Eur J Plant Pathol (2010) 127:305–309
DOI 10.1007/s10658-010-9600-6

Cucumber mosaic virus groups IA and II are represented among isolates from naturally infected lilies

Hanna Berniak · Maria Kamińska · Tadeusz Malinowski

Accepted: 1 March 2010 / Published online: 14 March 2010

Abstract Four cucumber mosaic virus isolates (named Cas, CB, P26 and Simp2) found in naturally infected lily plants were characterized on the basis of their serological properties and the results of analysis of RNA3 sequence fragments containing coat protein and movement protein genes. The properties of lily isolates were compared with those of eight other virus isolates originating from dahlia, delphinium, impatiens, honeysuckle, cucumber and redcurrant plants. On the basis of the reaction with group-specific monoclonal antibodies and RNA3 sequence analysis, two lily isolates (Cas and CB) were classified to group I of CMV, similarly to all previously reported virus isolates found in lily plants. Surprisingly, sequences of coat protein and movement protein genes of two other lily isolates (P26 and Simp2) showed more than 98% similarity to CMV group II isolates, and only 77% similarity to group I isolates. Results of ELISA with CMV group II-specific monoclonal antibodies confirmed the classification of isolate Simp2 as a member of group II. Isolate P26 reacted neither with CMV group I nor with group II-specific monoclonal antibodies. In order to explain the lack of reaction of P26 isolate with monoclonal antibodies, comparative analysis of predicted amino acid sequence of the coat protein was done. This revealed a mutation—change from alanine at position 138 to threonine, probably responsible for particular serological properties of isolate P26.

Keywords CMV grouping · *Lilium sp.* · Sequence analysis · Serology

H. Berniak (✉) · M. Kamińska
Department of Plant Protection,
Research Institute of Pomology and Floriculture,
Pomologiczna 18,
96-100 Skierniewice, Poland
e-mail: Hanna.Berniak@insad.pl

T. Malinowski
Department of Plant Physiology and Biochemistry,
Research Institute of Pomology and Floriculture,
Pomologiczna 18,
96-100 Skierniewice, Poland

Cucumber mosaic virus (CMV), the type member of genus *Cucumovirus* in the family *Bromoviridae*, is a positive-sense RNA plant virus with a tripartite genome. CMV has the widest host range among plant viruses encompassing more than 1,200 species in over 100 families of dicotyledonous and monocotyledonous plants (Palukaitis and García-Arenal 2003). Numerous CMV isolates described so far, can be divided into two major groups—I and II on the basis of serological data, nucleic acid hybridization, peptide mapping of the coat protein (CP) and/or sequence analysis of representative isolates (for review see: Palukaitis et al. 1992). More recently, further division of group I into groups IA and IB was proposed by Roossinck et al. (1999) based on phylogenetic analysis of the RNA3 5′ non-translated region (NTR) and CP gene.

Generally, there are no clear differences in the host range of isolates belonging to those groups, and most CMV strains can easily be transmitted into numerous herbaceous plants. The exception was a number of CMV isolates found in lily plants showing distinct biological properties. Most of them were not able to infect many species of tobacco and cucurbits (Jung et al. 2000; Ryu et al. 2002; Lee et al. 2007) and they induced mild systemic symptoms on other herbaceous test plants (Kamińska 1996; Yamaguchi et al. 2005; Lee et al. 2007). It was reported that lily isolates Li (Ryu et al. 2002) and Ly8 (Lee et al. 2007) were able to infect *Nicotiana tabaccum* cv. Xanti-nc and/or *N. glutinosa* systemically, but they did not induce even local infection on *Cucumis sativus* plants which is considered to be a good propagation host for the virus. Moreover, all previously described lily isolates were classified to group I of CMV, and their sequences were highly conserved regardless of lily species and/or variety as well as geographic origin (Chen et al. 2001). On the basis of these characteristics, the hypothesis was proposed that lily isolates constitute a unique pathological population, evolutionary adapted to lily plants (Chen et al. 2001; Ryu et al. 2002; Lee et al. 2007).

