Eur J Plant Pathol (2008) 122:447–450
DOI 10.1007/s10658-008-9283-4

Turnip ringspot virus recognised on Chinese cabbage in Russia

Igor Koloniuk · Josef Špak · Karel Petrzik

Received: 21 May 2007 / Accepted: 28 January 2008 / Published online: 22 February 2008

Abstract The nucleotide sequence of the 3′-terminal part of the RNA1 genome segment of the M12 isolate of comovirus *Turnip ringspot virus* (TuRSV) was established. This isolate originated in 1989 in Moscow (Russia) from Chinese cabbage with *Radish mosaic virus*-like symptoms. Comparison of the M12 RNA polymerase amino acid sequence with that of Radish mosaic virus (RaMV) revealed significant differences; these proteins are of different length and are only about 75% identical. On the other hand, the amino acid sequence of the M12 RNA polymerase was more than 94% identical with that of TuRSV recently described in Toledo (USA). We conclude that TuRSV occurs in Europe as well as in America and probably represents a new species of the genus *Comovirus*.

Keywords New virus · Phylogeny · RNA polymerase · Sequence comparison

I. Koloniuk · J. Špak · K. Petrzik (✉)
Department of Plant Virology,
Institute of Plant Molecular Biology, Biology Centre
of the Academy of Sciences of the Czech Republic v.v.i.,
Branišovská 31,
370 05 České Budějovice, Czech Republic
e-mail: petrzik@umbr.cas.cz

I. Koloniuk
e-mail: koloniuk@umbr.cas.cz

I. Koloniuk
Faculty of Sciences, University of South Bohemia,
Branišovská 31,
370 05 České Budějovice, Czech Republic

The genus *Comovirus* comprises fifteen definitive virus species with the last one added more-than 20 years ago (Brunt et al. 1982). Comoviruses are characterised by a bipartite + ssRNA genome encapsidated separately in isometric virions constructed from two polypeptide species. Most Comoviruses have a narrow host range with 11 of the 15 species being restricted to a few species of the family *Leguminosae*. The exceptions are *Andean potato mottle virus* infecting *Solanaceae*, *Squash mosaic virus* infecting *Cucurbitaceae*, *Ullucus virus C* infecting *Basellaceae* and *Radish mosaic virus* (RaMV) infecting different wild as well as cultivated *Brassicaceae* plants.

In 2006, partial sequences of another comovirus that infects *Brassicaceae* occurred in GenBank with the provisional name *Turnip ringspot virus* (TuRSV, accession numbers: DQ665367, EF191015). This virus found in Toledo (OH, USA), produced chlorotic ringspot and line pattern symptoms in turnip and was mechanically transmissible to plants in the *Brassicaceae* (Khandekar et al. 2007). Initially, it was considered to be a strain of RaMV. However, we revealed, that the nucleotide sequence of the published partial RNA polymerase gene (RdRp) differed significantly from that of the Czech RaMV1 isolate (Petrzik et al. 2005). In addition, hybridisation of TuRSV with many RaMV-specific primers failed, which indicated considerable genomic differences. We hypothesise that the Toledo isolate represents either a distant (recombinant) strain of RaMV or a new virus species.

RaMV is probably distributed worldwide. It was originally described in California (USA) in 1939. Later it was found in Japan, Europe (Brunt et al. 1996), Africa (Koenig and Fisher 1981) and more recently in Asia (Farzadfar et al. 2004). Until 2005, when the nucleotide sequence of the RdRp gene was published (Petrzik et al. 2005), no sequence data of RaMV was available for detection and molecular comparison.

During amplification and sequencing of part of the large capsid protein gene of our collection of RaMV isolates, we detected four isolates that differed considerably from the RaMV1 sequence, but were similar to the Toledo isolate sequence. All four isolates originated from symptomatic Chinese cabbage (*Brassica pekinensis*) plants collected at one location in Moscow, Russia (Špak and Kubelková 2000). One of these Moscow isolates (M12) was revived from the original lyophilized sample and propagated by mechanical inoculation on white mustard plants (*Sinapis alba*). The virus was purified by the PEG-6000/NaCl precipitation/centrifugation method of Klootwijk et al. (1977) and its RdRp gene was sequenced. RNA was isolated from the purified virus using the RNeasy Plant Mini kit (Qiagen). CDNA was synthesised using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's recommendations. A PCR-based primer walking approach was used to amplify the 3′-terminal part of RNA1. The amplification products were sequenced directly with amplification primers, or cloned in pCR4-TOPO vector (Invitrogen) and cycle sequenced with M13 primers and the BigDye ver.3.1 (Applied Biosystems, Warrington, UK) sequencing kit. The nucleotide sequence obtained was submitted to GenBank (accession number EF555589).

