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Sequence of the 3′-terminal region of the RNA of a mite transmitted potyvirus from *Hordeum murinum* L.

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Abstract

The 3026 nucleotides upstream of the 3′-polyadenylated tract of a mite transmitted virus from *Hordeum murinum L.* were cloned and sequenced, and portions of the sequence were expressed in *Escherichia coli.* Sequence comparisons with wheat streak mosaic virus (WSMV), Agropyron mosaic virus (AgMV) and Hordeum mosaic cirus (HoMV), three mite transmitted potyviruses, and potato virus Y (PVY), the type member of the genus *Potyvirus*, revealed that the virus is probably a potyvirus, but distinct from WSMV, AgMV, HoMV, and PVY. Serological tests further demonstrated these differences and that the virus is serologically related to another potyvirus, brome streak mosaic virus (BrSMV). We conclude that the virus should be named as the Hordeum isolate of BrSMV.

Introduction

The wheat streak mosaic virus (WSMV) is an important pathogen of wheat in several wheat growing countries [Milne, 1988]. As a mite transmitted virus is belongs to the newly proposed genus *Rymovirus* within the family *Potyviridae*, which comprises the largest and economically most important group of plant viruses [Barnett, 1992]. To this genus belong such viruses as Agropyron mosaic virus (AgMV), Hordeum mosaic virus (HoMV), oat necrotic mottle virus (ONMV), ryegrass mosaic virus (RgMV) and the type member, WSMV. Possible members include brome streak mosaic virus (BrSMV) and spartina mottle virus (SpMV) [Zagula et al., 1992]. The first report of WSMV for middle Europe came from Rabenstein and Stanarius (1981). They isolated the virus from *Hordeum murinum L.* as well as from *Bromus sterilis L.* in Germany. As it showed some differences from the 'type' strain properties, it was thought to be a new strain of WSMV [Rabenstein et al., 1982]. A similar potyvirus was isolated from *B. mollis L.* and *H. murinum* by Milicic et al. [1980] and later designated as BrSMV [Milicic et al., 1982]. Niblett et al. [1991] published the sequence of the 3′-end of the RNA of the American isolate WSMV-H81. Here we present the sequence of the 3′-region of the isolate from *H. murinum* and some serological data which lead to the conclusion, that it is a different virus rather than a strain of WSMV.

Materials and methods

Virus isolates

The mite transmissible potyvirus originally isolated from *H. murinum* [Rabenstein and Stanarius, 1981] has been maintained by mechanical inocu-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DJB Nucleotide Sequence Databases under the accession number X78485.

lation to barley (cv. 'Erfa') for more than ten years. The French isolate of BrSMV (11-Cal) was isolated from naturally infected barley by P. Signoret (Montpellier) and supplied by W. Huth (Braunschweig). RgMV and AgMV were isolated from *Lolium perenne* L. or *Agropyron repens* L., respectively [Rabenstein, 1981]. WSMV-PV-91 was obtained from the American Type Culture Collection and WSMV-CS from J. Vacke (Prague).

Virus purification
The potyvirus from *H. murinum* was purified from systemically infected leaves of barley cv. 'Erfa'. RgMV was propagated on *Lolium multiflorum* Lam. cv. 'Dilana'. AgMV, WSMV-PV-91, and WSMV-CS were purified from infected wheat cv. 'Alcedo'. Leaves infected with the different rymoviruses were harvested three to four weeks after inoculation and triturated in a blender with 0.1 M potassium citrate buffer pH 7.0 containing 0.1 % PMSF (phenylmethylsulfonyl fluoride). All other purification steps followed mainly the method described by Huth et al. [1984] except that carbon tetrachloride was substituted by 3 % Triton X-100 in distilled water (v:v).

Antisera
Polyclonal antisera against AgMV, BrSMV-H, RgMV, PVY and WSMV-CS were produced in rabbits using an immunisation scheme according to Richter [1992]. A polyclonal reference antiserum against WSMV-H81 was provided by S. A. Lommel (Raleigh). For some comparative investigations a polyclonal antiserum produced against turnip mosaic virus (TuMV-314) displaying a high degree of cross-reactivity with different members of aphid-borne potyviruses [Richter et al., 1994] was included.