In this study the serological properties and sequences of RNA3 fragments were compared for four CMV isolates found in lily plants (Cas, CB, P26, Simp2) and eight other virus isolates originating from delphinium (Del), honeysuckle (Wic and WicDS), impatiens (Imp), dahlia (D), cucumber (J and M) as well as redcurrant (Porz) plants. Biological properties of two lily isolates (Cas and P26), being the subject of this study, were partially characterized by Kamińska (1996), but the virus was neither sequenced nor serotyped.

CMV isolates were maintained in the plant virus collection at the Virology Laboratory, Research Institute of Pomology and Floriculture, Skierniewice, Poland. Isolate Del was kindly provided by Dr. J. Staniulis (Institute of Botany, Vilnius, Lithuania). An isolate of peanut stunt virus (PSV), obtained from Prof. H. Pospieszny (Institute of Plant Protection, Poznań, Poland) and tomato aspermy virus (TAV) from our virus collection were included for comparison. All isolates were maintained in *N. rustica* plants during experimental period.

Serological properties of the isolates were studied in DAS-ELISA using CMV-specific polyclonal antibodies (DTL and ToRS, Loewe Biochemica, Germany; M and Wic, Berniak et al. 2010) and group-specific monoclonal antibodies (CMV I and CMV II, Agdia, Biokom, Poland). Most of the tested isolates reacted with CMV-specific polyclonal antibodies (Table 1). The exceptions were: isolate P26 found in lily plant that did not react with M antibodies and isolate Porz from redcurrant that did not show a reaction with ToRS antibodies. Based on the reaction with monoclonal antibodies two CMV lily isolates Cas and CB as well as isolates originating from cucumber (J, M) were classified as members of group I. Another tested lily isolate (Simp2) and isolates D, Del, Imp, Porz, Wic and WicDS were classified to group II of CMV. Lily isolate P26 could not be classified in that experiment due to the lack of positive reaction with both monoclonal antibodies.

Total RNA was extracted from leaves of CMV-inoculated *N. rustica* plants using RNeasy Plant Mini Kit (Qiagen, Biokom, Poland). Fragments of viral RNA3 were amplified using cucumovirus-specific

Table 1 Reaction of CMV isolates with polyclonal and monoclonal antibodies in ELISA

CMV isolate	Polyclonal IgGs				Monoclonal IgGs	
	M	Wic	DTL	ToRS	CMV I	CMV II
Cas	+++	+++	++	++	+++	–
CB	+++	+++	++	++	+++	–
D	++	+++	+++	+++	–	++
Del	+++	+++	+	++	–	+++
Imp	++	+++	++	+++	–	+++
J	+++	+++	+++	+++	+++	–
M	+++	+++	++	++	+++	–
P26	–	++	+	+	–	–
Porz	++	+++	++	–	–	++
Simp2	++	+++	+	+	–	+++
Wic	++	+++	++	+++	–	++
WicDS	+	+++	+	+	–	±
PSV	–	–	±	±	–	–
TAV	–	–	–	–	–	–
N.rust.	–	–	–	–	–	–
buffer	–	–	–	–	–	–

ELISA results were scored as: '+++' for A_{405} values greater than 0.8, '++' for measurements of 0.4 to 0.8, '+' for measurements of 0.2 to 0.4, '±' for values ranging from 0.1 to 0.19 and '–' for values lowers than 0.1. *TAV* tomato aspermy virus, *PSV* peanut stunt virus, *N. rust.* healthy tobacco

(CPTALL5-CPTALL3, Choi et al. 1999) and CMV-specific (CMV1-CMV2, Wylie et al. 1993; D3F-D3R, Graves and Roossinck 1995; M1-M2, Lin et al. 2004; 3a780F-CP50R, Śliwa et al. 2008) primer pairs. Reverse transcription and amplification was performed using the Titan One Tube RT-PCR System (Roche Diagnostics, Poland) according to manufacturer's recommendation. Sequences of RNA3 fragments were read from both strands, directly from purified amplification products, using the same primers as for the original PCR and additionally designed primer cmv420r (Śliwa et al. 2008). Sequencing was performed in an AbiPrism 3100 Genetic Analyzer apparatus (Applied Biosystems, USA), in Maria Skłodowska Memorial Cancer Center and Institute of Oncology, Warsaw, Poland. Aligned nucleotide (nt) sequences of coat protein (CP) and movement protein (MP) genes and deduced amino acid (aa) sequences were analyzed and their identities determined using the Lasergene v. 7.1 software package (DNASTAR, USA).

