Eur J Plant Pathol (2011) 131:549–552
DOI 10.1007/s10658-011-9836-9

A classification of *Pepper yellow mosaic virus* isolates into pathotypes

Monika Fecury Moura · Tatiana Mituti · Julio M. Marubayashi · Ricardo Gioria · Romulo F. Kobori · Marcelo A. Pavan · Norberto da Silva · Renate Krause-Sakate

Accepted: 1 July 2011 / Published online: 22 July 2011

Abstract *Pepper yellow mosaic virus* (PepYMV) is the most important potyvirus infecting sweet pepper in Brazil. In this study, twenty isolates of PepYMV were obtained from commercial sweet pepper crops. To confirm virus identity, the coat protein gene was completely sequenced for eleven of these isolates, and partially sequenced for the other nine isolates. The amino acid identities obtained were above 93% when compared with the sequence of a characterized PepYMV isolate (AF348610). Extracts of *Nicotiana tabacum* cv. TNN plants infected with the different isolates were used to inoculate the differential series of *Capsicum* spp cultivars containing the genes $pvr2^1$, $pvr2^2$, $pvr2^3$, $pvr2^4$, and *Pvr4*. Using the same criteria established for *Potato virus Y* (PVY), fourteen isolates of PepYMV could be classified as known pathotypes described for PVY, that is: 1.2 (2 isolates), 1.3 (6) and 1.2.3 (6). The remaining six isolates, 1.3 (2) and 1.2.3 (4) could not be classified into the typical pathotypes of PVY because they were also virulent on Serrano Criollo de Morellos—334 (C.M 334) which carries the $pvr2^3$ and *Pvr4* genes. To classify the PepYMV into pathotypes and counter the biological diversity found in this species we propose the utilization of 2^x for the ability to overcome the correspondent allele of the *pvr2* locus and 4 for the capacity to break down the *Pvr4* gene. Using this criterion we could classify the PepYMV into five pathotypes: $2^1.2^2$; $2^1.2^3$; $2^1.2^2.2^3$; $2^1.2^3$. 4 and $2^1.2^2.2^3$. 4.

Keywords Potyvirus · Resistance · Sweet pepper

M. F. Moura · T. Mituti · J. M. Marubayashi · M. A. Pavan · N. da Silva · R. Krause-Sakate (✉)
Departamento de Produção Vegetal, Setor Defesa Fitossanitária, UNESP,
C.P. 237, 18603-970 Botucatu, SP, Brazil
e-mail: renatekrause@fca.unesp.br

R. Gioria · R. F. Kobori
Sakata Seed Sudamérica,
Bragança Paulista, SP, Brazil

Peppers (*Capsicum* spp) are frequently infected by viruses. Special attention must be given to the viruses belonging to the genus *Potyvirus* with ten different species described infecting pepper (Moury et al. 2005; Janzac et al. 2008). The most important is *Potato virus Y* (PVY), the only one distributed worldwide (Janzac et al. 2008).

In Brazil the incidence of PVY became rare in commercial sweet pepper fields after the introduction of PVY-resistant cultivars in the 1970s (Nagai and Smith 1968, Nagai and Costa 1972). However during the early 1990s the occurrence of an isolate that was able to overcome the resistance was reported and referred as PVY^M or $PVY^{1,2}$ (Boiteux et al. 1996). Later it was recognized as a new potyvirus species named *Pepper yellow mosaic virus*-PepYMV (Inoue-Nagata et al. 2002).

In Brazil PepYMV is the prevalent potyvirus in sweet pepper fields (Truta et al. 2004) and natural infection was also detected in chilli peppers (Inoue-Nagata et al. 2002) and tomato (Cunha et al. 2004). Heavy losses were associated with the presence of this virus in sweet pepper until the introduction of commercial sweet pepper hybrids probably containing the *Pvr4* gene. These hybrids were resistant to PVY pathotypes 0, 1 and 1,2 and PepYMV but in 2003/04 breakdown of resistance against PepYMV was observed in Brazil (Gioria et al. 2009).

