Arch Virol (2017) 162:3559–3562
DOI 10.1007/s00705-017-3521-z

ANNOTATED SEQUENCE RECORD

Molecular characterization of a beet ringspot nepovirus isolated from *Begonia ricinifolia* in Hungary

Szilvia Kis[1] · Pál Salamon[1] · Viktor Kis[2,3] · György Szittya[1]

Received: 30 May 2017 / Accepted: 31 July 2017 / Published online: 10 August 2017

Abstract A nepovirus was isolated from *Begonia ricinifolia* showing chlorotic ringspot and line pattern symptoms. The purified virus had spherical particles of ca. 30 nm and contained a single coat protein subunit of ca. 56 kDa. The complete nucleotide sequence of the bipartite viral genome was determined. RNA 1 is 7394 nucleotides long, flanked by 5' and 3' untranslated regions (UTR), and followed by a 3' poly-A tail. It contains a single 6810 nt long open reading frame (ORF), which is translated into a 255 kDa polyprotein composed of 2269 amino acids. The 4684 nt long RNA 2 has a 4053 nt long ORF which encodes a single polyprotein of 1350 amino acids with a molecular weight of 149 kDa. Sequence comparisons revealed that the virus isolated from *B. ricinifolia* has the highest sequence similarity to beet ringspot virus and should be considered as a strain of BRSV. This is the first report on the occurrence of BRSV in *B. ricinifolia* and the presence of this virus outside Scotland.

Nepoviruses (genus *Nepovirus*, family *Secoviridae*, suborder *Comovirinae*, order *Picornavirales*) are small icosahedral viruses (ca 28 nm in diameter) with a bipartite positive–strand, poly-A tailed RNA genome. Each of the two genomic RNA encodes a single large polyprotein. RNA 1 ranges in size from 7,2 kb to 8,4 kb and encodes the replication-related proteins. RNA 2 ranges in size from 3,7 kb to 7,3 kb and encodes the coat protein (CP) of 55-60 kDa and the movement protein (MP). Based on the phylogenetic relationships of the CPs, specific size of RNA 2, and cleavage site specificity of the viral protease, nepoviruses are divided into three subgroups (A, B and C) [1].

Beet ringspot virus (BRSV) was originally isolated in Scotland as a soil-borne virus, infecting a wide range of plant species including sugar beet, potato, turnip, wheat, oat, strawberry, many weeds and peach [2]. It was later thought to be a serotype of tomato black ring virus (TBRV-S) but based on the RNA sequence data the ICTV reclassified this virus as a distinct species within subgroup B of the nepoviruses [3]. This study deals with the molecular characterization of the first Hungarian isolate of BRSV (BRSV-Br1) discovered in a new natural host *Begonia ricinifolia* that showed unusual ring spot and line pattern symptoms (Fig.1A).

The Br1 isolate was transferred from symptomatic *B. ricinifolia* to *Nicotiana benthamiana* by sap inoculation and virions were purified from systemically infected *N. benthamiana* leaves [4]. Purified virions negatively stained with 2% uranyl acetate showed spherical particles ca. 30 nm in diameter with hexagonal outlines when examined by transmission electron microscopy (JEOL JEM-1011) (Fig. 1B). Proteins were prepared from virions [5] and separated by electrophoresis in 12% TGX Stain-Free™ FastCast™ Acrylamide Gels (Bio-Rad) in the presence of ProSieve QuadColor™ protein marker (4.6 kDa – 300 kDa) (Lonza). A single protein molecule of ca. 56 kDa characteristic of nepovirus CPs was visualized (Fig. 1C).