ELISA and Western blotting

Preparation of IgGs, alkaline phosphatase conjugates and DAS-ELISA were performed according to Clark and Adams [1977]. The plate trapped antigen (PTA) ELISA using the antiserum TuMV-314 was carried out as described by Richer et al. [1994]. Western blotting was performed in a semidry fashion on nitrocellulose Hybond C (Amersham). As a secondary antibody we used goat-anti rabbit-alkaline phosphatase conjugates (Sigma) and nitroblue tetrazoliumchloride/5-bromo-4-chloro-3-indolyl phosphate (Biomol) as a substrate.

Isolation, cloning and sequencing of the RNA

The RNA was isolated from the purified virus by means of proteinase K-digestion (500 µg virus in 500 µl 50 mM Tris-HC1, pH 7,8 with 1 % SDS and 0,1 mg/ml proteinase K, 1 h at 37 °C) followed by an extraction(1:1/vol:vol) with prewarmed (55 °C) phenol/chloroform. After centrifugation the aquaous phase was collected and 1/10 vol 3 M potassium acetate was added. The RNA was precipitated with 2,5 vol ethanol and the integrity checked electrophoretically in agarose gels after straining with ethidium bromide.

For cDNA synthesis AMV reverse transcriptase (Boehringer Mannheim) was used according to the manufacturers instructions. The reaction was primed with oligo$(dT)_{12-15}$. The second strand was synthesised using endonuclease free *Escherichia coli* DNA polymerase I, RNase H, *E. coli* DNA ligase and T4 DNA polymerase (all from Boehringer Mannheim) according to Gubler and Hoffman [1983]. The blunt ended cDNA was cloned either in pBluescript II SK+/EcoRV (Stratagene) or pUC 18/Sma I (Pharmacia). For sequencing, four clones of different sizes were chosen. They reacted in northern blot experiments with the viral RNA showing a band of the expected size (data not shown). The sequence reactions were performed for all four clones in both directions using different Sequenase kits (USB) and double stranded plasmids as templates. Subclones for sequencing were generated by cloning restriction fragments in pBluescript II SK+.

Results

The nucleic acid and deduced amino acid (aa) sequences of the 3'-end of the cloned viral RNA are presented in Fig. 1. It contains one large open reading frame which is terminated by a TAA stop codon at position 2827. Downstream of the coat

protein (cp) coding region is a 255 nucleotide (nt) untranslated sequence followed by a polyadenylated tract. In Fig. 2 a part of the deduced aa sequence of the cloned viral RNA is compared with the aa sequences of WSMV-H81 [Niblett et al., 1991] and potato virus Y (PVY) [Robaglia et al., 1989]. The percentage of overall homology of the sequences of both rymovirus isolates is very low. Assuming, that the possible protease cleavage sites are Q/G, Q/S or Q/A [Carrington and Daugherty, 1988], the cp of the sequenced virus isolate could be cleaved from the polyprotein at the Q/A site at aa 536. The resulting cp would have a molecular weight of 44,5 kD. This size is in good agreement with the electrophoretically estimated size of 45 kD for the cp of this virus isolate (Fig. 3). The possible cleavage site Q/S at aa 495 obviously has no relevance. The polymerases of potyviruses (NI_b), preceding the cp in the polyprotein, usually have a size of approximately 50 kD. In the aa sequence of the cloned virus isolate upstream from the putative cp/NI_b cleavage site only one other possible cleavage site, Q/S, is present. It is located at aa position 197. A cleavage at this site would result in a protein with a molecular weight of 38,7 kD. Otherwise the polymerase should be larger than 61 kD as no other possible protease cleavage sites exist in the sequenced part of the polyprotein.

