C}-to genome consists of 9,691 nucleotides and is 94,8% similar to that of PVY LYE84.2, an isolate of the PVY^{C} strain group. Using programs in RDP package, a putative recombination breakpoint was identified at approximately nucleotide position 2056 to 2632, corresponding to the HC-Pro/P3 coding region. The event seems to represent an introgression of a PVY^{O} strain group sequence and, in particular, of PVY-OZ, which is a non recombinant isolate of the O lineage. From this analysis PVY^{C}-to appeared to be a recombinant isolate. The virus has a very weak infectivity in pepper and possesses a CP coding region characterised by a PVY^{C2} restrictotype. Our results seem to support the hypothesis that PVY^{C}-to is a recombinant isolate of the PVY^{C2} strain group.

Key words: PVY, tomato, recombination, cloning, full genome sequence, phylogenetic analysis.

INTRODUCTION

Potato virus Y (PVY), the type member of the genus *Potyvirus*, family *Potyviridae*, is one of the most important pathogens of pepper, potato, tobacco and tomato crops, to which it is non persistently transmitted by many aphid species (De Bokx and Huttinga, 1981; Shukla *et al.*, 1994). Its host range includes also many solanaceous and non-solanaceous weeds (Kerlan, 2006).

Several distinct PVY strains have been recognised. Those from potato were historically classified as ordinary or common strains (PVY^{O}) and C strains (PVY^{C}), on the basis of hypersensitive reactions in potato cultivars bearing the Ny_{tbr} and the *Nc* resistance genes, respectively (Cockerham, 1970; De Bokx and Huttinga, 1981; Jones, 1990; Singh *et al.*, 2008). Other isolates were classified as necrotic strains (PVY^{N}) although they do not elicit hypersensitive resistance response in potato, but cause vein necrosis in tobacco. Changes in the severity of responses of field-grown potatoes have led to the identification of some new pathotypes of strains PVY^{N} and PVY^{O}, denoted PVY^{Z}, $PVY^{N\text{-}Wilga}$ and PVY^{NTN}. PVY^{Z} pathotypes are thought to be PVY^{O} strains able to overcome both Ny_{tbr} and *Nc* resistance genes (Jones, 1990; Singh *et al.*, 2008) while $PVY^{N\text{-}Wilga}$ patothypes are PVY^{N} strains serologically related to PVY^{O} but inducing less severe symptoms in potato than standard PVY^{N} strains, and necrotic vein banding in tobacco (Chrzanowska, 1991; Chachulska *et al.*, 1997; Blanco-Urgoiti *et al.*, 1998b). PVY^{NTN} pathotypes are the main causal agents of the so-called potato tuber necrotic ringspot disease (PTNRD). They were directly obtained from tubers exhibiting PTNRD and, apparently, originated from recombination between PVY^{O} and PVY^{N}. However, some non-recombinant isolates from North America (NA-PVY^{N} and NA-PVY^{NTN}) (Boonham *et al.*, 2002; Glais *et al.*, 2005; Lorenzen *et al.*, 2006; Schubert *et al.*, 2007; Singh *et al.*, 2008) and Japan (Ogawa *et al.*, 2008), caused PTNRD when inoculated to potatoes.

Pepper isolates of PVY have been classified as pathotypes 0; 1 and 1, 2; on the basis of their different ability to overcome recessive resistance *Pvr*2 alleles ($pvr2^{+}$, $pvr2^{1}$ $pvr2^{2}$) introgressed in pepper cultivars (Gebré Selassié *et al.*, 1985; Caranta *et al.*, 1997; Ruffel *et al.*, 2002). This ability was assigned to the central domain of the VPg coding region (Moury *et al.*, 2004; Ayme *et al.*, 2006). A third pathotype that arose during greenhouse manipulation, was named 1-3 by Luis-Arteaga *et al.* (1997).

Despite biological differences the strain grouping and naming of potato and pepper isolates of PVY is still an object of discussion (Singh *et al.*, 2008). Based on RFLP variations of the coat protein (CP) gene Blanco-Urgoiti *et al.* (1996) have proposed to group PVY isolates in three main clusters: potato PVY^{N} strains, potato PVY^{O} strains and isolates coming from hosts other than

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potato, denoted non potato PVY^{NP} strains. PVY^{C} strains could be actually grouped in two different genetic clusters, i.e PVY^{C1}, comprising non potato isolates able to infect both pepper and potato, and PVY^{C2}, grouping all isolates that are either unable to infect pepper mechanically or that infect it less efficiently than other isolates (Blanco-Urgoiti *et al.*, 1998a; Romero *et al.*, 2001).

Tobacco isolates have been characterised on the basis of symptoms induced in tobacco cultivars carrying or not the dominant *Rk* resistance gene to the root-knot nematode (Gooding, 1985; Gooding and Tolin, 1973) and have been grouped in three strains. Isolates of strain $M^{S}N^{R}$ induce necrosis only to tobacco plants carrying the *Rk* gene, while isolates of strain $M^{S}M^{R}$ cause mosaic, and those of strain $N^{S}N^{R}$ cause necrosis, regardless of the *Rk* gene (Gooding and Tolin, 1973). According to Tribodet *et al.* (2005) it seems that the HC-Pro coding region contains determinants for necrosis induction in tobacco.