✉ György Szittya
szittya.gyorgy@abc.naik.hu

[1] Department for Plant Biotechnology, National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute, Szent-Györgyi Albert ut 4, Gödöllő 2100, Hungary

[2] Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University of Sciences, Budapest, Hungary

[3] Present Address: Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine of the Hungarian Academy of Sciences, Szigony utca 43, Budapest 1083, Hungary

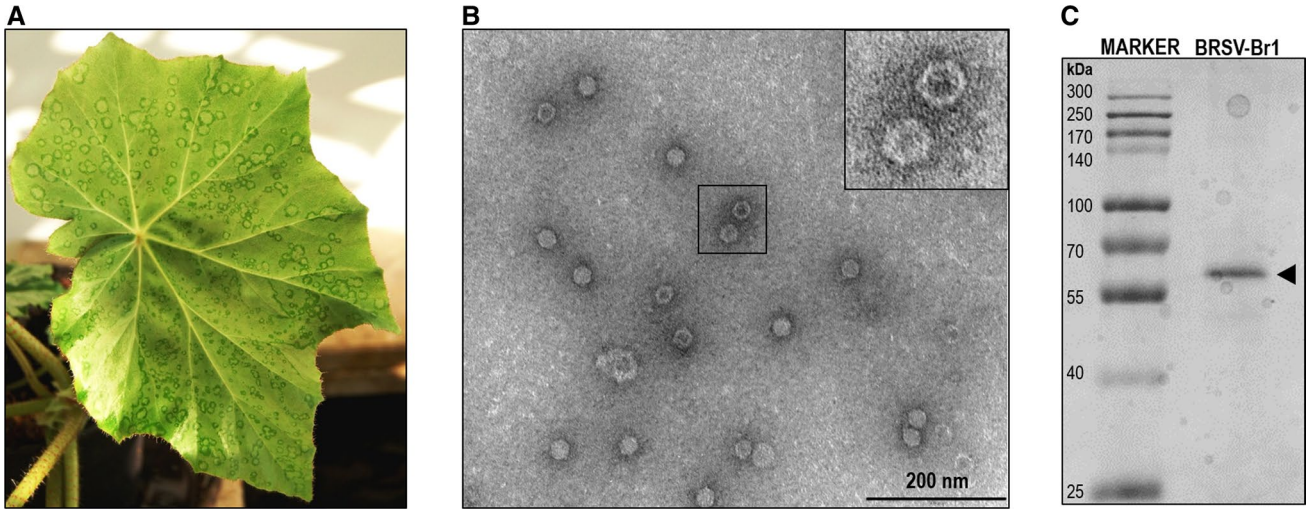

Fig. 1 (**A**) Ringspot and line pattern symptoms in *B. ricinifolia* infected by BRSV-Br1. (**B**) Transmission electron micrograph of negatively stained BRSV-Br1 particles. (**C**) PAGE analysis of the BRSV-Br1 coat protein

Total RNA was extracted from the diseased leaves of *N. benthamiana* using a phenol-chloroform extraction method [6] and used for first-strand cDNA synthesis with the High-Capacity cDNA reverse transcription kit (Applied Biosystems) with the primer oligo(dT) 18 following the manufacturer's protocols.

The complete nucleotide sequences of RNA 1 and RNA 2 (accession number MF141079 and MF141080, respectively) were determined using specific primers derived from the BRSV reference genome sequences deposited in the NCBI database (NC_003693 and NC_003694, respectively). The regions used for the design of specific primers were re-amplified and sequenced to rule out the presence of mutations. The 5'- terminal sequences of RNA 1 and RNA 2 were determined using 5' FirstChoice RLM-RACE kit (Ambion) with virus specific primers. All PCR amplifications were performed with high-fidelity Phusion DNA polymerase (Thermo Fisher Scientific) to ensure that the authentic genome sequence of BRSV-Br1 was obtained. Computer based analysis of the viral genome (Fig. 2A) and phylogenetic analyses (Fig. 2B) were conducted using the EMBOSS software package [7] and MEGA 7 [8], respectively.