Two regions of the cDNA were recloned in expression vectors and transformed into *Escherichia coli*, strain JM 101. For expressing the sequence starting from nt 1156 we used the plasmid pGEMEX-1 (PROMEGA). The resulting product is a fusion protein of a part of the putative NI_b and the complete cp linked to the plasmid encoded T7 gene 10 protein. For the shorter sequence, starting from nt 1849, we used the plasmid pGEX-2T (Pharmacia), which resulted in a fusion protein of a part of the cp with glutathione S-transferase. Both recombinant fusion proteins reacted with a polyclonal antiserum against the virus isolate from *H. murinum* (Fig. 3). An antiserum against WSMV-H81 did not react in western blot experiments and ISEM with this isolate (data not shown). The same was true for an antiserum, raised against WSMV-PV-91 (Fig. 3).

Using plant material infected with WSMV-PV-91 or the virus isolate from *H. murinum*, different sizes of the coat proteins were detected by specific antisera on western blots (Fig. 3). They ranged from 47 kD for WSMV-PV-91 to 44,5 kD for the sequenced isolate.

The virus under investigation and WSMV did not react with polyclonal antisera against PVY and TuMV both belonging the genus *Potyvirus* (data not shown). Further tests were done with antisera against different potyviruses of cereals by means of ELISA. The results are shown in Table 1. The polyclonal antisera reacted in DAS-ELISA only with their homologous antigens. Even Seifers et al. [1992] did not observe in PTA-ELISA cross reactions between AgMV and WSMV-H81 too. The potyvirus group specific antiserum TuMV-314 cross-reacted with AgMV and RgMV, but did not exhibit any reaction with the tested BrSMV and WSMV isolates.

Discussion

Results are presented, that the 'wheat streak mosaic virus', previously isolated in Germany by Rabenstein and Stanarius [1981] from *H. murinum*, has a sequence different from that published for WSMV-H81 and of AgMV and HoMV (R. French, pers. comm.), and it displays no serological relationships with other potyviruses. The strong reaction of the antiserum, produced against the sequenced poytvirus from *H. murinum*, with the French isolate of BrSMV(11-Cal) leads to the conclusion that the investigated virus is most likely an isolate of BrSMV. The results of the PTA-ELISA (Table. 1) support this hypothesis. Similar results were obtained by Huth et al. [1994]. They found no serological relationships between 11-Cal and an American isolate of WSMV and only 48% homology for the aa sequences of the cp. Both viruses, BrSMV and WSMV, differ in the sizes of their coat proteins what provides an additional evidence, that both isolates should be regarded as different viruses. Therefore we conclude that the potyvirus isolated from *H. murinum* and described by Rabenstein et al. [1982] is to be renamed in Hordeum isolate of BrSMV (BrSMV-H).

Unfortunately, no other sequence data are available for BrSMV for comparison. The BrSMV and PVY have a very high degree of homology especially in the core part of their putative poly-