Compared to other solanaceous species, tomato seems to be poorly selective with respect to symptoms induced by different PVY isolates (Abad and Jorda, 2000; Rosner *et al.*, 2000; Marchoux *et al.*, 2000; Morel *et al.*, 2000; Crescenzi *et al.*, 2005; Aramburu *et al.,* 2006). Plants infected by PVY^{O} or PVY^{C} show crinkling of young leaves often followed by necrotic mottling and necrosis of the veins on the lower leaf surface while fruits remain usually symptomless (Shukla *et al.*, 1994). By contrast, severe mosaic, often accompanied by interveinal yellow spots and whitish spots on fruits, is associated with PVY^{N} strains.

In 2006, tomato plants of cv. Caramba (De Ruiter Seeds, The Netherlands) showing unusual necrotic symptoms were observed in protected crops grown in the province of Foggia (Apulia, Southern Italy). Leaflets had necrotic spots on the upper epidermis that corresponded to translucent necrotic areas on the lower epidermis where some vein necrosis was also visible. Pale-yellow spots were scattered on the fruit surface which, after a short storage at room temperature, increased in number and size, became sunken and turned necrotic. Stems were not affected.

Preliminary electron microscope observations revealed the presence of potyvirus-like particles in symptomatic leaf tissues. Further tests based on molecular hybridization disclosed the presence of PVY but not of *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Tobacco mosaic virus* (TMV) and *Pelargonium zonate spot virus* (PZSV). According to recent suggestions on the naming of PVY strains (Singh *et al.,* 2008) we denoted this PVY isolate PVY^{C}-to.

Here we report the biological and molecular characterization of isolate PVY^{C}-to, provide evidence that it is a putative interlineage recombinant in the HC-Pro/P3 coding region [according to Ogawa *et al.* (2008), an interlineage recombinant is a PVY isolate with parents from major PVY lineages] and propose its allocation in the PVY^{C2} strain (Romero *et al.*, 2001) of the PVY^{C} strain group.

MATERIALS AND METHODS

Virus isolates and biological assays. PVY^{C}-to was transmitted to tobacco cv. Xanthi by mechanical inoculation using extracts made by crushing symptomatic tomato leaflets collected from one of the field plants, in 100 mM phosphate (Na_2-K) buffer, pH 7.2. PVY-SON41, a 0,1,2 pathotype (Gebré Selassié *et al.,* 1985) kindly supplied by Dr. B. Moury (INRA, Montfavet, France), was used as control. Both isolates were purified essentially according to Thompson *et al.* (1988).

For biological assays, 10 µl of a purified virus preparation at 100 ng/µl in 30 mM Na_2HPO_4 were rubbed on the leaves of two to four plants at the four leaf stage of the following: (i) tobacco cvs Xanthi and Samsun; (ii) *Chenopodium amaranticolor*; (iii) the local pepper cv. Friariello di Nocera highly susceptible to PVY; (iv) pepper hybrids Gold Queen (P^{0}) and Raggio ($P^{0,1}$) (Seminis, USA); (v) tomato cvs Caramba, UC82, Messapico and Diaz the latter two carrying the Sw-5 gene which provides resistance to ordinary strains of TSWV; (vi) the following potato varieties from Jones (1990) list, Cara, Desiree, Estima, King Edward, Maris Piper, Maris Bard, Pentland Crown, Pentland Ivory, kindly supplied by Dr. F. Dale and Mr. R. Wilson (Scottish Crop Research Institute, Dundee, UK). Mock-inoculated plants served as negative controls. After inoculation, all plants were grown in a glasshouse at 24±4°C with a 16 h photoperiod, and were monitored for leaf symptom expression every week for at least 60 days. Soon after blooming, tomato plants cv. Caramba infected by PVY^{C}-to were transplanted to large pots and moved to an insect-proof screenhouse to observe symptoms development on the fruits. Plants were tested for PVY infection by dot–blot hybridization with a digoxigenin-labelled PVY-specific RNA probe synthesised and used as previously described (Saldarelli *et al.*, 1996).

cDNA cloning and sequencing. PVY^{C}-to RNA was extracted from purified particles as described by Finetti-Sialer *et al.* (1997) and 1 µg RNA was annealed to 100 pmol of an oligo$(dT)_{15}$ primer and to 50 ng of random hexamers to be reverse transcribed using the cDNA Synthesis System (Roche, Germany) according to the manufacturer's protocol. The double-stranded cDNA was ligated into *Sma*I-digested, de-phosphorylated pUC18 plasmid with Ready-To-Go T4 DNA Ligase (Ge Healthcare Technologies, Italy) and used to transform competent *Escherichia coli* DH5α cells. Plasmids containing inserts of different size were used for DNA auto-

Table 1. Primer pairs used for cDNA synthesis and cloning of PVY^{C}-to genome.

Primer	Sequence (5'→3')	Position on PVY^{C}-to genome (accession No. EU482153)
PVY^{C}-to I For	AAATTAAAACAAATCAATACAACA	1-24
PVY^{C}-to I Rev	TTGCTGCTTCTCCCCTATGGA	1386-1406
PVY^{C}-to II For	AGCGTTCATGGCTTTAGTGATCA	3530-3552
PVY^{C}-to II Rev	TCGTGACAATCTCTAGGCAACCA	4502-4524
PVY^{C}-to III For	TACCTACAATGTACCGGGTAGCACT	6659-6683
PVY^{C}-to III Rev	TAGCGAGAACAACCATAAGAGAGT	7957-7980
PVY^{C}-to RACE I	ACCATGGCAATCCACATGTC	183-202
PVY^{C}-to RACE II	ACCGTCCTAGTTCAACAAG	132-151

mated sequencing in both directions (Primm, Italy). Additional pairs of primers (Table 1) were designed to amplify overlapping fragments to cover all the PVY^{C}-to genome. The 3'-terminal sequence of the genomic RNA was determined from at least three different clones while the 5' end region was cloned separately following instructions of the 5'/3'RACE Kit 2nd Generation (Roche, Germany) using 10 µM each of primer PVY^{C}-to RACE I (Table 1) to synthesize first strand cDNA and 10 µM of a second, nested primer PVY^{C}-to RACE II (Table 1) for the first PCR amplification. The final PCR product was ligated into a pGem-T Easy plasmid (Promega, USA) and used to transform competent *E coli* DH5α cells.