PVY isolates are classified into pathotypes, based on the differential resistance reaction of some particular *Capsicum* cultivars. The classification proposed by Gébré Selassié et al. (1985) is commonly used and accepted. According to this, PVY strains can be classified into pathotype 0, virulent on Yolo Wonder ($pvr2^{+}$); pathotype 1, virulent on this cultivar and on Yolo Y ($pvr2^{1}$); pathotype 1.2, virulent on those two varieties and on Florida VR-2 ($pvr2^{2}$), but not on Serrano Vera Cruz ($pvr2^{4}$). Additional to this classification pathotype PRW (Arteaga et al. 1997), lately renamed as P 1.3 by Arnedo-Andrés et al. (1998), is able to infect Puerto Rico Wonder ($pvr2^{3}$), but not Florida VR-2 and Serrano Vera Cruz. Finally, pathotype P1.2.3 infects Yolo Wonder, Yolo Y, Florida VR-2, Puerto Rico Wonder but not Serrano Vera Cruz and Serrano Criollo de Morellos-334 (CM-334) that contain the recessive gene $pvr2^{3}$ (Charron et al. 2008) and the dominant gene *Pvr4* (Arnedo-Andrés et al. 1998).

Due to the importance of PepYMV in sweet pepper crops in Brazil, the objective of this study was to evaluate the biological response of pepper genotypes carrying $pvr2^{1}$, $pvr2^{2}$, $pvr2^{3}$, $pvr2^{4}$, *Pvr4* genes to PepYMV isolates and to propose the utilization of the standard series of pepper cultivars to classify PepYMV into pathotypes.

Isolates of PepYMV were collected in the most important sweet pepper field areas of São Paulo State, Brazil during the period of October 2007 to November 2008. The preliminary analysis to detect the potyvirus presence was an indirect ELISA test with specific anti-potyvirus antiserum (protocol according to Agdia Inc., Elkhart, IN). To verify mixed infection, leaf extracts, prepared in 0.02 M phosphate buffer pH 7.0, were rub-inoculated on *Nicotiana tabacum* cv. TNN, *Cucurbita pepo* cv. Caserta, *Solanum lycopersicum* cv. Santa Clara, *Datura stramonium* and *Datura metel*. The symptoms were evaluated 30 days after mechanical inoculation.

Selected isolates (Table 1) were inoculated onto *N. tabacum* cv. 'TNN' plants for maintenance and total RNAs extracted, as described by Bertheau et al. (1998) and also using the total RNA Purification kit from Norgen. The primer pair PepNib (5′ GWTSGYYGMMTTGGATGATG 3′) and PepUTR (5′ AGTAGTACAGGAAAAGCC 3′) were designed based on sequences of PVY and PepYMV. The amplification resulted in a DNA fragment of 960 nucleotides corresponding to the complete coat protein gene. The reaction was performed in a one step RT-PCR with the PCR-Master Mix kit (Promega) using μl of total RNA and 1 unit of AMV reverse transcriptase (Promega). The cycle was of 42°C/30 min followed by 95°C/2 min and 39 cycles of 92°C/1 min, 53°C/1 min and 72°C/1.5 min, and 72°C/10 min. Nucleotide sequences directly obtained from

Table 1 List of PepYMV isolates, locality were sample was collected in São Paulo State and proposed pathotype using the differential series of *Capsicum* spp described by Gebré-Selassie et al. (1985) and Arnedo-Andrés et al. (1998). *Complete coat protein sequence available on GenBank. ** Magali R and Rubia R breakdown isolate of PepYMV cited by Gioria et al. (2009)

