

# Molecular analyses of the coat protein region of different viruses on Poaceae belonging to the *Potyviridae*

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**Summary** — The coat protein region of the RNA of 3 different viruses all infecting Poaceae and belonging to the family *Potyviridae* were analyzed. Brome streak mosaic virus (BrSMV) and Agropyron mosaic virus (AgMV) are described as mite-transmissible, while cocksfoot streak virus (CfSV) is transmissible by aphids. The coat protein is important for the transmission of *Potyviridae* and the degree of identity between their coat protein sequences is used to indicate a relationship within the *Potyviridae*. Alignments of the coat protein region of BrSMV and several other members of the *Potyviridae* show a homology of 47% to a mite-transmissible rymovirus, but only 23–30% to aphid-transmissible potyviruses. Alignments of CfSV and different aphid-transmissible potyviruses show about 50% homology, respectively. All these results agree with the classification of *Potyviridae* based on their coat protein region. Alignments of AgMV to mite-transmitted viruses show a homology of only about 25%. This means either a low relationship between mite-transmitted *Potyviridae* or AgMV is possibly not transmissible by mites.

**brome streak mosaic virus / Agropyron mosaic virus / cocksfoot streak virus / coat protein / classification of *Potyviridae***

**Résumé** — Analyse moléculaire de la région de la protéine capsidique de divers virus appartenant aux *Potyviridae* et infectant les poacées. La région de la protéine capsidique du RNA de 3 virus différents infectant les poacées et appartenant à la famille des *Potyviridae* a été analysée. Le virus de la mosaïque striée du brome (BrSMV) et le virus de la mosaïque du chiendent (AgMV) sont transmis par acariens, alors que le virus de la striure du dactyle (CfSV) est transmis par aphides. La protéine capsidique est importante pour la transmission des *Potyviridae* et le degré de similitude entre les séquences de leurs protéines capsidiques est utilisé pour évaluer les relations entre ces virus. La comparaison des séquences des régions de la protéine capsidique du BrSMV révèle une homologie de 47% avec celle d'un rymovirus transmissible par acariens, mais seulement 23–30% avec des potyvirus transmissibles par aphides. Entre le CfSV et différents potyvirus transmissibles par aphides, la comparaison révèle une homologie de 50% environ. Tous ces résultats sont en accord avec la classification des *Potyviridae* basée sur la région couvrant la protéine capsidique. La comparaison de l'AgMV à des virus transmissibles par les acariens révèle une homologie de seulement 25%. Cela signifie soit une faible parenté entre les *Potyviridae* transmissibles par acariens, soit que l'AgMV n'est pas transmissible par les acariens.

**virus de la mosaïque striée du brome / virus de la mosaïque du chiendent / virus de la striure du dactyle / protéine capsidique / classification des *Potyviridae***

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## INTRODUCTION

The virus family *Potyviridae* is characterized by properties like particle morphology and vector-transmission. Based on their RNA-genome and their vectors different genera exist in the *Potyviridae*. The members of the genus *Potyvirus* are transmitted by aphids and have a single-stranded (ss) RNA-genome, while viruses that also have an ssRNA-genome but are transmissible by mites belong to the genus *Rymovirus*. Members of the genus *Bymovirus* are transmissible by fungi and have a bipartite genome.

A great deal of molecular analysis was done with *Potyviridae* infecting dicots but little information exists about *Potyviridae* infecting Poaceae, particularly mite-transmissible rymoviruses.

Two viruses, brome streak mosaic virus (BrSMV) and Agropyron mosaic virus (AgMV) are described as mite-transmissible. BrSMV is transmitted by the mite *Aceria tulipae* (Huth *et al*, 1995), while AgMV is transmissible by another mite *Abaracus hystrix* (Slykhuis, 1969). A third virus, cocksfoot streak virus (CfSV) is not transmissible by mites but by aphids (Catherall, 1971) and was also selected for molecular analyses and the comparison of sequence data. BrSMV was first isolated from *Bromus mollis* in Yugoslavia by Milicic *et al* (1980, 1982), but the distribution of the virus in Europe is not really known so far. AgMV and CfSV can infect several Poaceae and both viruses are described as widespread in Europe (Catherall, 1971; Slykhuis, 1973).