Phylogenetic and recombination analyses. Bioedit (Hall, 1999) and Clustal W (Thompson *et al.*, 1994) were used to align the PVY^{C}-to full-length sequence with those of 23 PVY isolates from GenBank and with that of *Plum pox virus* (PPV, accession No. X81083) as outgroup. Phylogenetic relationships among the aligned sequences were inferred with the neighbour-joining method (Saitou and Nei, 1987), using the Jukes Cantor distance model (1969) with the distance data matrix bootstrap resampled 1000 times (Felsenstein, 1985). The Treecon software (Van de Peer and De Wachter, 1997) was used to construct and display the resulting trees.

Recombination and detection analysis was done using the recombination detection program version 3 (RDP3), i.e. RDP (Martin *et al.*, 2005), GENECONV (Padidam *et al.*, 1999), BOOTSCAN (Salminen *et al.*, 1995), MAXCHI (Maynard-Smith, 1992), CHIMAERA (Posada and Crandall, 2001) and SISCAN (Gibbs *et al.*, 2000). Automated analysis was carried out using the default RDP3 settings and only potential recombination events independently identified by two or more methods were taken into consideration. The isolate accessions numbers are reported in Fig. 1.

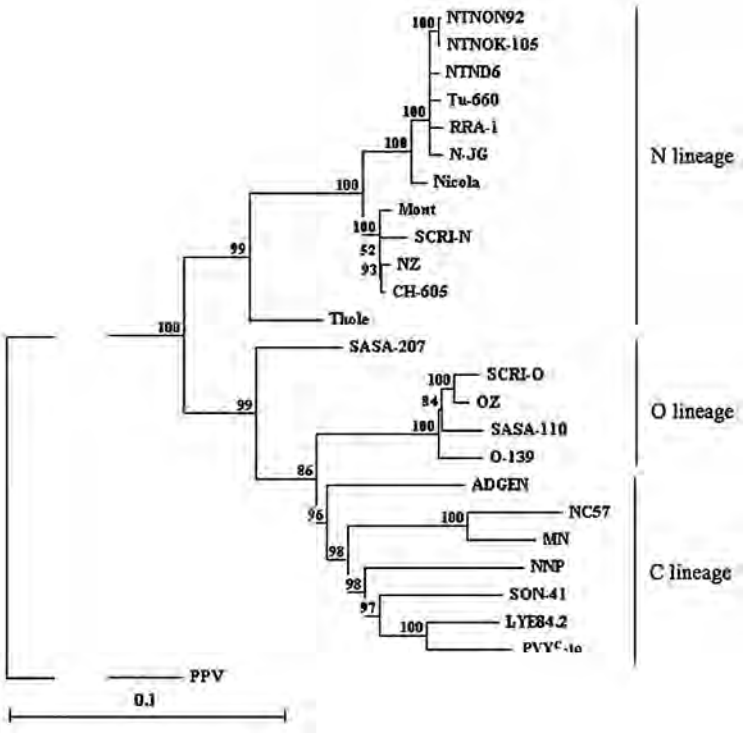

Fig. 1. Phylogenetic relationships between PVY^{C}-to and the following available PVY sequences (GenBank accession Nos. in parentheses): Adgen-C (AJ890348), LYE84.2 (AJ439545), M^{S}N^{R} (AF463399), SON41 (AJ439544), O-139 (U09509), SASA-110 (AJ585195), SCRI-O (AJ585196), Oz (EF026074), CH-605 (X978895), Mont (AY884983), Nz (AM268435), N-Jg (AY166867), NTND6 (AB331515), NTNOK105 (AB331516), NTNON92 (AB331519), RRA-1 (AY884984), Tu660 (AY166866), Nnp (AF237963), SASA-207 (AJ584851), SCRI-N (AJ585197), Thole (M95491), NC-57 (DQ309028) and Nicola (AJ890346). The tree was generated using the whole sequence data. Group classification was deduced from available literature and is reported to the right of vertical bars. Numbers at each node indicate bootstraps (only values >50 are shown). Horizontal branch length is drawn to scale. The bar indicates 0.1nt replacements per site. The sequence of *Plum pox virus* (GenBank accession no X81083) was used as an outgroup.

Table 2. Response of test plants to mechanical inoculation of PVY^C-to and PVY-SON41.