The strategy of Comovirus replication involves translation of the polyprotein with subsequent cleavage by virus-specific protease. The exact cleavage motif has only been identified for CPMV, (Wellink et al. 1986). For the other comoviruses, the cleavage sites were proposed according to the amino acid (aa) homology with this virus. The cleavage motifs recognised by the protease are Q/G, S, D, M, A, T, N, F or C in different viruses. The Q/H motif found at the beginning of RdRp in the M12 isolate also occurred in RaMV (Petrzik et al. 2005) and correlated best with the multiple alignments with the other comoviruses. The length of RdRp in comoviruses varies from 703 to 713 aa. In the M12 isolate it was 712 aa long and the relative molecular mass of this protein was about 81.5 K. All motifs (Ia–VIII) proposed by Koonin et al. (1991) for positive-strand viral RNA polymerases are located in the central part of this gene (Fig. 1).

RaMV has two serological sub-groups, one comprising European isolates and the other comprising isolates from California and Japan (Brunt et al. 1996). We compared the sequence of the RdRp of the M12 isolate with those of the representatives of both subgroups, i.e. the Czech RaMV1 isolate and the Japanese RaMV-J isolate (Komatsu et al. 2007). The overall amino acid sequence identity of the M12 RdRp gene was 75.4% (175 of 712 positions are different) with RaMV1 and 76.1% (170 differences) with RaMV-J. This is on the border of the comovirus species demarcation criteria (less than 75% homology of the polymerase aa sequence; Le Gall et al. 2005). On the other hand, the pairwise RaMV1/RaMV-J identity is 95% (35 of 707 positions are different) and the aa sequence of the M12 isolate is 94.4% identical (22 of 396 positions are different) to that of the partially sequenced Toledo (To) isolate of TuRSV (GenBank AC No. DQ665367). There are no further TuRSV isolates from different locations currently available to study intra-species variability. Nevertheless, in a diversity study of another comovirus *Bean pod mottle virus* (BPMV), the overall nucleotide sequence identity between strains K-G7 and K-Ha1 has been established to be 85.5% and 86.9% for RNA-1 and RNA-2, respectively. Furthermore, the percent deduced aa sequence identity of a 653 nt RNA-2 fragment in pairwise comparisons of seven different BPMV isolates was at least 98.6% (Gu et al. 2002). Our comparison of aa identity of the polymerase protein of all five isolates of BPMV available in public databases (AAM73715, AAM73718, AAW69769, AW69768, NP_612349) revealed a minimum identity of 94.4%. A similar comparison of the RdRp aa sequence of five *Broad bean wilt virus* (genus *Fabavirus*; family *Comoviridae*) isolates showed a minimum of 91.1% aa identity (data not shown). These nucleotide and amino acid identities suggest that the common intra-species identity of comoviruses and related fabaviruses is about 90% at the amino acid level. This is very different from the 75.4% and 76.1% identity between the M12 isolate and the RaMV1 and RaMV-J strains, respectively.

```
                                                                                                           100
TuRSV M12   HAQQYFDYLAFEEREGDGIARIATLKKGIHIPVPTKTSLVETPAEMHLDTPCDKYPSILSNTDPRLEVSGVTDYDPFKAGILKYQNPMGELDQDLLQEVC
RaMV 1      ...KF....P.A.K..E..EKV.M.Q......L....A.....V.W..............SD........H.............KD...VV.NK..I...

                                                                      Ia
                                                                                                           200
TuRSV M12   DEIEETWRDCQEEFETFEEVSLEEAINGIKGMEYMERIPMATSEGFPHILTRSHGEKGKIRFVEGDGEDLTLLPNTSVTEALDIMEEHLEHEVPTLIGIE
TuRSV To    ---------------------------------------------------------------------------------------------------.
RaMV 1      E...QS.L..K..G.........Q....VRDVD..D...L..........S...............S.AMM.I.G...A...E...AQ..N.........

                 I                 II                        III                    IV
                                                                                                           300
TuRSV M12   CPKDEKLPYRKIFEKPKTRCFSILPMEYNLLVRRRFLKFVRFIMRRRDILPCQVGINPYGMEWTDLAARLKSKGNNILCCDYSSFDGLLSKQVMAAMASM
TuRSV To    ............................................K................................V.................T....
RaMV 1      ........H............................T.......N.NV..................M...R......................K..SD.

                                                          V                                       VI
                                                                                                           400
TuRSV M12   INSFCGGDTSIKKKRENLLMACCSRYAICKSTVWRVECGIPSGFPLTVICNSIFNEILIRYSFKAIMREQKVPDLIPISFDKYVGMVTYGDDNLLSVSEV
TuRSV To    ............R..................D....................................K....G..S.......................
RaMV 1      .......SE.S.R...H.............NS..K...............................L.S.....MVSV...E..S..V............

                                                       VII             VIII
                                                                                                           500
TuRSV M12   VKPFFDGKRLKEFLAKHKVVITDGKDKTSPYLLFRRLEDCDFLKRGFKKDRSGFFWNSPEEKESLWAQLHYINVTNLEQHEAYKTNLVNVLRELYMWDIN
TuRSV To    ...Y...........................................R..KN.I...A........................................V.
RaMV 1      I..Y...........TLRIT..........F.Q..C............N--R.LY.DA.............V.AN...K.........T.....F...KK

                                                                                                           600
TuRSV M12   ECAALRKKALQRVSWLLPSDLPTVAQIEKWYASCRGRYVPDSADSINFLLDQEHLGPLLAPQGVQRGVRITDQVRTVNLAHENHTTRKPGELWILCQTLY
TuRSV To    ..S..........E..V...........E...T.....M..............................L.........................-----
RaMV 1      ...E..R.....I...V....Q......A...GN..K.L...S...SM..QK.N.........E...IE.MPR...A......FRDA.DD.V.V....M.

                                                                                                           700
TuRSV M12   PHSMLPEGVRAINWPLGTGRGGLPTTSWVEENVKRPTSEIRKTISAALKRGEQIVFATRDNILPCNMLAVLFLVIEGSIKVETSNMIISAVIQQCKTLGY
RaMV 1      ..GR.....I.V...V.........ST.MD..F......L..KLKS..DN.KKL.....EG.....IM......L.KKM.P.E..V.L..A.L...S...

                     712
TuRV M12    LVRECDFAFFAT
RaMV 1      .P.......
```