Code	Locality in São Paulo State	Pathotype
234-23q*	Iacanga	2^{1} .2^{3} .4
237-23 t*	Iacanga	2^{1} .2^{3} . 4
240-29c*	Iacanga	2^{1} .2^{2} .2^{3}
249-29 l*	Iacanga	2^{1} .2^{3}
251-29n*	Iacanga	2^{1} .2^{3}
254-6q*	Pirajú	2^{1} .2^{2} .2^{3}
257-6 t*	Pirajú	2^{1} .2^{2} .2^{3} .4
265-35a	Pirajú	2^{1} .2^{2}
268-32a*	Pirajú	2^{1} .2^{2} .2^{3} .4
348*	Lins	2^{1} .2^{3}
389-2c*	Mogi-Mirim	2^{1} .2^{2}
273-6o	Pirajú	2^{1} .2^{2} .2^{3}
295	Pirajú	2^{1} .2^{2} .2^{3}
304	Pirajú	2^{1} .2^{2} .2^{3} . 4
347	Lins	2^{1} .2^{3}
367	Reginópolis	2^{1} .2^{3}
382	Reginópolis	2^{1} .2^{2} .2^{3}
388-1c	Mogi-Mirim	2^{1} .2^{3}
403	Mogi-Mirim	2^{1} .2^{2} .2^{3} .4
PepYMVLins**	Lins	2^{1} .2^{2} .2^{3}

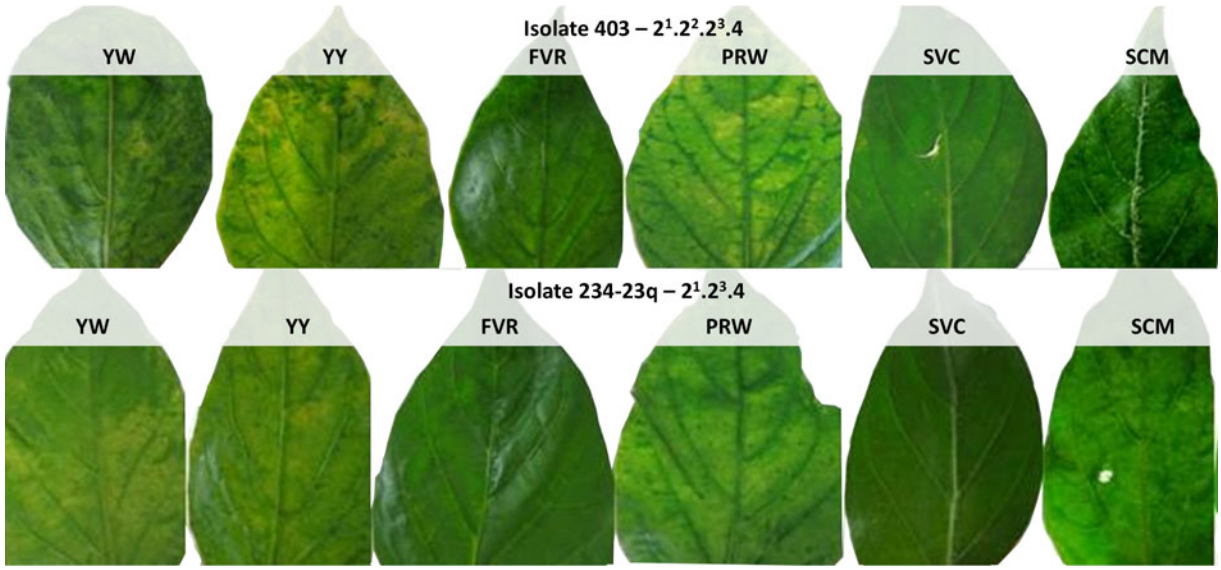

Fig. 1 Reaction observed in the differential series of *Capsicum*: Yolo Wonder (YW), Yolo Y (YY), Florida VR-2 (FVR2), Puerto Rico Wonder (PRW), Serrano Vera Cruz (SVC) and Serrano Criollo de Morelos 334 (CM-334) by the isolates 403 and 234-23Q of PepYMV classified as pathotype $2^1.2^2.2^3.4$ and $2^1.2^3$. 4, respectively

purified PCR products were compared with those deposited in GenBank using the Blastn (http://www.ncbi.nlm.nih.gov/BlAST) and Clustal W (http://www.ebi.ac.uk/Tools/clustalw/index.html) programs.

