Short communication

Complete Genome Sequence of a *Brome Mosaic Virus* Isolate from the Czech Republic

Sebastien GADIOU and Jiban Kumar KUNDU

Department of Virology, Division of Plant Health, Crop Research Institute, Prague, Czech Republic

Abstract: An isolate of *Brome mosaic virus* (BMV) was originally isolated from *Agropyron repens* and maintained in *Hordeum vulgare.* The full-length genome of this isolate (BMV-CZ) was sequenced. Phylogenetic analysis revealed that BMV-CZ shared a minimum of 95.6% sequence identity, localized in the 5′-UTR of RNA-1 with the other BMV isolates from the database, and a maximum of divergence of 30.8% with *Broad bean mottle virus* localized in the 5′-UTR of RNA-3. This is the first sequence report of full-length BMV from the Czech Republic.

Keywords: BMV; sequence analysis; variability

The family *Bromoviridae* is composed of five genera: *Bromovirus*, *Alfamovirus*, *Ilarvirus*, *Cucumovirus* and *Oleavirus*. *Brome mosaic virus* (BMV) is a member of *Bromovirus*, a genus with single-stranded positive RNA genome (Roossinck *et al.* 2005). The BMV genome is mainly composed of three RNA particles: RNA-1, RNA-2 and RNA-3. The three RNAs are capped by 5′-end and 3′-end sequences mimic tRNA structure. RNA-1 (3.2 kb) encodes the 1a protein (109 kDa), which contains both an N-proximal methyltransferase domain and a C-proximal helicase-like domain. RNA-2 (2.9 kb) encodes the 2a protein (94 kDa), a RNA-dependent RNA polymerase responsible for replication of the viral genome (Sullivan & Ahlquist 1997). RNA-1 and RNA-2 are also involved in RNA replication. RNA-3 is encoded for the coat protein (CP) and the movement protein (MP) (Ahlquist *et al.* 1990). Here we describe a full-length genome sequence of a BMV isolate originally collected by Dr. J. Vacke (CRI, Prague) from couch grass (*Agropyron repens*) and maintained in barley (*Hordeum vulgare*) plants. The sequence analysis showed a close relationship with the other BMV isolates, the maximum divergence in the coding region was localized in the open reading frame (ORF)-3b coding for the coat protein.

The BMV isolate (BMV-CZ) was originally found in *A. repens* from Ruzyně-Prague and mechanically transmitted to *H. vulgare* by Dr. Vacke (CRI, Prague) in 2002. cDNA was obtained by reverse transcription using M-MLV reverse transcriptase (Promega, Madison, USA) according to the manufacturer's instructions using the specific reverse primer of each fragment (Table 1). PCR conditions were as follows: denaturation at 95°C for 3 min; followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 90 s; with a final extension at 72°C for 10 min. PCR fragments were obtained using the primer pairs: BromRev antisense 5-TGGTCTCTTTTAGAGATTTACAG-3 for RNA-1, RNA-2 and RNA-3; RNA1F1 sense 5-GTAGACCACGGAACGAGGTC-3 for RNA-1; RNA2F1 sense 5-GTAAACCACGGAACGAGG-

Table 1. Percent identities of BMV-CZ isolate with those of other bromoviruses

Viruses	Isolates*	RNA-1			RNA-2			RNA-3			
		5'UTR	Replication protein 1a	3'UTR	5'UTR	Polymerase 2a	3'UTR	5'UTR	Movement protein 3a	Coat protein 3b	3'UTR
Brome mosaic virus – BMV	Fescue	95.6	97.9/**98.9**	99.0	97.2	97.9/**98.4**	98.7	98.9	98.3/**97.9**	96.6/**96.8**	95.7
	Russian	95.6	98.3/**99.9**	99.0	95.8	98.3/**98.9**	99.6	98.9	98.1/**97.9**	96.5/**96.8**	95.7
	KU1	95.6	97.7/**98.5**	99.5	94.4	98.2/**98.6**	98.7	98.9	98.2/**97.9**	95.8/**95.7**	95.2
Cowpea chlorotic mottle virus – CCMV	R	70.6	66.4/**70.0**	62.9	71.8	60.4/**61.5**	58.8	38.5	56.5/**53.0**	64.4/**71.1**	57.0
	T	70.6	66.1/**69.7**	61.4	71.8	60.3/**61.1**	58.8	38.5	56.2/**53.0**	64.2/**70.1**	57.0
Cassia yellow blotch virus – CYBV	KU1	73.5	62.6/**63.5**	67.1	66.2	57.0/**54.2**	62.7	35.2	53.0/**49.5**	55.0/**55.1**	60.0
Spring beauty latent virus – SBLV	KU1	58.8	63.7/**67.1**	59.0	59.2	63.7/**65.3**	53.5	42.9	57.4/**50.2**	64.0/**59.4**	50.4
Broad bean mottle virus – BBMV	BBMV	51.6	62.9/**65.7**	52.3	53.2	56.0/**35.7**	52.2	30.8	51.6/**46.7**	52.2/**45.5**	51.2