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 -  E  T  E  I  T  P  Y  G  E  N  E  E  L  L  W  R  H  R  I    19
CAGAGACTGAAATAACACCATACGGAGAGAACGAAGAGCTACTCTGGCGTCATCGCATAA   60
 T  T  E  V  G  D  C  G  A  T  M  V  A  L  S  D  Q  K  I  V    39
CCACTGAGGTTGGTGACTGTGGCGCAACAATGGTAGCCCTCAGCGACCAGAAGATCGTTG  120
 G  F  H  S  L  G  G  I  S  M  N  Y  F  V  P  V  T  Q  E  L    59
GATTTCACAGCCTGGGTGGAATTAGCATGAACTACTTCGTTCCAGTCACACAGGAGTTAT  180
 L  D  F  L  N  S  K  T  E  K  P  L  V  P  W  R  F  S  E  D    79
TGGACTTCTTGAACTCAAAAACCGAGAAACCGCTAGTGCCATGGAGATTCAGTGAAGATC  240
 Q  V  D  V  G  G  L  Y  I  H  N  D  F  D  K  F  P  F  V  K    99
AAGTCGACGTTGGTGGTTTGTATATCCACAACGATTTTGATAAATTTCCATTCGTCAAAA  300
 T  I  Q  K  L  V  G  F  Q  N  G  H  M  I  K  Y  C  G  E  G   119
CAATTCAGAAACTAGTTGGGTTTCAGAATGGCCACATGATAAAATATTGTGGTGAAGGAT  360
 F  T  P  V  A  R  S  E  N  R  L  S  R  Q  H  V  I  S  G  Q   139
TCACGCCGGTGGCACGCTCGGAGAATCGATTGTCAAGACAGCACGTTATTAGCGGACAGC  420
 R  E  S  F  I  H  F  V  E  A  S  A  K  W  R  P  I  V  T  P   159
GAGAGAGCTTTATACACTTCGTGGAAGCTAGTGCGAAATGGAGACCGATAGTCACGCCGA  480
 M  L  G  R  L  Q  P  S  A  L  N  R  E  A  Y  Y  K  D  V  L   179
TGCTAGGACGATTGCAACCATCAGCACTGAACCGCGAAGCGTACTACAAGGACGTGTTGA  540
 K  Y  D  K  P  I  R  L  G  T  V  H  E  E  A  F  Q /S  A  V   199
AGTACGATAAGCCAATAAGACTAGGAACAGTACACGAGGAAGCTTTCCAGAGTGCGGTCA  600
 I  N  V  I  R  I  L  E  N  A  G  F  E  R  G  G  V  K  A  C   219
TTAACGTGATTCGAATTCTTGAGAATGCTGGCTTTGAGCGAGGCGGTGTCAAGGCATGCT  660
 F  D  Y  G  K  I  F  N  D  L  N  L  D  A  A  M  G  A  L  Y   239
TCGACTACGGGAAAATCTTCAACGATTTAAACCTTGATGCAGCCATGGGTGCTCTATACG  720
 A  G  K  K  K  D  Y  F  V  E  A  T  D  E  E  I  E  E  M  F   259
CTGGAAAGAAGAAGGACTACTTCGTGGAGGCTACAGATGAGGAGATTGAGGAAATGTTCT  780
 L  R  S  A  G  K  I  C  A  N  G  H  G  V  W  S  A  L  L  K   279
