Eur J Plant Pathol (2008) 122:443–445
DOI 10.1007/s10658-008-9280-7

Characterization of two distinct Polish isolates of *Pepino mosaic virus*

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Received: 25 April 2007 / Accepted: 24 January 2008 / Published online: 26 February 2008

Abstract In Poland in 2002 and 2005 two different isolates of *Pepino mosaic virus* signed PepMV-SW and PepMV-PK were obtained. Both isolates were compared on the basis of their symptomatology on a series of plant species. In addition, the isolates were characterized by the nucleotide sequence analysis of the triple gene block, coat protein and a part of the polymerase genes. The studies showed that the Polish isolates differ from each other and belong to two strains. PepMV-SW was highly similar to European isolates, showing extensive sequence identity, ca. 99%. Pairwise comparisons of PepMV-PK with other PepMV isolates from the GenBank database showed that the highest nucleotide sequence identity was with two isolates: Ch2 from Chile and US2 from the USA.

Keywords Genetic diversity · Host range · *Potexvirus*

Pepino mosaic virus (PepMV), a member of genus *Potexvirus*, was first described in 1980 on pepino (*Solanum muricatum*) in Peru (Jones et al. 1980) and afterwards it was isolated from different wild species of the genus *Lycopersicon* (Soler et al. 2002). Meantime, PepMV has been reported in several European countries and in North America as an agent of a viral disease of tomato crops (French et al. 2001; Vlugt van der et al. 2000).

Sequencing data from European tomato isolates show very high genome nucleotide sequence identity ca. 99%. More recently, distinct PepMV isolates from tomato have been reported in the USA (Maroon-Lango et al. 2005) and also from tomato seeds originating from Chile (Ling 2006) showing only 79–82% identity with European tomato isolates. In Poland in 2002 and 2005, two different isolates of the *Pepino mosaic virus* signed PepMV-SW and PepMV-PK were obtained from tomato (Pospieszny et al. 2003; Pospieszny and Borodynko 2006). Both isolates were collected from greenhouses. The PepMV-SW isolate was obtained from tomato plants showing symptoms of distortion and leaf mosaic whilst PepMV-PK was isolated from tomato fruits exhibiting symptoms of marbling. This paper presents results of comparative studies of both the Polish PepMV isolates in terms of their host range, symptomatology and nucleotide sequences of full length coat protein (CP), triple gene block (TGB) and a part of the RNA-dependent RNA polymerase gene (RdRp).

Crude sap of *Nicotiana benthamiana* plants infected with PepMV-SW and PepMV-PK isolates was inoculated mechanically to test plants. Three plants of each species were inoculated. The experiments were conducted at least three times and monitored for up to 1 month for symptom development. The presence of the virus in all test plants was

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Table 1 Host range and symptoms caused by Polish isolates of PepMV on different plant species

Plants	Symptoms	
	PepMV-SW	PepMV-PK
Chenopodium quinoa	Lchl	(Lchl)
Nicotiana tabacum cv. Xanthi nc	(Lchl), M	(s)
N. tabacum cv. Samsun	(Lchl), M	(s)
N. tabacum cv. White Burley	(Lchl), IY	IY
N. benthamiana	Lchl, M, SM	Lchl, M
N. affinis	Lchl, M	(s)
N. debneyi	Lchl, M	Lchl, MM
N. glutinosa	Lchl, M, SM	M, SM
N. clevelandii	Lchl, M, SM	Lchl, M, SM
Solanum melongena	(Lchl), M	s, MM
Solanum tuberosum	–	–
Lycopersicon esculentum	s, M, (B)	s, (MM)
Datura inoxia	(Lchl), M	Ldnl, s, MM
Capsicum annuum	–	–
Nicandra physaloides	(Lchl), M	s, MM
Petunia hybrida	–	–
Physalis floridana	Lchl, M, SM, (D)	–

Lchl Local chlorotic lesions, *Ldnl* local diffusion necrotic lesion, *M* mosaic, *MM* mild mosaic, *SM* sever mosaic, *D* deformations, *IY* interveinal yellowing, *B* bubbling, *s* symptomless, *()* occasional appearance, – no infection

evaluated by the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test or the reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated from infected plants of *N. benthamiana*, using the method described by Chomczyński (1993). RNA was dissolved in 40µl of sterile water, 2µl of which were used for cDNA synthesis. The reverse transcription step was performed with the polidT_{22} primer and M-MuLV RT (Fermentas). Three overlapping cDNA fragments, covering the entire coat protein gene, triple gene block and part of the polymerase gene were further amplified by the polymerase chain reaction (PCR), using *Taq* DNA polymerase (Fermentas) and appropriate primers (Pagan et al. 2006).