Host	Cultivar	Symptoms	
		PVY^C-to	PVY-SON41
Nicotiana tabacum	Xanthi	NcLL SM, LB	VC
N. tabacum	Samsun	MM	SM
Solanum lycopersicum	Caramba	LD	MM,rec
S. lycopersicum	Diaz	LD	MM, rec
S. lycopersicum	Messapico	LD	MM, rec
S. lycopersicum	UC82	LD	MM, rec
Chenopodium amaranticolor		NcLL	ClLL
Capsicum annuum	Raggio ($P^{0,1}$)	Ni	MM
C. annuum	Gold queen (P^0)	Ni	MM
C. annuum	Friariello di Nocera	MM, rec	SM, LD
Solanum tuberosum	Cara (Na, Nv, Nc, Ny_{tbr})	NcLL	Ni
S. tuberosum	Desiree (na, nv, nc, ny_{tbr})	MM	Ni
S. tuberosum	Estima (na, nv, nc, ny_{tbr})	SM-VN	MM
S. tuberosum	King Edward (Na, Nv, Nc, ny_{tbr})	NcLL	NcLL
S. tuberosum	Maris Bard (na, Nv, Nc, Ny_{tbr})	NcLL	NcLL
S. tuberosum	Maris Piper (Na, Nv, Nc, ny_{tbr})	NcLL	NcLL
S. tuberosum	Pentland Crown (na, Nv, nc, Ny_{tbr})	VN	VN
S. tuberosum	Pentland Ivory (na, Nv, Nc, Ny_{tbr})	NcLL	Ni

SM, severe mosaic; MM, mild mosaic; LD, severe leaf distorsion; VC/VN, systemic veinal chlorosis/necrosis; Cl/Nc LL, chlorotic/necrotic local lesions, LB, leaf bubbling; Ni, not infected; rec, recovery; in brackets, genotypes; *S. tuberosum* genotypes are according to Jones (1990)

RESULTS AND DISCUSSION

Table 2 summarizes the symptoms obtained in experimental plants mechanically inoculated with PVY^C-to in comparison with PVY-SON41. On the whole, symptoms shown by the different hosts in response to PVY^C-to infection appeared more severe than those elicited by PVY-SON41.

The disease phenotype observed in the field was partially reproduced in tomato cv. Caramba by mechanical inoculation of PVY^C-to under controlled conditions. Plants were infected systemically and showed mosaic and severe leaf distorsion but only few necrotic spots that became visible both on the upper and lower side of the leaves. PVY-SON41 infection in tomato was mostly symptomless or caused a very mild discolouration of the young leaves that gradually disappeared as the blade expanded.

Symptoms elicited in potato cultivars carrying the *Nc* gene consisted of necrotic local lesions that developed between 10 and 15 days post inoculation. The upper non-inoculated leaves of these varieties were negative when tested by dot blot hybridization with a PVY-specific RNA probe, confirming virus confinement in the inoculated leaves. Cvs Desiree, Estima and Pentland Crown that do not carry the *Nc* gene were infected systemically. Top necrosis was never observed. Cvs Cara, Desiree and Pentland Ivory were not infected by PVY-SON41, probably because of inoculum failure. Tobacco plants infected with PVY-SON41 showed a milder mosaic than those infected by PVY^C-to but none of the two PVY isolates induced vein necrosis. PVY^C-to did not infect the hybrid pepper genotypes Gold Queen and Raggio used in this study and this was confirmed in three independent experiments, by back inoculation from pepper to tobacco, and by molecular hybridization. In the cv. Friariello di Nocera PVY^C-to induced a very mild systemic mosaic that disappeared as the plant aged. On the contrary, PVY-SON41 was able to infect systemically the hybrid pepper genotypes Queen and Raggio. This was in agreement with the designation of PVY-SON41 as 0,1,2 pathotype, with respect to its infectivity to resistant pepper varieties. PVY-SON41 infection in cv. Friariello di Nocera was characterized by severe systemic mosaic and leaf distortion. Thus, pepper seemed a suitable host to distinguish PVY^C-to from PVY-SON41.

The complete PVY^C-to genome sequence (GenBank accession No. EU482153) consists of 9,691 nt, excluding the 3' poly(A) tail, and contains a single large open reading frame (ORF), starting at nt 186 and ending at nt 9,362 and UTR at 5' and 3' termini that are 185 and 329 nt-long, respectively. The single ORF has the coding capacity for a polyprotein of 3,059 amino acids (aa) whose putative cleavage sites for the viral-encoded proteinases yield all the characteristic potyviral proteins with esti-