TACGAAGCGCTGGGAAAATCTGTGCAAATGGGCACGGAGTGTGGTCAGCTCTCCTAAAGG  840
 A  E  L  R  P  A  E  K  V  A  A  N  K  T  R  T  F  T  S  A   299
CTGAGTTACGCCCAGCTGAGAAGGTTGCAGCCAACAAGACGAGAACATTCACATCAGCAC  900
 P  I  D  I  L  F  G  A  K  A  V  V  D  D  F  N  K  Q  F  Y   319
CCATCGACATCTTATTTGGAGCAAAGGCTGTGGTAGACGATTTCAATAAGCAATTCTATA  960
 K  R  H  L  L  G  P  W  T  V  G  I  N  K  F  N  K  G  W  D   339
AGCGGCATCTACTAGGTCCTTGGACTGTTGGCATAAACAAGTTCAACAAGGGCTGGGACT 1020
 L  L  A  R  S  L  M  R  Y  E  W  F  I  D  A  D  G  S  Q  F   359
TACTTGCACGATCGTTGATGCGCTATGAATGGTTTATTGATGCAGACGGATCACAATTCG 1080
 D  S  S  I  T  P  L  L  M  N  A  V  L  T  I  R  L  Y  F  M   379
ACAGCTCAATAACGCCGTTGCTCATGAATGCTGTTCTGACAATAAGGTTGTACTTCATGG 1140
 E  R  D  D  I  T→E  L  M  L  R  N  L  Y  T  Q  I  I  S  T   399
AGCGAGACGATATCACTGAGCTCATGTTGAGGAATTTGTACACTCAAATCATCAGTACAT 1200
 C  M  L  A  E  D  G  L  I  V  Q  K  H  R  G  N  N  S  G  Q   419
GCATGCTCGCGGAAGATGGCTTAATAGTGCAAAAGCATCGAGGAAACAATAGTGGTCAAC 1260
 P  S  T  V  V  D  N  T  L  C  L  M  I  A  M  E  Y  A  R  Q   439
CAAGCACAGTTGTGGACAATACGCTGTGCTTGATGATAGCAATGGAATATGCGCGCCAAA 1320
 R  A  I  S  D  G  H  L  N  M  Q  M  R  Y  V  C  N  G  D  D   459
GAGCCATTAGTGATGGGCACCTCAACATGCAGATGCGATATGTGTGTAATGGTGACGACT 1380
 L  L  I  N  A  N  E  E  A  K  E  V  V  Q  N  K  Y  E  Q  Y   479
TGCTAATCAATGCGAATGAAGAAGCAAAGGAAGTAGTTCAAAACAAGTATGAACAATACA 1440
 I  K  E  L  E  L  N  Y  C  F  D  D  A  F  Q /S  I  E  G  V   499
TAAAGGAACTTGAACTAAATTATTGCTTCGATGATGCATTCCAGAGCATTGAAGGTGTTG 1500
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Fig. 1. Nucleotide sequence of the cloned potyviral RNA with deduced amino acid sequence.
underlined, bold faced types – possible protease cleavage sites, mentioned in the text; → – first amino acid of expressed recombinant proteins.