The coat protein, a functional protein proteolytically processed from the polyprotein encoded by the potyviral RNA, is responsible for the encapsidation of the viral RNA but is also important for the transmission of the *Potyviridae*, for the aphid-transmissible potyviruses as well as for the mite-transmissible rymoviruses or the fungus-transmissible bymoviruses. The degree of identity between their coat protein sequences indicates the relationship of different viruses of the *Potyviridae* (Shukla and Ward, 1988, 1989). According to this classification, viruses with a homology of more than 40% belong to one genus in the *Potyviridae*, while a lower homology indicates that the compared viruses belong to different genera in the *Potyviridae*.

## MATERIAL AND METHODS

The isolates of the 3 viruses were propagated in wheat or cocksfoot after mechanical inoculation. Purification

was done according to the method described by Huth *et al* (manuscript in preparation). Subsequently, the RNA was removed from virions by incubation for 45 min with proteinase K followed by phenol extraction and precipitation (Maiss *et al*, 1988). The viral RNA was used as template for oligo(dT) primed cDNA synthesis using the Riboclone cDNA synthesis system (Promega). RNA was reverse-transcribed using AMV reverse transcriptase, and the dsDNA end was made blunt by T4-DNA polymerase. Subsequently, the cDNA was ligated to the *Hind*III cut phagemid vector pT7T3 (19U; Pharmacia) and cloned into *Escherichia coli* NM522 (Pharmacia). For CfSV RT-PCR was carried out using oligo(dT) and a specific oligonucleotide complementary to a conserved region of the N1b gene. The DNA fragment generated was digested and subsequently cloned into pT7T3. The cDNA clones were selected by restriction mapping as well as by dot-blot hybridisation. The sequencing of the selected clones was conducted by the dideoxynucleotide chain termination method (Sanger *et al*, 1977). For sequencing mainly ssDNA templates were used, which were prepared with the helper phage M13K07 (Pharmacia). Each nucleotide was determined either by sequencing at least two independent cDNA clones or by sequencing both strands of a cDNA clone. The sequence data were assembled and analysed using the GCG program package (Deveraux *et al*, 1984). Nucleotide and protein sequences of BrSMV, AgMV and CfSV were analysed by DNASIS. For the comparison of protein sequences of different viruses, ALIGN was used and the phylogenetic tree was constructed using CLUSTAL with standard parameters.

## RESULTS

Three cDNA clones representing the 3' terminal region of the genomic RNA of BrSMV including a poly(A)tail were sequenced. Two clones covered at their 5' end the amino acid motif GDD, which is located in the N1b protein and is characteristic for RNA-dependent RNA polymerases. This led to the conclusion that, besides a part of the N1b, the entire coat protein gene was also cloned and sequenced.

The clones of AgMV and CfSV, which were sequenced, also contain the a poly(A)tail as well as the amino acid motif GDD located in the N1b protein. So the coat protein regions of all three viruses have been cloned and fully sequenced.

For a comparison with other members of the *Potyviridae*, amino acid sequences were selected for all 3 viruses, which start at the putative cleavage site between N1b and CP and cover the entire coat protein up to the 3' end of the coat protein gene.

The amino acid sequence of the coat protein regions of the 3 investigated viruses were com-

pared with the similar protein regions of several *Potyviridae* such as the mite-transmitted wheat streak mosaic virus (WSMV; Niblett *et al.*, 1991) or the aphid-transmitted plum pox virus (PPV; Maiss *et al.*, 1989), potato virus Y (PVY; Robaglia *et al.*, 1989) and tobacco etch virus (TEV; Allison *et al.*, 1986). These results are shown in table I. Alignments of the deduced amino acid sequence of the putative coat protein regions of BrSMV, AgMV and CfSV with those of other *Potyviridae* show different degrees of identity correlated with vector-transmissibility.