The lengths of CP and MP ORFs of tested isolates were identical with those of previously described CMV reference strains: the CP ORF consisted of 657 nt, thus encoded a coat protein of 218 amino acids, whereas the length of MP ORF was 840 nt which corresponds to 279 aa in the movement protein. Tested isolates differed in their lengths of RNA3 IGR, which was: 283 nt for D isolate, 289 nt for isolates CB and Imp, 292 nt for Cas, Porz and WicDS isolates, 293 nt for Del and P26 isolates and 294 nt for isolate Wic.

A phylogenetic relationship of tested isolates was estimated using the neighbour-joining method in MEGA software v. 4.0.2 (Tamura et al. 2007). Analysis of CP ORF and amino acid sequences (Fig. 1), performed for studied isolates together with CMV group IA, IB and II reference strains and two other members of genus *Cucumovirus* (PSV and TAV), confirmed the ELISA data indicating that isolates Cas, CB, J and M belong to group I, whereas isolates D, Del, Imp, Porz, Simp2, Wic and WicDS belong to group II. The problematic lily isolate P26, which did not react with CMV group-specific monoclonal antibodies, clustered together with group II isolates. Within group I, tested isolates showed a close relationship with group IA representatives, as they formed a separate cluster. Identical results of the isolates classification were obtained when MP nucleotide and amino acid sequences were analyzed (data not shown).

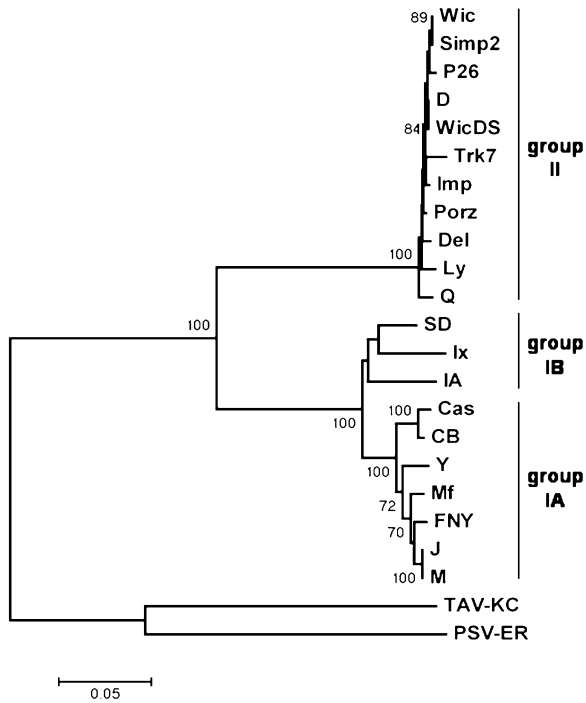

Fig. 1 Phylogenetic analysis of the aa sequences of the CMV CP. The evolutionary history was inferred using the neighbor-joining method in MEGA software v. 4.0.2. GenBank accession numbers of studied Cas, J, M, Imp, P26, Wic, D, Porz, Del, CB, WicDS and Simp2 isolates were: DQ018286, DQ018287, DQ018288, DQ018289, DQ018290, DQ018291, DQ018292, DQ639763, EU191025, EU191026, EU191027 and FJ621495, respectively. CMV reference strains used for RNA3 sequence comparison were: Fny, Mf, Y from group IA (GenBank acc. no. D10538, AJ276481, D12499, respectively), IA, Ix and SD from group IB (acc. no. AB042294, U20219, AB008777), as well as Ly, Q and Trk7 from group II (acc. no. AF198103, M21464, L15336). Isolates of TAV (strain KC; acc. no. AJ237849) and PSV (strain ER; acc. no. U15730) were included as out-groups

Detailed analysis of the CP and MP ORFs revealed high nucleotide sequence identity between Cas and CB isolates originating from lily plants (99.1% and 98.8%, respectively). Deduced amino acids sequences of the MP of Cas and CB isolates shared 99.6% similarity, with only one amino acid change in the protein sequence. Amino acids sequences of the coat protein of those isolates were identical. The CP nucleotide sequence identity of Cas and CB isolates in relation to two other tested group I isolates (M and J) was slightly lower (97.3%). The sequences of tested isolates were compared with sequences deposited in GenBank using the BLASTN algorithm (Altschul et al. 1997), accessed through the home page of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.