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 E  F  M  S  H  K  F  M  L  R  N  G  I  Y  I  P  K  L  A  R   519
AGTTTATGTCACATAAATTCATGCTGCGAAATGGCATCTACATACCCAAGCTAGCCCGGC 1560
 H  R  I  V  A  I  L  E  W  Q  R  S  A  E  P  Q /A  I  K  S   539
ACCGGATAGTGGCGATTCTGGAATGGCAACGGAGTGCGGAACCTCAAGCTATTAAGAGCG 1620
 A  I  L  A  A  C  V  E  A  F  G  Y  D  D  S  T  E  L  I  R   559
CTATTCTAGCAGCGTGCGTGGAAGCATTCGGTTATGACGATTCAACTGAATTGATTCGTG 1680
 E  Y  A  I  S  L  E  P  V  W  G  S  F  L  P  T  D  G  E  I   579
AGTACGCAATATCTTTGGAGCCAGTTTGGGGTTCTTTCTTACCAACCGATGGTGAGATCG 1740
 E  Q  L  Y  L  E  G  I  A  K  Q  E  V  A  R  C  L  A  G  V   599
AACAACTCTACCTCGAAGGAATCGCGAAACAGGAGGTAGCTCGCTGCTTAGCTGGTGTTG 1800
 D  D  V  C  K  F  E  S  A  A  P  G  T  N  E  A  V→D  E  M   619
ATGATGTGTGCAAGTTTGAGAGTGCAGCACCAGGAACTAATGAAGCCGTTGACGAGATGC 1860
 L  K  A  A  G  D  D  E  A  L  A  R  A  N  A  T  S  T  S  D   639
TGAAAGCTGCTGGTGATGATGAAGCATTGGCACGAGCAAATGCCACGTCCACTAGTGATA 1920
 T  I  P  P  A  R  N  V  G  A  D  T  T  T  P  A  K  A  N  P   659
CCATACCACCAGCGCGAAACGTGGGAGCGGACACAACAACACCAGCCAAGGCCAACCCAC 1980
 P  S  G  R  R  P  S  S  R  S  L  I  D  N  S  I  G  G  N  G   679
CATCAGGAAGACGTCCCTCGTCTAGAAGCTTAATAGATAATTCTATTGGGGGAAACGGTG 2040
 V  Q  D  V  A  D  R  T  S  G  I  V  F  P  V  P  T  R  K  S   699
TCCAGGATGTCGCGGATCGAACAAGCGGTATTGTGTTTCCAGTACCAACAAGAAAGAGCA 2100
 T  S  L  Y  L  T  P  K  V  K  L  R  A  T  T  E  E  L  R  K   719
CAAGTCTTTATCTAACGCCTAAGGTAAAGCTGAGGGCAACAACTGAAGAATTGAGAAAGT 2160
 Y  E  S  T  S  L  N  P  Q  Q  I  D  L  R  Y  S  T  Q  Q  E   739
ACGAAAGTACTTCCCTGAATCCTCAGCAAATAGACTTGAGGTACTCAACACAGCAAGAGC 2220
 L  N  D  W  I  K  A  S  A  D  G  L  G  Q  T  E  E  A  F  I   759
TTAATGATTGGATCAAAGCATCTGCTGATGGGTTGGGCCAAACTGAAGAAGCCTTCATTG 2280
 D  N  I  L  P  G  W  I  V  H  C  I  V  N  T  T  S  S  E  N   779
ACAATATTCTGCCTGGTTGGATAGTACACTGTATCGTTAATACAACCAGTTCGGAAAACC 2340
 R  K  A  G  S  W  R  C  V  T  N  A  G  T  A  D  E  E  Q  V   799
GGAAAGCTGGCTCTTGGCGATGTGTAACAAATGCTGGTACGGCTGATGAGGAGCAAGTGC 2400
 L  Y  D  I  E  P  M  Y  S  A  A  N  P  T  M  R  A  I  M  R   819
TCTATGATATCGAACCGATGTACTCAGCAGCGAATCCGACAATGCGTGCTATCATGCGGC 2460
 H  F  S  D  L  A  G  L  V  I  A  E  S  F  K  Q  G  R  P  L   839
ATTTTAGCGACTTGGCCGGTCTGGTGATAGCGGAGAGCTTTAAGCAAGGGCGCCCACTCA 2520
 I  P  K  G  Y  I  K  A  G  V  L  D  A  S  S  A  A  R  A  C   859
TACCAAAGGGATATATAAAAGCGGGAGTCCTAGATGCCAGTAGCGCGGCACGAGCATGCG 2580
 D  F  V  V  R  D  R  H  D  T  A  T  F  V  Q  V  Q  N  Q  V   879
ACTTTGTCGTGCGCGATAGACACGACACAGCTACGTTTGTGCAAGTGCAAAATCAGGTCT 2640
 L  V  N  R  V  S  G  I  T  N  W  L  F  A  Q  Q  C  L  A  L   899
TAGTTAATCGCGTTAGCGGTATAACTAACTGGCTGTTTGCACAGCAATGCCTAGCGCTGG 2700
 V  L  T  R  T  W  R  A  M  T  L  R  C  R  R  G  H  P  Q  P   919
TGCTAACGAGGACATGGCGCGCCATGACGCTCAGATGCCGCAGAGGGCATCCACAACCTA 2760
 R  L  S  A  S  F  L  E  V  S  T  S  C  R  A  R  R  R  L  G   939
GGTTGAGCGCGAGCTTTCTAGAAGTCTCGACGAGTTGTCGAGCACGCCGGCGTCTGGGGG 2820
 V  R  S  §                                                    942
TTCAAAGGTAAAACTCGTAATAGAGTTTTCCTCGCGGGACAATGGCATTGGACCAGGAA 2880