Fig. 1 Comparison of the amino acid sequence of the *Turnip ringspot virus* isolate M12 (TuRSV M12), isolate Toledo (TuRSV To) (DQ665367) and RaMV1 RNA polymerase protein, respectively. Only variable positions are shown. Conserved polymerase motifs Ia–VIII are indicated

Based on this comparison, we conclude that M12 and To are isolates of one virus, TuRSV, which differs from RaMV. The exact taxonomic classification of TuRSV will not be possible until the whole genome has been sequenced.

Multiple alignments of amino acid sequences were obtained with CLUSTALW and the phylogenetic and molecular evolutionary analyses were conducted using the neighbour-joining method in MEGA version 3.1 (Kumar et al. 1994). The amino acid sequences of comoviruses were obtained from GenBank: *Andean potato mottle virus* (APMoV; AAA42422), *Bean pod mottle virus* (BPMV; NP_734070), *Cowpea mosaic virus* (CPMV; NP_734057), *Cowpea severe mosaic virus* (CPSMV; NP_734062), *Radish mosaic virus* (RaMV; AAY32935, BAF75830), *Red clover mosaic*

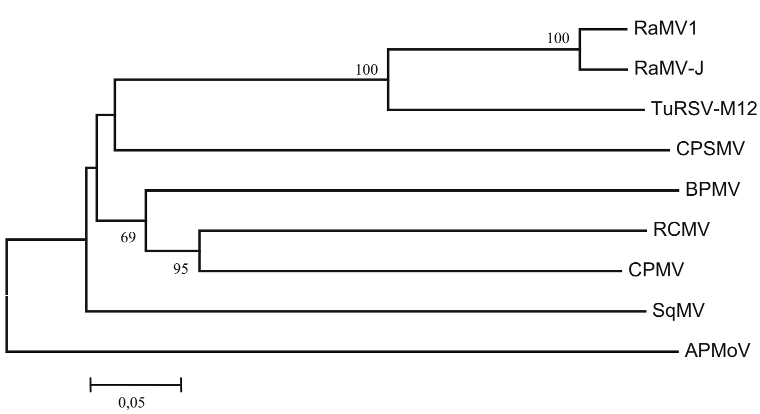

Fig. 2 Phylogenetic tree of comoviruses based on amino acid sequence comparison of RNA-dependent RNA polymerase. Bootstrap values higher than 50 are denoted in the tree. Abbreviations and GenBank accession numbers: *APMoV*, *Andean potato mottle virus*, AAA42422; *BPMV*, *Bean pod mottle virus*, NP_734070; *CPMV*, *Cowpea mosaic virus*, NP_734057; *CPSMV*, *Cowpea severe mosaic virus*, NP_734062; *RaMV*, *Radish mosaic virus*, AAY32935, BAF75830; *RCMV*, *Red clover mosaic virus*, NP_734030; *SqMV*, *Squash mosaic virus*, NP_734012; *TuRSV*, *Turnip ringspot virus*, this paper

virus (RCMV; NP_734030) and *Squash mosaic virus* (SqMV; NP_734012). The phylogenetic analysis based on the amino acid sequences of the RdRp placed the TuRSV-M12 isolate close to the RaMV branch (Fig. 2).