Isolates classified as PepYMV were mechanically inoculated on pepper genotypes utilized for PVY pathotype classification (Gébré Selassié et al. 1985; Arnedo-Andrés et al. 1998). Extracts from inoculated plants were tested by indirect ELISA with anti-potyvirus (Agdia) antiserum to confirm infection.

The amino acid identities obtained were above 93% when compared with the sequence of a characterized PepYMV isolate (AF348610). These sequences were deposited in GenBank (accession numbers: HQ594518, HQ594519, HQ594520, HQ594521, HQ594522, HQ594523, HQ594524, HQ594525, HQ594526, HQ594527). For the other isolates, described on Table 1, partial sequences also showed identities above 93% with PepYMV (not shown).

A variety of resistant phenotypes was observed on the differential series of *Capsicum* spp. Based on the same classification used for PVY isolates, two PepYMV isolates were classified as pathotype 1.2, six as pathotype 1.3 and six as pathotype 1.2.3. Two others isolates overcome the $pvr2^1$ and $pvr2^3$ alleles but also the *Pvr4* gene present on Serrano Criollo de Morellos—334 (C.M-334). The remaining four isolates have the ability to break down the $pvr2^1$, $pvr2^2$,

Table 2 Proposed classification of PepYMV isolates into pathotypes using the differential series of *Capsicum* spp described by Gebré-Selassie et al. (1985) and Arnedo-Andrés et al. (1998). R: resistante (no symptoms observed) and S: susceptible.*New pathotypes observed for PepYMV

Genotypes of *Capsicum* spp.	Pathotypes					
	Genes	2^1 .2^2	2^1 .2^2 .2^3	2^1 .2^3	2^1 .2^3 .4*	2^1 .2^2 .2^3 . 4*
Yolo Wonder	$pvr2^+$	S	S	S	S	S
Yolo Y	$pvr2^1$	S	S	S	S	S
Florida VR-2	$pvr2^2$	S	S	R	R	S
Puerto Rico Wonder	$pvr2^3$	R	S	S	S	S
Serrano Veracruz	$pvr2^4$	R	R	R	R	R
Serrano Criollo de Morelos 334	*Pvr4*, $pvr2^3$	R	R	R	S	S

pvr2^3 alleles and the *Pvr4* gene from CM-334 (Fig. 1). The PepYMV-Lins isolate, able to breakdown Magali R and Rubia R hybrids (Gioria et al. 2009) has the capacity to overcome *pvr2*^{1}, *pvr2*^{2} and *pvr2*^{3} present on Puerto Rico Wonder, but not CM-334 which besides being homozygous for *pvr2*^{3} has additionally *Pvr4* that limits the infection by this isolate (Table 1).

Our results indicate that the differential series of *Capsicum* is useful to classify the majority of isolates of PepYMV into pathotypes. To cover the biological diversity of PepYMV, however, additional sources of resistance genes have to be located. To represent the biological diversity found in PepYMV we propose the utilization of 2^x, where x is the number of the specific allele of *pvr2* locus overcome and number 4 for the capacity to break down resistance do to *Pvr4* gene from CM-334. Using this criterion we could classify the PepYMV into five pathotypes: $2^1.2^2$; $2^1.2^3$; $2^1.2^2.2^3$; $2^1.2^3$. 4 and $2^1.2^2.2^3$. 4 (Table 2). This system may also be useful for PVY since Janzac et al. 2010 described a PVY variant, selected in the laboratory, virulent on *Pvr4* pepper plants. Despite its low competitiveness in susceptible cultivars, this opens the possibility to find a natural PVY isolate able to overcome the *Pvr4* gene. We also need to be aware that nine different alleles for the *pvr2* gene were identified by Charron et al. (2008) and different spectrums of virulence were observed between isolates of PVY already classified into pathotypes.

Utilization of pepper cultivars with genetic resistance to potyviruses is one of the most practical, economical and environmentally secure strategies to reduce important losses caused by this group of viruses. To increase the durability of a resistance gene, breeders needs to consider the biological variability of the virus. As PepYMV has become prevalent in sweet pepper in Brazil (Truta et al. 2004), this study proposes the classification of PepYMV into pathotypes using the same standard series described by Gébré Selassié et al. (1985) for PVY (Table 2). This classification system allows the evaluation of the PepYMV population using resistance genes available in pepper and also can be very helpful for breeders to select the most effective sources of resistance.