For both isolates a 500 bp region of the 3′ end of RdRp gene was amplified using Pep3 and Pep4 primers and an 845 bp region, including the complete CP gene was amplified using PepCP-D and PepCP-R primers. To amplify a 1,317 bp region encompassing the complete TGB region of the PepMV-PK isolate PepUSTGB-D and PepUSTGB-R primers were used. The TGB region of PepMV-SW was amplified using TGB-D and TGB-R primers. The obtained RT-PCR products were purified using the Qiaex II gel extraction kit (Qiagen) and then cloned using the pGEM-T-Easy Kit (Promega). *Escherichia coli* DH5α (Invitrogen) was transformed with the ligated vector, and plasmid DNA was isolated using the Qiagen Miniprep Kit (Qiagen). Three randomly selected clones were sequenced on an ABI automatic sequencer. The obtained nucleotide sequences data were submitted to the GenBank database and given the accession numbers: EF408821 for PepMV-PK and EF408822 for PepMV-SW. Nucleotide and amino acid sequences were compared with the sequences available in the GenBank database. The analysis was done on a 500 bp fragment at the 3′ end of the RdRp

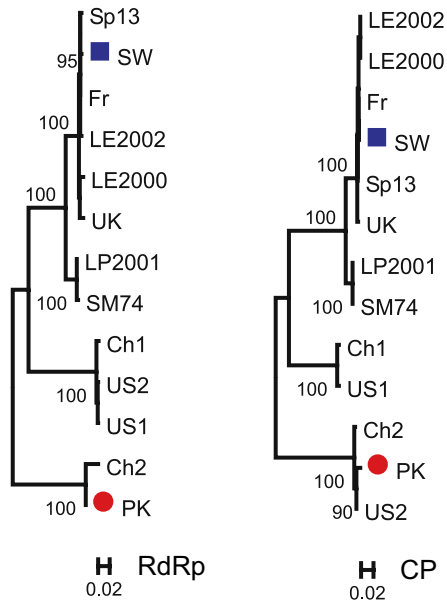

Fig. 1 Neighbour-joining trees basis on nucleotide alignments for two genomic regions of Polish isolates of the *Pepino mosaic virus* (PepMV): the RNA-dependent RNA polymerase gene (RdRp) and the coat protein gene (CP). The Polish isolates: PepMV-SW and PepMV-PK are marked with *blue* and *red colours*, respectively. Sequences of other PepMV isolates obtained from public data bases were included. Tomato isolates were from France (Fr AJ438767), the UK (UK AF340024), Spain (Sp13 AF484251; LE2000 AJ6066359; LE2002 AJ606360), USA (US1 AY509926; US2 AY509927) and Chile (Ch1 DQ000984; Ch2 DQ000985). Peruvian isolates were from *Lycopersicon peruvianum* (LP2001 AJ606361) and *Solanum muricatum* (SM74 AM109896). The *numbers* at each branch point indicate bootstrap values, branch values shown are also >90%. The *scale bar* represents, for the horizontal branch lengths, genetic distances of 0.02

gene and on the full length of TGB1, TGB2, TGB3 and CP genes. Multiple sequence alignments were performed using ClustalW. The alignments were used to calculate pairwise genetic distances by Kimura's two-parameter method. Phylogenetic analysis was carried out by the neighbour-joining method implemented with Mega version 3.1. Bootstrap values for phylogenetic comparisons are based on 1,000 pseudoreplicates.

On the basis of the host range and phylogenetic analyses, we showed that the Polish isolates are very distinct and belong to different strains. Most of the mechanically inoculated plant species reacted differently to the Polish PepMV isolates (Table 1). The major differences appeared in *Nicotiana tabacum* cvs Xanthi nc. and Samsun, and *Nicotiana affinis* which were infected occasionally without symptoms, and in *Physalis floridana* that was not infected by PepMV-PK in contrast to PepMV-SW. Minor differences were observed for *N. tabacum* cv. White Burley, *Solanum melongena*, *Lycopersicon esculentum*, *Nicandra physaloides* and *Nicotiana glutinosa*. Overall, the PepMV-SW isolate caused distinct symptoms in infected plants, whilst PepMV-PK induced a very mild mosaic or did not produce symptoms. The biological differences between both Polish PepMV isolates were confirmed by the sequence alignments of the five regions of their genomes. The nucleotide distance matrix for PepMV-PK and PepMV-SW indicated that the nucleotide identity percentages were as follows: 77% for the CP genes, 75% for the RdRp, and 75%, 81% and 88% for the TGB1, TGB2 and TBG3, respectively. PepMV-SW isolate showed very high identities (98–100%) to the European tomato strain. On the other hand, the PepMV-PK isolate is highly similar to the US2 strain, more particularly to Ch2 than to the US2 isolate. The comparison of PepMV-PK and Ch2 revealed the following nucleotide identities: 99% for the CP genes, 98% for the RdRp, 98%, 99% and 98% for the TGB1, TGB2 and TBG3, respectively. A close phylogenetic relationship between Ch2 and PepMV-PK may suggest their common origin. Both our and other phylogenetic analyses (Ling 2006) of PepMV isolates from the GenBank database showed a tendency to generate four distinct groups (strains): European tomato, Peruvian, US1 (Ch1, US1) and US2 (US2, Ch2 and PepMV-PK). At the amino acid level similar results were obtained, the only difference was that the Peruvian isolates clustered with tomato European isolates. The different positions of the US2 isolate in the RdRp and CP trees is due to differences in nucleotide sequence of the 3′ end of the RdRp gene of the US2 and both PepMV-PK and Ch2 isolates (Fig. 1). It has been shown before that the variations in the PepMV genome were concentrated in this area between the second half of replicase and first half of TGB 1 (Ling 2006). Besides PepMV-PK, in 2006 and 2007, we have found several isolates similar to the US2 strain. Unfortunately, it can indicate that similar genetic variants have been introduced to Poland. Very recently both the Peruvian and the US2 strains have also been detected in Spain (Pagan et al. 2006). The above data indicate a growing distribution of the US2 strain in Europe.

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