TuRSV occurs in Europe and America and although related, we conclude RaMV and TuRSV are different virus species of the genus *Comovirus*.

Acknowledgements Authors thank Dr. S.M. Leisner (University of Toledo, OH, USA) for kindly providing the Toledo isolate of TuRSV. This work was supported by the grant of the Grant Agency of the Czech Republic No.: 522/07/0053, and the project of the Academy of Sciences of the Czech Republic No.: AV0Z50510513.

References

Brunt, A. A., Barton, R. J., Phillips, S., & Jones, R. A. C. (1982). *Ullucus virus C*, a newly recognised comovirus infecting *Ullucus tuberosus* (Basellaceae). *Annals of Applied Biology*, *101*, 73–78.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., & Zurcher, E. J. (1996). (eds.) *Radish mosaic comovirus*. Plant Viruses Online: Descriptions and lists from the VIDE database. Version: 20th August 1996. http://image.fs.uidaho.edu/vide/descr662.htm

Farzadfar, S., Pourrahim, R., Golnaraghi, A. R., Jalali, S., & Ahoonmanesh, A. (2004). Occurrence of *Radish mosaic virus* on cauliflower and turnip crops in Iran. *Plant Disease*, *88*, 909.

Gu, H., Clark, A. J., de Sá, P. B., Pfeifer, T. W., Tolin, S., & Ghabrial, S. A. (2002). Diversity among isolates of *Bean pod mottle virus*. *Phytopathology*, *92*, 446–452.

Khandekar, S., Rajakaruna, P., & Leisner, S. (2007). Identification and host relations of *Turnip ringspot virus*, a novel comovirus discovered in Ohio. *Phytopathology*, *97*, S57.

Klootwijk, J., Klein, I., Zabel, P., & van Kammen, A. (1977). *Cowpea mosaic virus* RNAs have neither m7GpppN nor mono-, di- or triphosphates at their 5′ ends. *Cell*, *11*, 73–82.

Koenig, R., & Fisher, H. U. (1981). A Moroccan *Radish mosaic virus* isolate from turnip. *Plant Disease*, *65*, 758–760.

Komatsu, K., Hashimoto, M., Maejima, K., Ozeki, J., Kagiwada, S., Takahashi, S., et al. (2007). Genome sequence of a Japanese isolate of *Radish mosaic virus*: the first complete nucleotide sequence of a crucifer-infecting comovirus. *Archives of Virology*, *152*, 1501–1506.

Koonin, E. V., Choi, G. H., Nuss, D. L., Shapira, R., & Carrington, J. C. (1991). Evidence for common ancestry of a chestnut blight hypovirulence-associated double-stranded RNA and a group of positive-strand RNA plant viruses. *Proceedings of the National Academy of Sciences of the United States of America*, *88*, 10647–10651.

Kumar, S., Tamura, K., & Nei, M. (1994). MEGA: molecular Evolutionary Genetics Analysis software for microcomputers. *Computer Applications in the Biosciences*, *10*, 189–191.

Le Gall, O., Iwanami, T., Karasev, A. V., Jones, T., Lehto, K., Sanfaçon, H., et al. (2005). Comoviridae. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Deselberger, & L. A. Ball (Eds.) *Virus taxonomy, classification and nomenclature of viruses, VIIIth Report of the International Committee on the Taxonomy of Viruses* (pp. 807–818). London: Elsevier/Academic.

Petrzik, K., Holá, M., & Špak, J. (2005). Complete nucleotide sequence of *Radish mosaic virus* RNA polymerase gene and phylogenetic relationships in the genus Comovirus. *Acta Virologica*, *49*, 271–275.

Špak, J., & Kubelková, D. (2000). Serological variability among European isolates of *Radish mosaic virus*. *Plant Pathology*, *49*, 295–301.

Wellink, J., Rezelman, G., Goldbach, R., & Beyreuther, K. (1986). Determination of the proteolytic processing sites in the polyprotein encoded by the bottom-component RNA of *Cowpea mosaic virus*. *Journal of Virology*, *59*, 50–58.