Acknowledgements We thank FAPESP for the Financial Support and CAPES for the granted to the first author.

References

Arnedo-Andrés, A. M., Arteaga, L. M., & Ortega, R. (1998). Response of Serrano Criollo de Morelos-334 to PVY pathotypes. *Capsicum and Eggplant Newsletter*, 105–109.

Arteaga, L. M., Andres, A. M., & Ortega, G. R. (1997). New Potato virus Y pathotype in pepper. *Capsicum and Eggplant Newsletter, 16,* 85–86.

Bertheau, Y. D., et al. (1998). DNA amplification by polymerase chain reaction (PCR). In M. C. M., Perombelon, & Van Der Wolff, J. M. (Eds.), Methods for the detection and quantification of *Erwinia carotovora* subsp. *atroseptica* on potatoes. *Scottish Crop Research Institute Occasional Publication*.

Boiteux, L. S., Cupertino, F. P., Silva, C., Dusi, A. N., Monte-Nesich, D. C., Van Der Vlugt, R. A. A., et al. (1996). Resistance to potato virus Y (pathotype 1–2) in *Capsicum annuum* and *Capsicum chinense* is controlled by two independent major genes. *Euphytica, 87*, 53–58.

Charron, C., et al. (2008). Natural variation and functional analysis provide evidence for coevolution between plant eIF4E and potyviral VPg. *The Plant Journal, 54*, 56–68.

Da Cunha, L. C. V., De Resende, R. O., Nagata, T., & Inoue-Nagata, A. K. (2004). Distinct features of *Pepper yellow mosaic virus* isolates from tomato and sweetpepper. *Fitopatologia Brasileira, 29*, 663–667.

Gébré Selassié, K., Marchoux, G., Delecolle, B., & Pochard, E. (1985). Variabilité naturelle des souches du virus Y de la pómme de terre dans les cultures de piment du Sud-Est de la France. Caractérisation et classification en pathotypes. *Agronomie, 5*, 621–630.

Gioria, R. et al. (2009). Breakdown of resistance in sweet pepper against Pepper yellow mosaic virus in Brazil. *Scientia Agrícola*, 66.

Inoue-Nagata, A. K., Fonseca, M. E. N., Resende, R. O., Boiteux, L. S., Monte, D. C., Dusi, A. N., et al. (2002). Pepper yellow mosaic virus, a new potyvirus in sweet-pepper, Capsicum annuum. *Archives of Virology, 147*, 849–855.

Janzac, B., Fabre, M. F., Palloix, A., & Moury, B. (2008). Characterization of a new potyvirus infecting pepper crops on Ecuador. *Archives of Virology, 153*, 1543–1548.

Janzac, B., Montarry, J., Palloix, A., Navaud, O., & Moury, B. (2010). A point mutation in the polymerase of Potato virus Y confers virulence toward the Pvr4 resistance of pepper and a high competitiveness cost in susceptible cultivar. *Molecular Plant Microbe Interaction, 23*, 823–830.

Moury, B., Palloix, A., Caranta, C., Gognalons, P., Souche, S., Gébré-Selassie, K., et al. (2005). Serological, molecular and pathotype diversity of Pepper veinal mottle virus and Chili veinal mottle virus. *Phytopathology, 95*, 227–232.

Nagai, H., & Costa, A. S. (1972). Four new pepper varieties resistant to virus Y in Brasil. *Meeting on Genetics and Breeding of Capsicum*, 283–287.

Nagai, H., & Smith, P. G. (1968). Reaction of pepper varieties to naturally occurring viruses in California. *Plant Disease Reporter, 52*, 928–930.

Truta, A. A. C., et al. (2004). Identidade e propriedades de isolados de potyvírus provenientes de Capsicum spp. *Fitopatologia Brasileira, 29*, 160–168.