The alignment of the coat protein regions of BrSMV and WSMV shows a homology of 47%. This suggests a close relationship between these 2 viruses, which are both transmissible by mites. The homology of BrSMV to the different aphid-transmissible potyviruses is quite smaller. Only a limited homology of 23 to 30% was observed. Alignments of the coat protein regions of CfSV and different aphid-transmissible potyviruses show about 50% homology, respectively.

The alignments of AgMV to the other *Potyviridae* show a homology of about 40% to aphid-transmitted viruses. In contrast, to mite-transmitted viruses a homology of only about 25% is observed.

## DISCUSSION

The genome of potyviruses is a positive-sense ssRNA. In contrast to potyviruses, bymoviruses have a bipartite genome. Rymoviruses have a ssRNA genome like potyviruses. BrSMV is the first rymovirus for which a complete nucleotide sequence has been reported, and the sequence data indicate a genome organization similar to that of potyviruses (Götz and Maiss, 1995).

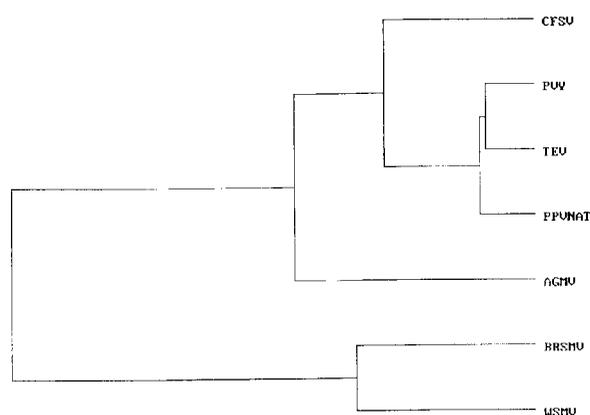
**Table I.** Percentage amino acid sequence identity of the predicted coat protein of BrSMV, AgMV and CfSV and other members of the *Potyviridae* including the mite-transmitted WSMV and several aphid-transmitted potyviruses.

	BrSMV	AgMV	CfSV
BrSMV			
AgMV	25.7		
CfSV	23.2	40.8	
WSMV	47.1	23.9	24.8
PPV	23.7	41.8	47.2
PVY	26.2	46.3	50.0
TEV	29.2	48.5	49.6

The results of the alignments agree with the classification based on the coat protein region. Shukla and Ward (1988) described 38–75% identity between different aphid-transmitted potyviruses. The sequence comparison of the aphid-transmitted CfSV to other potyviruses fits into this range. The homology of BrSMV and WSMV to the aphid-transmitted potyviruses is considerably lower suggesting that they do not belong to the same genus. However, the degree of identity of 47% between BrSMV and WSMV indicates both viruses as members of one genus, the rymoviruses, which includes viruses transmissible by mites. In addition, a close relationship between these 2 viruses is not surprising, because they are transmissible not only by mites but by the same mite species *Aceria tulipae* (Slykhuis, 1955; Huth *et al.*, 1995).

The degree of relationship between different members of the *Potyviridae* is also shown in the phylogenetic tree (fig 1). The different potyviruses have different degrees of relationship, but they are more closely related to each other than to AgMV, BrSMV or WSMV, respectively. On the other hand, BrSMV is more similar to WSMV than to any other potyvirus. The dendrogram suggests 2 groups of viruses. In the first group all of the viruses are transmissible by aphids belonging to the genus potyvirus. BrSMV and WSMV belong to another group, representing the mite-transmissible rymoviruses. In contrast, AgMV hardly fits into this group.

The described vector transmissibility of AgMV does not agree with the alignment of *Potyviridae* based on coat protein sequence data. This leads to the conclusion that either a low relationship exists between mite-transmitted *Potyviridae* transmissible by different species of mites or that AgMV is possibly not transmissible by mites.



**Fig 1.** Phylogenetic tree. Relationship of members of the *Potyviridae* based on their coat protein amino acid sequence (BrSMV, AgMV, CfSV, WSMV, PPV, PVY and TEV).

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