Numbers in bold type represent the percent of amino acid identities

*Accession number of the isolates:

BMV: Fescue – RNA-1 DQ530423, RNA-2 DQ530424 and RNA-3 DQ530425; Russian – RNA-1 X02380, RNA-2 X01678 and RNA-3 J02042; KU1 – RNA-1 X58456, RNA-2 X58458 and RNA-3 X58459;

CCMV: R- RNA-1 AF325736, RNA-2 AF325737 and RNA-3 AF325738; T- RNA-1 AF325739, RNA-2 AF325740 and RNA-3 AF325741;

CYBV: KU1- RNA-1 AB194806, RNA-2 AB194807 and RNA-3 AB194808;

SBLV: KU1- RNA-1 AB080598, RNA-2 AB080599 and RNA-3 AB080600;

BBMV: RNA-1 M65138, RNA-2 M64713 and RNA-3 M60291

TTC-3 for RNA-2 and BromF1 sense 5-GTAAAATACCAACTAATTCTCGTTCG-3 for RNA-3 and cloned into the pGEM-T Easy Vector (Promega, Madison, USA) to obtain the full sequence. Two independent clones were sequenced for each fragment (Macrogen, Korea). The final contigs were edited with Sequencher 4.8 (Gene Codes Inc, Ann Arbor, USA) and the full sequences of RNA segments (RNA-1, RNA-2 and RNA-3) were successfully produced. Sequences of the virus isolate presented here and those sequences from the GenBank database (National Center for Biotechnology Information-NCBI, USA) were aligned using Clustal-X (Thompson *et al.* 1997) and phylogenetic tree was obtained by Neighbour-Joining (NJ) (Saitou & Nei 1987) method implemented in Clustal-X. Sequence identities were calculated by MEGA 3.1 software (Kumar *et al.* 2004). The average number of nucleotide substitutions per site between the BMV-CZ and the three other isolates (Fescue, Russian and KU1) was calculated by DNAsp software (Nei 1987) on full genome sequences. The genome sequences of the BMV-CZ isolate were deposited in the GenBank database under the following accession