BRSV-Br1 RNA 1 is 7394 nt long and contains a single 6810 nt long ORF, which is translated into a 2269 amino acid long polyprotein with a molecular weight of 254,6 kDa. The RNA 1 encoded polyprotein (P1) is predicted to be cleaved by the viral proteinase into five mature proteins. These were identified based on the conserved sequence motifs [1] as the: proteinase cofactor (Pro-cof), NTP binding protein (NTP-b), viral genome-linked protein (VPg), proteinase (Pro) and RNA-dependent RNA polymerase (RdRp) (Fig. 2A). The complete sequence of RNA 2 is 4684 nt long. It contains a 4053 nt long ORF which encodes a single polyprotein of 1350 amino acids with a molecular weight of 149 kDa. Based on homology to other subgroup B nepoviruses [1], the RNA 2 encoded polyprotein (P2) is predicted to be processed into mature protein 2A, the putative movement protein (MP) and the coat protein (CP) (Fig. 2A).

Sequence comparison of P1 and P2 with other members of the genus *Nepovirus*, revealed that the isolate Br1 show 94% and 92 % identity with the corresponding polyproteins of BRSV. Phylogenetic analysis of the predicted amino acid sequence of the putative CP also confirmed that it is most closely related to BRSV and belongs to subgroup B of the genus *Nepovirus* (Fig. 2B). Based on the molecular and phylogenetic data we conclude that isolate Br1 is a strain of BRSV and propose to name it BRSV-Br1.

