# Generation of an infectious *Beet mosaic virus* (BtMV) full-length clone based on the complete nucleotide sequence of a German isolate

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## ABSTRACT

*Beet mosaic virus* (BtMV) is a member of the genus *Potyvirus* within the large and economically important family *Potyviridae*. BtMV occurs worldwide in major beet-growing areas, especially in temperate regions. The host range of BtMV includes all cultivated sugar beet and near relatives. BtMV infects mainly plants in the families *Chenopodiaceae*, *Solanaceae* and *Leguminosae*. It shows clearly visible mosaic disease symptoms on the leaves, whereas the infected plants are often of normal size. Damage and yield reduction due to BtMV infection has been reported for *Beta vulgaris*.

Limited information is available about its molecular properties and variability. The aim of this study was to determine the complete nucleotide sequence of a German isolate of BtMV (BtMV-G) and to compare the sequence with other potyvirus sequences. In addition an infectious full-length clone of BtMV-G was constructed in order to provide a possibility to study the virus multiplication cycle and to obtain an improved understanding of the molecular biology of potyviruses.

Ribonucleic acid was extracted from purified BtMV-G (DSMZ; PV-0065) or BtMV-G infected *Nicotiana benthamiana* plants and used as a template for cDNA synthesis. BtMV-specific oligonucleotides were designed and used together with a 26mer oligonucleotide, containing a random hexamer sequence at its 3'-end, for synthesis and amplification of cDNA fragments by reverse transcription-polymerase chain reaction (RT-PCR). The 5'-terminus of the genome was determined by reverse transcription of viral RNA using a specific primer, tailing of the cDNA with dGTP and PCR. All PCR fragments were cloned into the pGEM®-T Easy vector and subsequently the complete sequence of BtMV-G was determined. In addition, four cDNA clones generated by RT-PCR were used to assemble an infectious full-length clone of BtMV-G in a plasmid harbouring an enhanced *Cauliflower mosaic virus* 35S promoter.

The BtMV-G genome comprises 9592 nucleotides (nt) and contains one large open reading frame encoding a polyprotein of 3085 amino acid residues. The 5'- and 3'-untranslated regions were determined with 166 and 171 nt, respectively. Nine putative proteolytic cleavage sites were identified in the polyprotein resulting in ten mature proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, NIa, VPg, NIb and CP, which are typical for all

members of the genus *Potyvirus*. Alignment of the predicted polyprotein sequence with a sequence of a BtMV isolate from the U.S.A. (BtMV-Wa) as well as with other potyviruses revealed amino acid sequence motifs typical of potyviruses. However, some motifs located in the HC-Pro, CI and NIb of BtMV-G contained different amino acids in comparison with other potyviruses. The highly conserved amino acid motif in the HC-Pro "Lys-Ile-Thr-Cys" involved in aphid transmission is diverged to the less common "Lys-Met-Ala-Cys" motif. Phylogenetic analysis clearly showed BtMV-G as a distinct member of the genus *Potyvirus*, sharing the highest amino acid sequence identity (55%) with *Peanut mottle virus* (PeMoV). The phylogenetic tree grouped BtMV-G, BtMV-Wa and PeMoV in one cluster located in the neighbourhood of the *Bean common mosaic virus* cluster.

The BtMV-G full-length clone leads to infectious virus in *N. benthamiana* after particle bombardment. Inoculated plants showed a delayed symptom development compared to the BtMV-G wild-type virus. Subsequent mechanical inoculation of *N. benthamiana* with BtMV-G generated from the full-length clone revealed indistinguishable symptoms from the wild-type virus. However, in *Atriplex hortensis* cv. 'Rheinische' BtMV-G generated from the full-length clone caused only small yellow blots on leaves compared with severe symptoms and stunting of the plants caused by the wild-type virus. In addition, BtMV-G from the infectious clone was not able to cause symptoms on some cultivars susceptible to the wild-type virus like *Spinacia oleracea* and *Beta vulgaris* (8T0015). It has still to be investigated, which genes of BtMV-G derived from the infectious full-length clone are involved in the different symptom expression.

The infectious cDNA clone of BtMV-G provides a powerful tool to study virus replication and could contribute towards a better understanding of the molecular biology of BtMV.