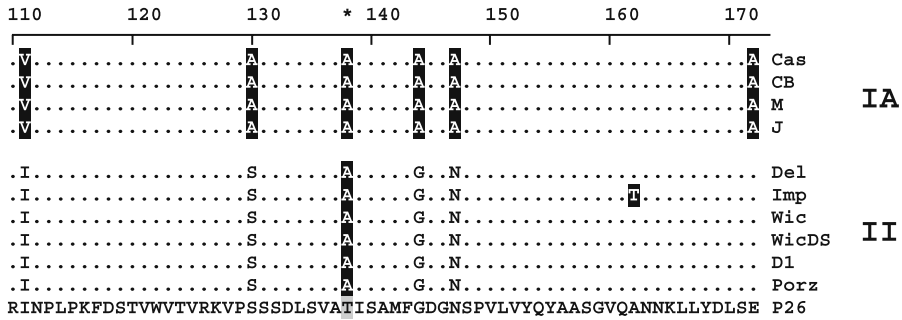

Fig. 2 Alignment of deduced amino acid (aa) sequences of coat protein fragment of the studied CMV isolates, corresponding to R^{110}–E^{173} of isolate P26. Dots represent aa identical in all studied isolates. Amino acid residues different from the ones in P26 isolate are shown in inverted colour (white on black). Star indicates position of aa mutation A/T^{138}, probably involved in the interaction with CMV-II specific monoclonal antibody

gov). The comparison revealed that isolates Cas and CB were highly similar to those of previously described CMV isolates found in different lily cultivars in Korea, the Netherlands and Taiwan (acc. no. AJ131615, AJ131617, AJ131619, AJ495841, AJ296154).

Two other CMV lily isolates, P26 and Simp2, showed high CP and MP nucleotide sequence identity to each other (99.5%) as well as to six tested group II isolates originating from other ornamental plant species (98.8–99.5%). Only 77.6% to 77.1% sequence identity was observed between these two isolates and all previously described CMV isolates originally found in lily plants. The comparison of sequences of P26 and Simp2 with sequences deposited in GenBank revealed that they were most similar to CMV isolates originating from alstroemeria, pepper and tomato plants isolated in Australia, Japan and the Netherlands (acc. no. AB176847, AJ276587, AJ3043997, AJ585519). Thus, our results provide negative verification of the hypothesis about limitation of the population of CMV isolates naturally infecting lily plants to a subset of group I.

In order to explain the lack of reaction of P26 isolate with group II-specific monoclonal antibodies, comparative analysis of the predicted CP amino acid sequence has been done for the isolates of known serological characteristics. Among group II isolates, four amino acid substitutions appeared and only one of them was unique for P26 isolate: change from alanine at position 138 to threonine ($A^{138} \rightarrow T^{138}$) (Fig. 2). It is not clear to us how this substitution translated into different antigenic properties. A direct participation of the aa at position 138 in the specific epitope seems unlikely. Alanine at position 138 appears in the CP of CMV isolates belonging to both groups. Additionally, this residue does not fall into any one of the six recognised antigenic motifs indicated by He et al. (1998) for CMV isolate Fny.

The more probable mechanism may be indirect—replacing hydrophobic alanine, present in most CMV-II group isolates with hydrophilic threoine apparently may have influenced the conformation of neighbouring amino acid residues. Smith et al. (2000) determined the structure of CMV-Fny (group I isolate) coat protein to 3.2 Å resolution using X-ray crystallography, and reported that amino acids 138, 144 and 147 were located very close one to each other on αEF structure in the 3-D model of CP subunits. Since glycine at position 144 (G^{144}) and asparagine at position 147 (N^{147}) are present in the group II isolates (including P26), but not in group I isolates, we speculate that these residues may be critical for group II recognition by monoclonal antibody. Replacing hydrophobic A^{138} with hydrophilic T^{138} may have changed the conformation of the whole αEF structure, and in particular it may have reduced the interaction of G^{144} and N^{147} with group II-specific monoclonal antibodies. We cannot exclude, however, the possibility that some post-transcriptional modifications may also be responsible for particular properties of isolate P26 coat protein.