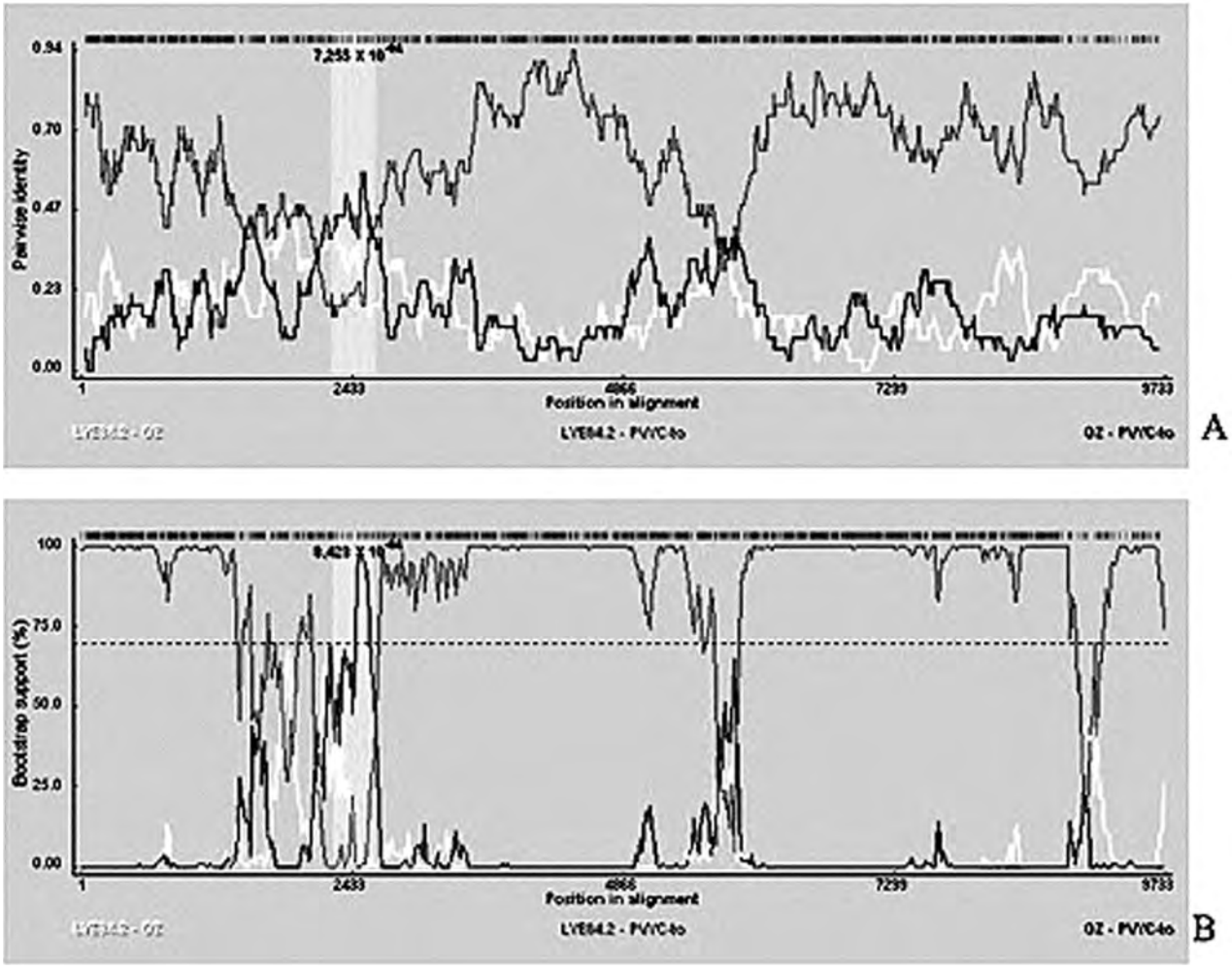

Fig. 2. A. identification of a putative 576 nt-long recombinant region in the PVY^{C}-to genome (black line) highlighted by RDP (P = 7,255 x 10^{-4}) approximately located between nt 2056 and 2632 i.e. at the junction point between the coding region of HC-Pro and P3 proteins, window size 30 nt. The sequences of LYE 84.2 (white line) and OZ (grey line) represent the likely parental sequences of PVY^{C}-to. B. the same putative recombinant region was identified by BOOTSCAN (P = 8,423 x 10^{-4}), each window comparison covered a region of a window size of 200 nt with a step size of 20 nt.

mated size of 272, 465, 365, 52, 634, 52, 188, 244, 519 and 267 aa for P1, HC-Pro/P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP, respectively. At the nucleotide level, PVY^{C}-to showed identity with other PVY isolates ranging from 82% (PVY-Tu-660 and PVY-N-JG) to 94.8% (PVY-LYE84.2). Genetic distances are indicated in the neighbour-joining phylogenetic tree shown in Fig. 1 that is based on the full-length nucleic acid sequences. This analysis, clearly identifies PVY^{C}-to as a PVY^{C} group strain and shows that it is very similar to isolate PVY-LYE84.2.

Since recombination has been previously reported for PVY, we examined the PVY^{C}-to for any evidence of recombination. Three of the five programs used to this aim identified a major recombination breakpoint at approximately nucleotide position 2056 to 2632 (Fig. 2). The 576 nt-long putative recombination site involved HC-Pro/P3 coding region and was detected by RDP (P =7.2 × 10^{-4}), BOOTSCAN (P = 8,423 × 10^{-4}) (Fig. 2) and 3Seq ($P = 2.4 \times 10^{-5}$) (not shown). A recombination breakpoint has been previously reported by Glais *et al.* (2002) in the HC-Pro/P3 region, thus supporting the hypothesis that PVY^{C}-to might be a true recombinant.

The RDP package was also used to search the most closely related regions (referred to as "parental sequences" by Ogawa *et al.*, 2008) to the identified recombination site of PVY^{C}-to (Fig. 3). The event seems to represent an introgression of a PVY^{O} strain sequences and, in particular of a PVY isolate similar to PVY- OZ that is a non-recombinant isolate of the O lineage (Ogawa *et al.*, 2008). From this analysis, PVY^{C}-to appeared to derive from two parental sequences from major lineages O and C, thus representing an interlineage recombinant isolate.

Recombination analysis did not detect any significant recombinant breakpoint in the remaining part of the PVY^{C}-to genome, including the CP coding sequence. Phylogenetic analysis inferred by using the CP coding