TGTAATGTGAAAGAGCAAACTTGGTCAAACGAGCGTAACAAAAGCGCAGCAAAATGGAAA 2940

GTCGGGCGTTATTCCAGGGCGCGTGTTTCGCCACTTTTGTGGAGGCGCACGTTTCATGGC 3000

GTTTCTTTCGGCTACTGTTGAGCAATA$_n$                               3026
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Fig. 1. Continued.

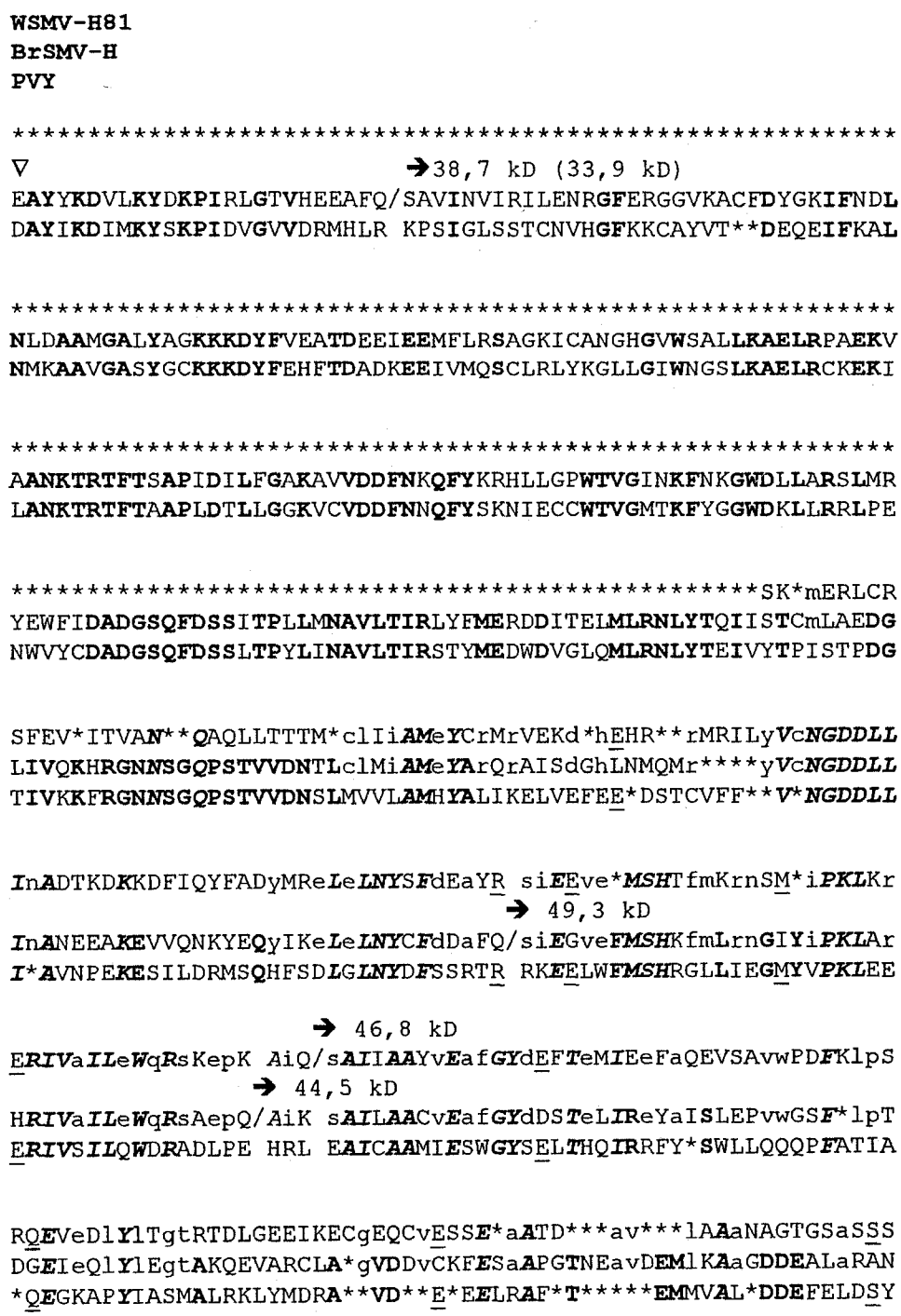

Fig. 2. Comparison of the deduced amino acid sequences of WSMV [Niblett et al., 1991], the sequenced virus (starting from aa 172, Fig. 1) and PVY [Robaglia et al., 1989].
Common amino acids of the viral proteins are marked by the following letters: WSMV/BrSMV – lower case; WSMV/PVY – underlined; BrSMV/PVY – bold; all three viruses – italic, bold; ➔ – putative protease cleavage sites with deduced sizes of proteins; ∇– aa position 172 of BrSMV.

Fig. 2. Continued.

merases (Fig. 4). The conserved sequence consensus motif {S(T)GXXXTXXXNS(T)-18 to 37 aa-GDD}, which is typical for a variety of both animal and plant positive stranded RNA virus RNA-dependent polymerases [Kramer and Agros, 1984], is almost completely present in the BrSMV deduced polymerase in a position similar to that of other potyviruses including PVY (Fig. 2). This motif could not be found for WSMV. The MVWCIENG motif in the cp, typical for viruses of the genus *Potyvirus* is not present in either WSMV or BrSMV. But both show the RAT motif, which is common for some potyviruses. The DAG motif in the cp, typical for aphid transmitted viruses, could not be found. A motif associated with the mite transmittability has not been proposed, as sequence date for other mite transmitted viruses are lacking.