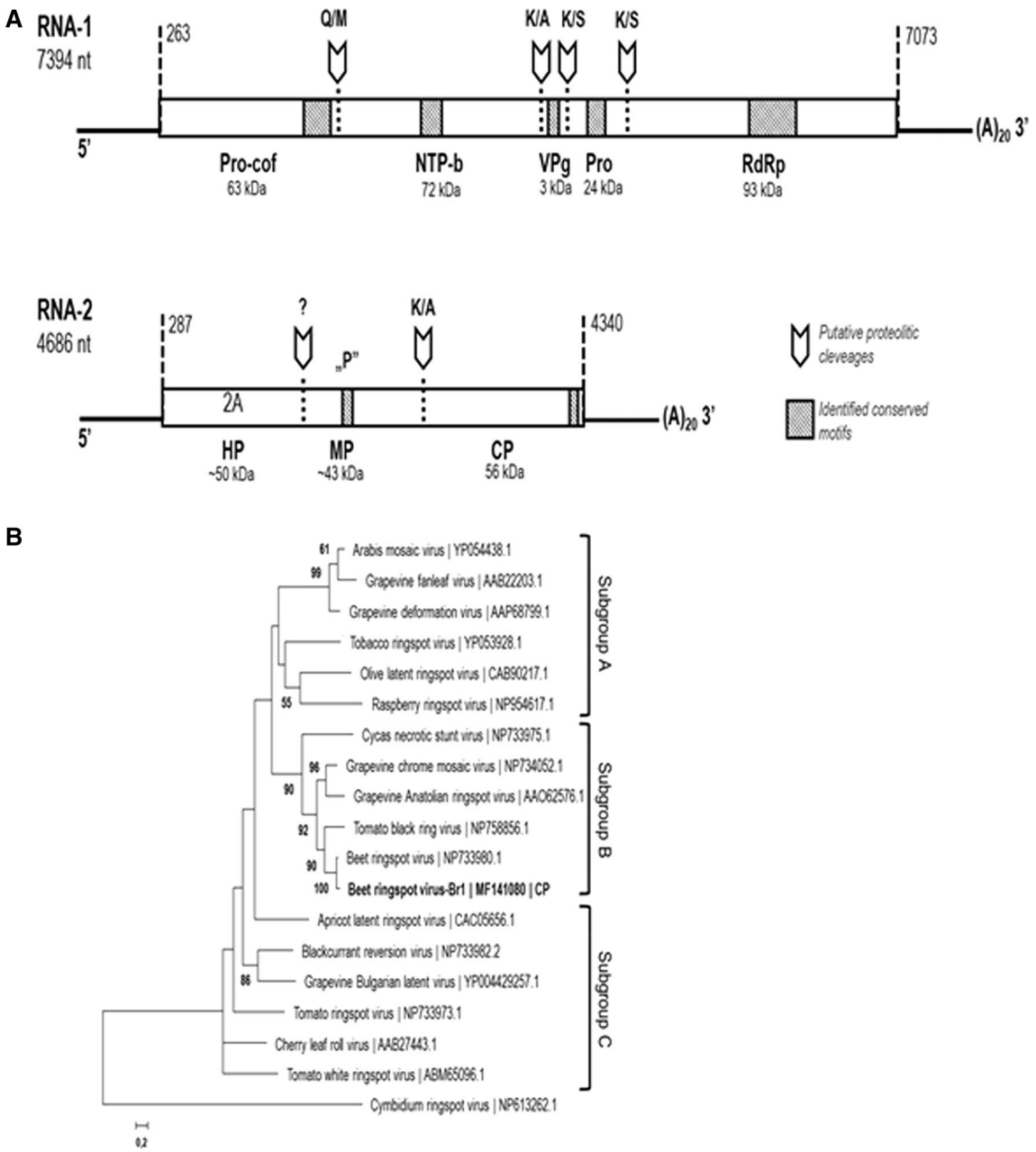

Fig. 2 (**A**) Schematic representation of the genome organization of BRSV-Br1. Grey boxes indicate ORFs, with the names of predicted gene products and M_rs below them. Polyprotein putative cleavage sites that release the specific proteins are indicated (dotted lines) with the sequence of the cleaved dipeptide. The positions of the identified conserved motifs are represented by striped boxes. The putative cleavage sites were deduced by the similarity of the dipeptide alongside the upstream sequence using previously identified or inferred sites from other nepoviruses, fitting the taxonomy demarcation of subgroup B of nepoviruses. Question marks in RNA 2 means that we were unable to identify the exact putative cleavage site to separate 2A from MP and its location and M_rs is an estimation. (**B**) Maximum-likelyhood phylogenetic tree showing the relationship of BRSV-Br1 and other nepoviruses based on alignments of CPs. Branch lengths are proportional to the genetic distances. Cymbidium ringspot virus (genus *Tombusvirus*) was used as outgroup. Numbers on branches indicate percantage of bootstrap support out of 1000 bootstrap replications. Bootstrap percentages greater than 50 % are shown

Acknowledgements The authors are grateful for the helpful comments of A. Auber and P. Gyula.

Compliance with ethical standards

Funding This work was supported by Hungarian National Research, Development and Innovation Office grant K119701. S.K. was funded by the Young Researcher Career Development Program of the Ministry of Agriculture, Hungary.

Conflict of interest All authors declare that they have no conflict of interest.

Human and animal rights statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Fuchs M, Schmitt-Keichinger C, Sanfaçon H (2017) Chapter two—A renaissance in nepovirus research provides new insights into their molecular interface with hosts and vectors. Adv Virus Res 97:61–105
2. Harrison BD (1957) Soil transmission of Beet ringspot virus to peach (*Prunus persica*). Nature 180:1055–1056
3. Pringle CR (1998) Virus taxonomy-San Diego 1998. Arch Virol 143:1449–1459
4. Pinck L, Fuchs M, Pinck M, Ravelonandro M, Walter B (1988) A satellite RNA in grapevine fanleaf virus strain F13. J Gen Virol 69:233–239
5. Wang W, Vignani R, Scali M, Cresti M (2006) A universal and rapid protocol for protein extraction from recalcitrant plant tissues for proteomic analysis. Electrophoresis 27:2782–2786
6. Szittya G, Salamon P, Burgyán J (2000) The complete nucleotide sequence and synthesis of infectious RNA of genomic and defective interfering RNAs of TBSV-P. Virus Res 69:131–136
7. Rice P, Longden I, Bleasby A (2000) EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet. 16:276–277
8. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874