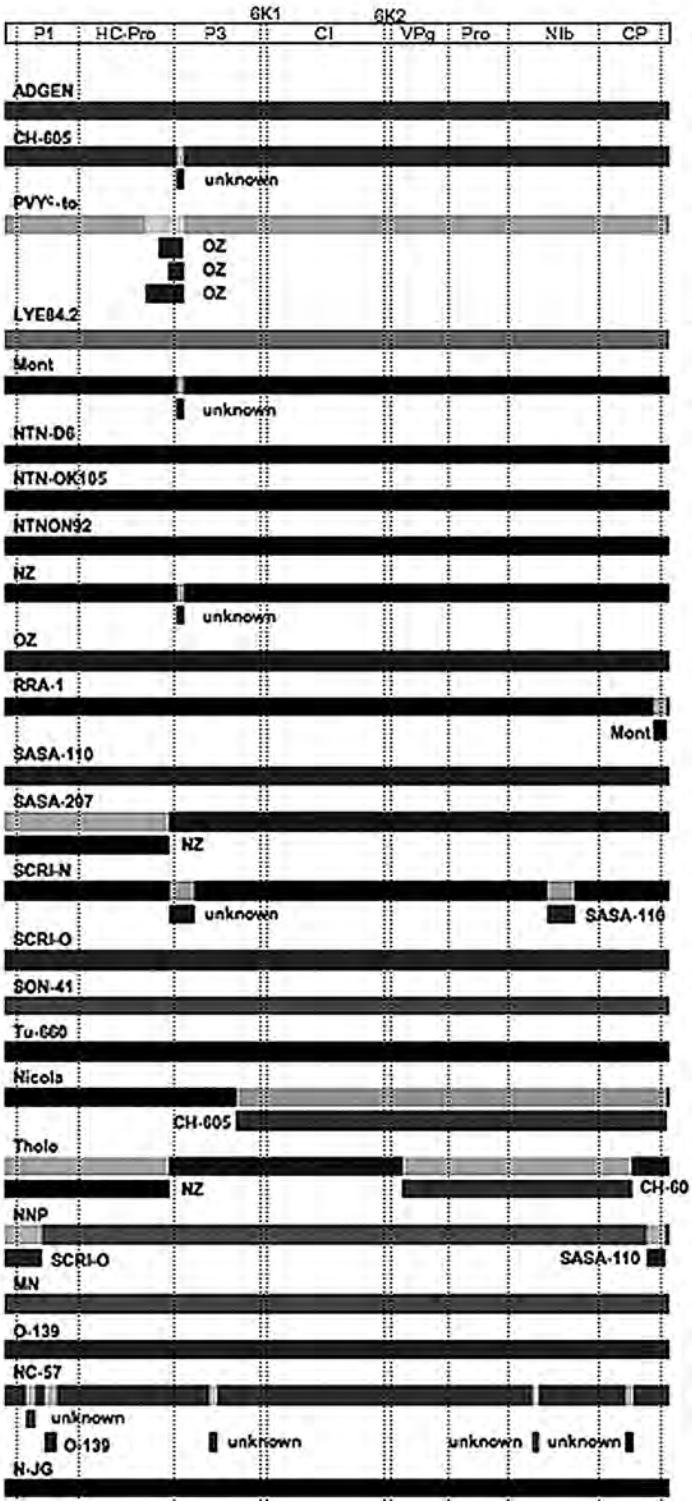

Fig. 3. Maps (not drawn to scale) depicting recombinant events detected amongst PVY sequences used in this study. Vertical dotted lines indicate approximate position of PVY ORFs while small rectangles underlying those representing the genome of different PVY strains indicate recombination fragments obtained from data analysed using RDP and BOOTSCAN programs. The analysis indicates PVY-OZ as the most similar sequence to the recombinant breakpoint identified in the PVY^{C}-to genome, around the HC-pro/P3 coding region.

sequences yielded a tree that was identical to the whole genome tree reported in Fig. 1 (not shown).

Our results provide substantial evidence that most of the PVY^{C}-to genome is of the PVY^{C} strain type, including the CP coding region. As already mentioned, the variability of this region to endonuclease restriction analysis can produce restrictotypes (*sensu:* Blanco-Urgoiti *et al.*, 1996) that have been proposed for a better taxonomic definition of the PVY^{C} strain group (Romero *et al.,* 2001). PVY^{C}-to produced a restrictotype (not shown) that was similar to that of pepper isolates C23 and C30 described by Romero *et al.* (2001) and classified as PVY^{C2} genetic strains. *In silico* analysis showed that PVY^{C}-to CP coding region did not contain any *Eco*RV restriction site, while it contained three *Dde*I, one *Hinf*I, two *Rsa*I and two *Taq*I restriction sites (Fig. 4). The CP sequences of PVY C23 and C30 isolates are not available in any database which impaired a thorough comparison.

On the whole, the present results seem to support the hypothesis that PVY^{C}-to is an interlineage recombinant isolate of the PVY^{C} group and, probably, of the PVY^{C2} genetic strain. The disease was not exactly reproduced by mechanical inoculation under experimental conditions but this may depend on difference in age at the time when plants were infected as well as to the environmental and growing conditions, as pointed out in other instances (Armburu *et al.*, 2006). The possibility that another virus among those usually found in Apulian tomato crops showing necrotic symptoms (Gallitelli *et al.*, 2004) could be present in the same plant in mixed