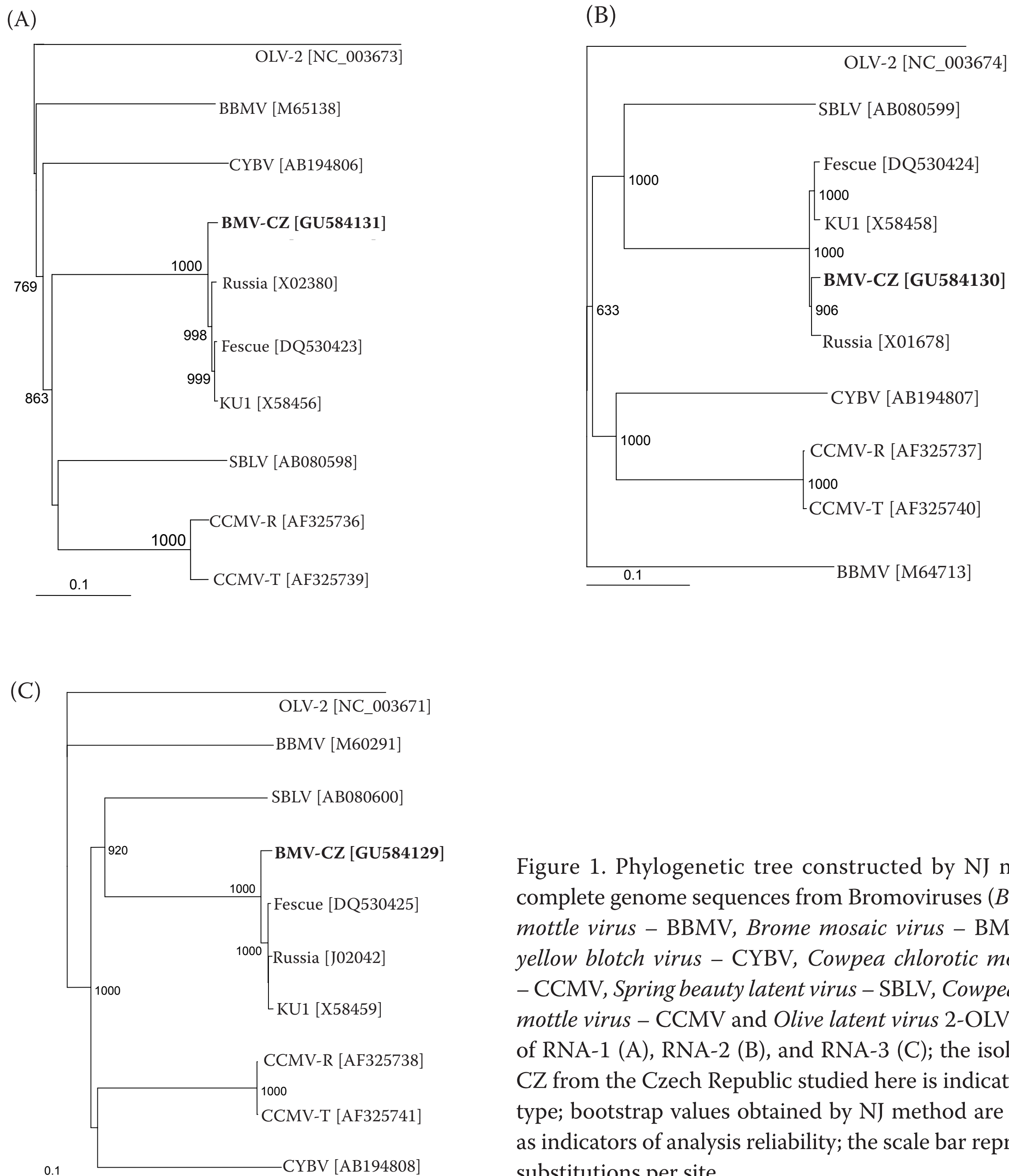

Figure 1. Phylogenetic tree constructed by NJ method of complete genome sequences from Bromoviruses (*Broad bean mottle virus* – BBMV, *Brome mosaic virus* – BMV, *Cassia yellow blotch virus* – CYBV, *Cowpea chlorotic mottle virus* – CCMV, *Spring beauty latent virus* – SBLV, *Cowpea chlorotic mottle virus* – CCMV and *Olive latent virus* 2-OLV2*)* isolates of RNA-1 (A), RNA-2 (B), and RNA-3 (C); the isolate BMV-CZ from the Czech Republic studied here is indicated in bold type; bootstrap values obtained by NJ method are presented as indicators of analysis reliability; the scale bar represents 0.1 substitutions per site

numbers: RNA-1 (GU584131), RNA-2 (GU584130) and RNA-3 (GU584129).

From an isolate of BMV maintained in our laboratory, the full genomes of its three RNAs were sequenced and genetic analyses were performed. The three RNAs showed typical organization. The RNA-1 (3234 bp) was organized in 3'- and 5'-UTR with the ORF-1a coding for the replication protein 1a with a start codon in nt position 75 and stop codon in 2960. The replication protein 1a was characterized by a sequence of 961 amino acids previously described and containing the methyltransferase- and helicase-like domains (Ahola & Ahlquist 1999). There was high homology at the amino acid level of protein 1a (> 98.9%) with the other BMV isolates, and greater variability in the 5'-UTR (Table 1). The RNA-2 (2866 bp) with the ORF-2a coded for polymerase 2a (Smirnyagina *et al.* 1996) showed high sequence identities with the other BMV isolates. Two coding proteins were identified on RNA-3 (2108 bp) as already described in other records. The first, the movement protein 3a (912 bp long), was implicated in long distance movement and cell-cell propagation (Takeda *et al.* 2004) and the second was the coat protein of the BMV 570 bp in length (Choi & Rao 2000). The sequence identities with the other isolates of BMV (Fescue, Russian and KU1) were 97.9% and 96.8% on the amino acid level for the movement and coat proteins, respectively (Table 1). The phylogenetic analysis revealed that BMV-CZ was closely related to the other BMV isolates and clearly separated from the other Bromoviruses (Figure 1). The bootstrap support for the branches indicated that BMV-CZ might be considered a separate strain of BMV. The NJ analysis strongly separated (> 90%) BMV-CZ from the other isolates as did the Maximum Parsimony analysis (not shown). The identities showed high sequence homologies between the BMV isolates (BMV-CZ, Fescue, Russian and KU1) in different parts of the genome with ranges of 95.6–99.0%, 94.4–99.6% and 95.2–98.3% in RNA-1, RNA-2 and RNA-3, respectively (Table 1). In the coding