To our knowledge, this is the first report on CMV group II isolates occurring in lily plants.

Acknowledgments This study was supported by the Ministry of Science and Higher Education (Project No. 2P06R 047 26).

References

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., et al. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389–3402.

Berniak, H., Malinowski, T., & Kamińska, M. (2010). Characterization of polyclonal antibodies raised against two isolates of *Cucumber mosaic virus*. *Journal of Plant Pathology*, 92, 233–236.

Chen, Y. K., Derks, A. F. L. M., Langeveld, S., Goldbach, R., & Prins, M. (2001). High sequence conservation among cucumber mosaic virus isolates from lily. *Archives of Virology, 146*, 1631–1636.

Choi, S. K., Choi, J. K., Park, W. M., & Ryu, K. H. (1999). RT-PCR detection and identification of three species of cucumoviruses with a genus-specific single pair of primers. *Journal of Virological Methods, 83*, 67–73.

Graves, M. V., & Roossinck, M. J. (1995). Characterization of defective RNAs derived from RNA3 the Fny strain of cucumber mosaic cucumovirus. *Journal of Virology, 69*, 4746–4751.

He, X., Liu, S., & Perry, K. L. (1998). Identification of epitopes in cucumber mosaic virus using a phage-displayed random peptide library. *Journal of General Virology, 79*, 3145–3153.

Jung, H. J., Ueda, S., Ryu, K. H., Lee, S. Y., & Choi, J. K. (2000). A novel strain of *Cucumber mosaic virus* isolated from *Lilium longiflorum*. *Plant Pathology Journal, 16*, 306–311.

Kamińska, M. (1996). Virus infection of lilies in Poland. *Phytopathologia Polonica, 11*, 51–58.

Lee, J. A., Choi, S. K., Yoon, J. Y., Hong, J. S., Ryu, K. H., Lee, S. Y., et al. (2007). Variation in the pathogenicity of lily isolates of *Cucumber mosaic virus*. *Plant Pathology Journal, 23*, 251–259.

Lin, H. X., Rubio, L., Smythe, A. B., & Falk, B. W. (2004). Molecular population genetics of *Cucumber mosaic virus* in California: Evidence for founder effects and reassortment. *Journal of Virology, 78*, 6666–6675.

Palukaitis, P., & García-Arenal, F. (2003). Cucumber mosaic virus. No. 400 in: Descriptions of Plant Viruses. Asssociation of Applied Biologists, Warwick, U.K.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G., & Francki, R. I. B. (1992). Cucumber mosaic virus. *Advances in Virus Research, 41*, 281–348.

Roossinck, M. J., Zhang, L., & Hellwald, K. H. (1999). Rearrangements in the 5′ nontranslated region and phylogenetic analyses of cucumber mosaic virus RNA3 indicate radial evolution of three subgroups. *Journal of Virology, 73*, 6752–6758.

Ryu, K. H., Park, H. W., & Choi, J. K. (2002). Characterization and sequence analysis of a lily isolate of *Cucumber mosaic virus* from *Lilium tsingtauense*. *Plant Pathology Journal, 18*, 85–92.

Śliwa, H., Kamińska, M., & Malinowski, T. (2008). Detection and identification of Cucumber mosaic virus isolate from red currant 'Rosetta'. *Acta Horticulturae, 780*, 55–60.

Smith, T., Chase, E., Schmidt, T., & Perry, K. L. (2000). The structure of Cucumber mosaic virus and comparison to Cowpea chlorotic mottle virus. *Journal of Virology, 74*, 7578–7586.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*, 1596–1599.

Wylie, S., Wilson, C. R., Jones, R. A. C., & Jones, M. G. K. (1993). A polymerase chain reaction assay for cucumber mosaic virus in lupin seeds. *Australian Journal of Agricultural Research, 44*, 41–51.

Yamaguchi, N., Seshimo, Y., Yoshimoto, E., Ahn, H., Ryu, K. H., Choi, J. K., et al. (2005). Genetic mapping of the compatibility between a lily isolate of *Cucumber mosaic virus* and satellite RNA. *Journal of General Virology, 86*, 2359–2369.