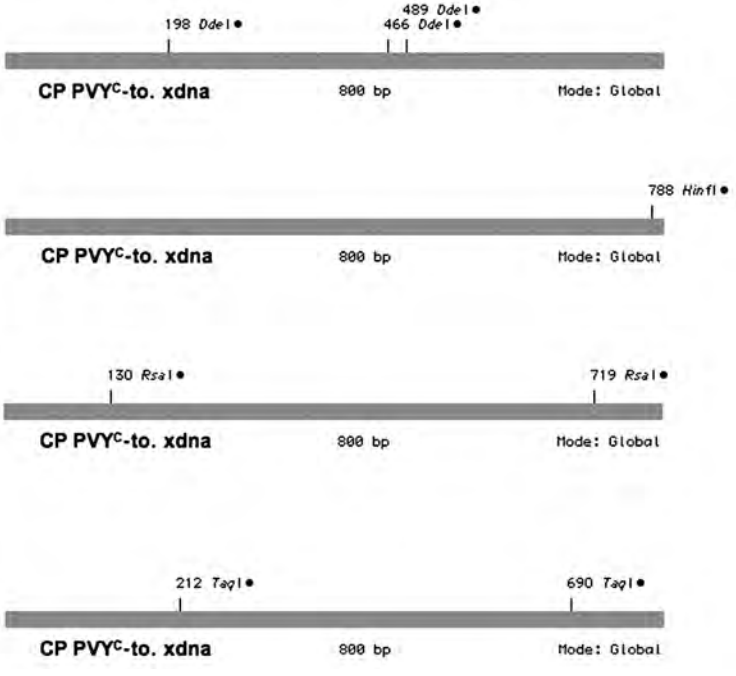

Fig. 4. *In silico*-produced restrictotype of the CP coding region of the PVY^{C}-to genome. Restriction sites were identified using the program DNA strider 1.4f6.

infection was ruled out by dot blot analysis carried out for CMV, TMV, TSWV and PZSV and by electron microscope observations. Further analyses are needed to find a connection, if any, between genomic sequence and disease phenotype elicited by PVY^{C}-to in tomato

To our knowledge this is the first complete characterization of a PVY isolate necrotic to tomato found in Italy. A PVY isolate, denoted PVY-LF02, inducing necrosis in tomato was found in hydroponic cultures grown in Calabria (Southern Italy). The virus was characterized at a very preliminary level which did not led to any conclusive taxonomic association with any of the PVY groups (Fanigliulo *et al.*, 2005).

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REFERENCES

Abad P., Jorda C., 2000. Characterization of potato Y potyvirus isolates from tomato crops in Islas Canarias (Spain). *Bulletin OEPP/EPPO Bulletin* **30**: 281-287.

Ayme V., Souche S., Caranta C., Jacquemond M., Chadoeuf J., Palloix A., Moury B., 2006. Different mutations in the genome-linked protein VPg of *Potato virus Y* confer virulence on the *pvr23* resistance in pepper. *Molecular Plant-Microbe Interactions* **19**: 557-563.

Aramburu J., Galipienso L., Matas M., 2006. Characterization of potato virus Y isolates from tomato crops in northeast Spain. *European Journal of Plant Pathology* **115**: 247-258.

Blanco-Urgoiti B., Sanchez 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. *Archives of Virology* **141**: 2425-2442.

Blanco-Urgoiti B., Sanchez F., Perez de San Roman C., Dopazo J., Ponz F., 1998a. Potato virus Y group C isolates are a homogeneous pathotype but two different genetic strains. *Journal of General Virology* 7**9**: 2037-2042.

Blanco-Urgoiti B., Tribodet M., Leclere S., Ponz F., Perez de San Roman C., Legorburu F.J., Kerlan C., 1998b. Characterisation of potato virus Y (PVY) isolates from seed potato batches. Situation of the NTN, Wilga and Z isolates. *European Journal of Plant Pathology* **104**: 811-819.

Boonham N., Walsh K., Hims M., Preston S., North J., Barker I., 2002. Biological and sequence comparisons of Potato virus Y isolates associated with potato tuber necrotic ringspot disease. *Plant Pathology* **51**: 117-126.

Caranta C., Lefebvre V., Palloix A., 1997. Polygenic resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. *Molecular Plant-Microbe Interactions* **10**: 872-878.

Chachulska A.M., Chrzanowska M., Robaglia C., Zagorski W., 1997. Tobacco veinal necrosis determinants are unlikely to be located within the 5'and 3' terminal sequences of the Potato virus Y genome. *Archives of Virology* **142**: 765-779.

Chrzanowska M., 1991. New isolates of the necrotic strain of potato virus Y (PVY^{N}) found recently in Poland. *Potato Research* **34**: 179-182.

Cockerham G., 1970. Genetical studies on resistance to potato viruses X and Y. *Heredity* **25**: 309-348.

Crescenzi A., Fanigliulo A., Comes S., 2005. Characterisation of Potato virus Y isolate PVY-LF02 inducing necrosis in tomato. *Acta Horticulturae* **695**: 331-337.

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

Fanigliulo A., Comes S., Pacella R., Harrach B., Martin D.P., Crescenzi A., 2005. Characterisation of Potato virus Y nnp strain inducing veinal necrosis in pepper: a naturally occurring recombinant strain of PVY. *Archives of Virology* **150**: 709-720.

Felsenstein J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783-791.

Finetti Sialer M., Di Franco A., Gallitelli D., 1997. Tomato necrotic yellows induced by a novel strain of *Alfalfa mosaic virus. Journal of Plant Pathology* **79**: 115-120.

Gallitelli D., Guario A., Sumerano P., Finetti-Sialer M., Di Franco A., Papanice M.A.,Vovlas C., Di Geronimo A., Lasorella V., 2004. Indagine sulla eco-epidemiologia di virus delle ortive in Provincia di Brindisi. *Informatore Fitopatologico* **54**: 53-58.

Gebré Selassié K., Marchoux G., Delecolle 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.

Gibbs M.J., Armstrong J.S., Gibbs A.J., 2000. Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics* **16**: 573-58. (http://www.anu.edu.au/BoZo/software/)

Glais L., Tribodet M., Kerlan C., 2002. Genomic variability in potato potyvirus Y (PVY): evidence that PVY^{NW} and PVY^{NTN} variants are single to multiple recombinants between PVY^{O} and PVY^{N} isolates. *Archives of Virology* **147**: 363-378.