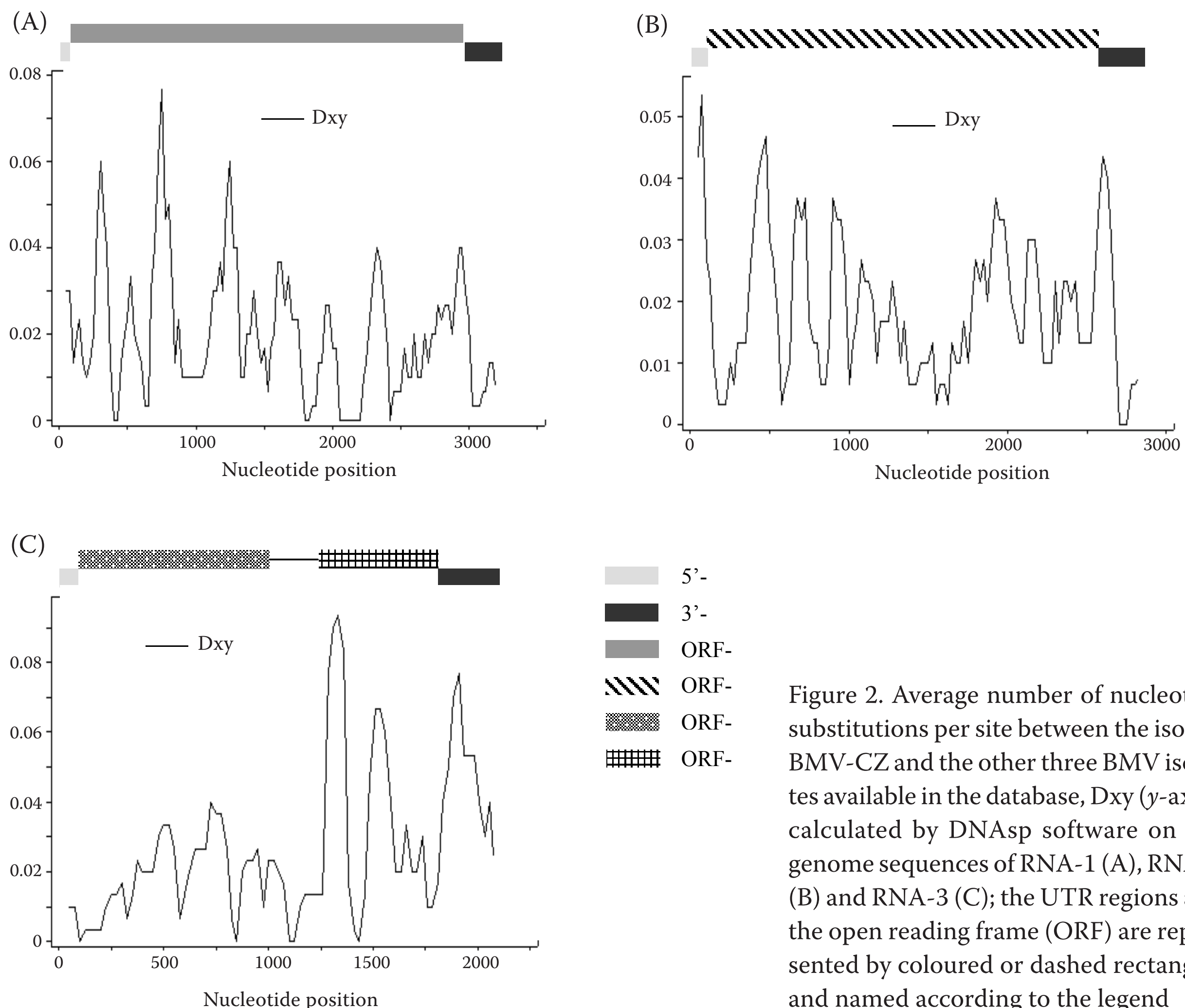

Figure 2. Average number of nucleotide substitutions per site between the isolate BMV-CZ and the other three BMV isolates available in the database, Dxy (*y*-axis); calculated by DNAsp software on full genome sequences of RNA-1 (A), RNA-2 (B) and RNA-3 (C); the UTR regions and the open reading frame (ORF) are represented by coloured or dashed rectangles and named according to the legend