Glais L., Tribodet M., Kerlan C., 2005. Specific detection of the PVYN-W variant of Potato virus Y. *Journal of Virological Methods* **125**: 131-136.

Gooding G.V., 1985. Relationship between strains of Potato virus Y and breeding for resistance, cross protection, and interference. *Tobacco Science* **29**: 99-104.

Gooding G.V., Tolin S.A., 1973. Strains of potato virus Y af-

fecting flue-cured tobacco in the South eastern United States. *Plant Disease Reporter* **57**: 200-204.

Hall T.A., 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

Jones R.A.C., 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *Annals of Applied Biology* **117**: 93-105.

Jukes T.H., Cantor C.R., 1969. Evolution of protein molecules. In: Munro H.N. (ed), Mammalian Protein Metabolism, pp. 21-132. Academic Press, New York, NY, USA.

Kerlan C., 2006. Potato Virus Y. *AAB Descriptions of Plants Viruses* No. 414.

Lorenzen J.H., Meacham T., Berger P.H., Shiel P.J., Crosslin J.M., Hamm P.B., Kopp H., 2006. Whole genome characterization of *Potato virus Y* isolates collected in the western USA and their comparison to isolates from Europe and Canada. *Archives of Virology* **151**: 1055-1074.

Luis-Arteaga M., Arnedo M., Gil R., 1997. New Potato virus Y pathotype in pepper. *Capsicum and Eggplant Newsletter* **16**: 85-86.

Marchoux G., Gebré-Selassié K., Gognalons P., Luis-Arteaga M., 2000. Le virus Y de la pomme de terre s'adapte à d'autres solanacées. *Phytoma* **533**: 45-47.

Martin D., Williamson C., Posada D., 2005. RDP2: recombination detection and analysis from sequence alignment. *Bioinformatics* **21**: 260-262.

Maynard-Smith J., 1992. Analyzing the mosaic structure of genes. *Journal of Molecular Evolution* **34**: 126-129.

Morel C., Gognalons P., Guilbaud L., Caranta C., Gebré-Selassié K., Marchoux G., Jacquemond M., 2000. Biological and molecular characterisation of two tomato strains of potato virus Y (PVY). *Acta Physiologiae Plantarum* **22**: 336-343.

Moury B., Morel C., Johansen E., Guilbaud L., Souche S., Ayme, V., Caranta C., Palloix A., Jacquemond M., 2004. Mutations in *Potato virus Y* genome-linked protein determine virulence towards recessive resistances in *Capsicum annuum* and *Lycopersicon hirsutum*. *Molecular Plant-Microbe Interactions* **3**: 322-329.

Ogawa T., Tomitaka Y., Nakagawa A.,, Ohshima K., 2008. Genetic structure of a population of *Potato virus Y* inducing potato tuber necrotic ringspot disease in Japan; comparison with North American and European populations. *Virus Research* **131**: 199-212.

Padidam M., Sawyer S., Fauquet C.M., 1999. Possible emergence of new geminiviruses by frequent recombination. *Virology* **265**: 218-225.

Posada D., Crandall K.A., 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. *Proceedings of the National Academy of Sciences USA.* **98**: 13757-13762.

Romero A., Blanco-Urgoiti B. , Soto M.J., Fereres M.J., Ponz, F., 2001. Characterization of typical pepper-isolates of PVY reveals multiple pathotypes within a single genetic strain. *Virus Research* **79**: 71-80.

Rosner A., Lachman A., Pearlsman L., Maslenin L., Antignus Y., 2000. Molecular characterisation and differential diagnosis of a necrotic PVY isolate in tomato. *Annals of Applied Biology* **137**: 253-257.

Ruffel S., Dussault M.H., Palloix A., Moury B., Bendahmane A., Robaglia C., Caranta C., 2002. A natural recessive resistance gene against *Potato virus Y* in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). *Plant Journal* **32**: 1067-1075.

Saitou N., Nei M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Saldarelli P., Barbarossa L., Grieco F., Gallitelli D., 1996. Digoxigenin-labelled riboprobes applied to phytosanitary certification of tomato in Italy. *Plant Disease* **80**: 1343-1346.

Salminen M.O., Carr J.K., Burke D.S., McCutchan F.E., 1995. Identification of breakpoints in intergenotypic recombinants of HIV type 1 by Bootscanning. *AIDS Research And Human Retroviruses* **11**: 1423-1425.

Schubert J., Fomitcheva V., Sztangret-Wisniewskab J., 2007. Differentiation of *Potato virus Y* strains using improved sets of diagnostic PCR-primers. *Journal of Virological Methods* **140**: 66-74.

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

Singh R.P., Valkonen J.P.T., Gray S.M., Boonham N., Jones R.A.C., Kerlan C., Schubert J., 2008. Discussion paper: The naming of Potato virus Y strains infecting potato. *Archives of Virology* **153**: 1-13.

Thompson S., Fraser R.S.S., Barden K.L., 1988. A beneficial effect of trypsin on the purification of Turnip mosaic virus (TuMV) and other potyviruses. *Journal of Virological Methods* **20**: 57-64.

Thompson J.D., Higgins D.G., Gibson T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673-4680.

Tribodet M., Glais L., Kerlan C., Jacquot E., 2005. Characterization of Potato virus Y (PVY) molecular determinants involved in the vein necrosis symptom induced by PVYN isolates in infected *Nicotiana tabacum* cv. Xanthi. *Journal of General Virology* **86**: 2101-2105.

Van de Peer Y., De Watcher R., 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Computer Applications in Biosciences* **13**: 227-230.

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