region the highest amino acid homology was in the replication protein 1a between BMV-CZ and the Russian isolate (up to 99.9%) and the most divergent was in the ORF-3b encoding for the coat protein with a divergence of 4.3%. The precise comparison of the BMV-CZ isolate with the other BMV isolates highlighted that diversity was distributed along the genomes of RNA-1 and RNA-2 (Table 1 and Figure 2). In RNA-3 the coat protein region presented greater diversity in the N-terminal region in comparison with the C-terminal (Figure 2). In BMV, four RNA-interacting domains (RID I–IV) were described (Calhoun & Rao 2008). Within these domains there were no discrepancies between BMV-CZ and the previously described isolates. Furthermore, an arginine-rich RNA-binding motif (ARM) found at the N-terminal region of BMV coat protein has been characterized; it contains 25 amino acids and is highly basic due to the presence of a conserved ARM characteristic of RNA binding proteins (Choi & Rao 2000). Our isolate had the following amino acid sequence in this region: STSGTGKMTRAQRRAAARRNPR**AVK**. The bold characters represent the changes compared the Russian isolate (Acc. No. J02042). Our study provided a new full-length genome sequence of BMV from the Czech Republic.

Acknowledgements*.* This work was supported by a grant from the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002700604. The English of the manuscript was edited by International Science Editing http://www.internationalscienceediting.com.

References

Ahlquist P., Allison R., Dejong W., Janda M., Kroner P., Pacha R., Traynor P. (1990): Molecular-biology of Bromovirus replication and host specificity. In: Pirone T.P., Shaw J.G. (eds): Viral Genes and Plant Pathogenesis. Springer, New York, 144–155.

Ahola T., Ahlquist P. (1999): Putative RNA capping activities encoded by Brome mosaic virus: methylation and covalent binding of guanylate by replicase protein 1a. Journal of Virology, **73**: 10061–10069.

Calhoun S.L., Rao A.L.N. (2008): Functional analysis of brome mosaic virus coat protein RNA-interacting domains. Archives of Virology, **153**: 231–245.

Choi Y.G., Rao A.L.N. (2000): Molecular studies on bromovirus capsid protein VII. Selective packaging of BMV RNA4 by specific N-terminal arginine residues. Virology, **275**: 207–217.

Kumar S., Tamura K., Nei M. (2004): MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics, **5**: 150–163.

Nei M. (1987): Molecular Evolutionary Genetics. Columbia University Press, New York.

Roossinck M.J., Bujarski J., Ding S.W., Hajimorad R., Hanada K., Scott S., Tousignant M. (2005): Family *Bromoviridae*. Elsevier Academic Press, San Diego, 1049–1058.

Saitou N., Nei M. (1987): The Neighbor-Joining Method – a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, **4**: 406–425.

Smirnyagina E., Lin N.S., Ahlquist P. (1996): The polymerase-like core of brome mosaic virus 2a protein, lacking a region interacting with viral 1a protein *in vitro*, maintains activity and 1a selectivity in RNA replication. Journal of Virology, **70**: 4729–4736.

Sullivan M.L., Ahlquist P. (1997): cis-acting signals in bromovirus RNA replication and gene expression: Networking with viral proteins and host factors. Seminars in Virology, **8**: 221–230.

Takeda A., Kaido M., Okuno T., Mise K. (2004): The C terminus of the movement protein of Brome mosaic virus controls the requirement for coat protein in cell-to-cell movement and plays a role in long-distance movement. Journal of General Virology, **85**: 1751–1761.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. (1997): The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, **25**: 4876–4882.

Received for publication June 4, 2010
Accepted after corrections October 11, 2010

Corresponding author:

Jiban Kumar Kundu, Ph.D., Výzkumný ústav rostlinné výroby, v.v.i., odbor rostlinolékařství, oddělení virologie, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 402, e-mail: jiban@vurv.cz