The overall degree of aa homologies is higher between BrSMV and PVY than between PVY and WSMV (Fig. 4).

From our data we conclude that the BrSMV should be considered to be a member of the *Potyviridae*: the structure of the genome (one large open reading frame, cp located at the 3′ end, putative polymerase gene upstream from cp gene, RNA polyadenylated) is typical for a potyvirus and the aa sequence of its polymerase shows extended homologies to that of PVY. Further evidence is the structure and size of the BrSMV particles and the induction of pinwheel inclusions in infected plant cells [Rabenstein el al., 1982]. The structures of the cytoplasmatic inclusions induced by the isolate of *H. murinum* and the structures described by Milicic et al. [1980] are identical. They are different from those of WSMV, AgMV and HoMV which are on their part indistinguishable from each other [Langenberg, 1991].

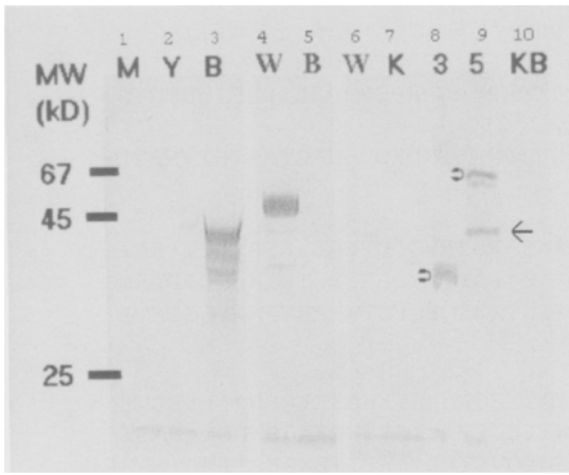

Fig. 3. Video image of western blots probed with polyclonal antisera against BrSMV (lanes 1–3, 6–9) and WSMV (lanes 4–5).
Designation of lanes: M – marker (it was temporarily stained with Ponceau S); Y – tobacco infected with PVY; B – wheat infected with BrSMV; W – wheat infected with WSMV; K – healthy control plants of wheat; 3 – expression proteins of BrSMV in pGEX2-T, the arrowhead ➲ marks the fusion protein band; 5 – expression proteins of BrSMV in pGEMEX-1, the arrowhead ➲ marks the main fusion protein band; the cp, cleaved in *E. coli* from the fusion protein, is marked by ←; KB – control with E. coli/pGEMEX-1 expression proteins.

According to the size of the cp and the transmission of the virus by mites it should belong to genus *Rymovirus* of *Potyviridae*. The aa sequences of the core parts of the cp of both streak viruses lack homologies, in this region typical for potyviruses [Puurand et al., 1994]. The conserved sequence consensus motif LGV/M found at the C-terminus of the cp of almost all potyviruses [e.g. Niblett et al., 1991; Puurand et al., 1994; Grumet and Fang, 1990; Wetzel et al., 1991] is present in the BrSMV cp (LGV) but only incomplete (NGV) in the WSMV. Shukla and Ward (1988) have predicted that potyviruses can be differentiated into distinct members or strains on the basis of the aa homology of their cp. The degree of homology between distinct members ranges from 38 % to 71 %, whereas strains are 83 % to 99 % homologous. The overall degree of homology reaches for PVY/BrSMV 13 % (beginning the comparison at the proposed NI_b/cp cleavage site of BrSMV) and 16 % for BrSMV/WSMV. For these reasons we assume that the BrSMV possibly belongs to a separate subgroup. It is phylogenetically closer to the genus *Potyvirus* than WSMV. This suggestion is supported by the serological comparisons, which did not show any cross reactions between BrSMV and other potyviruses of cereals.

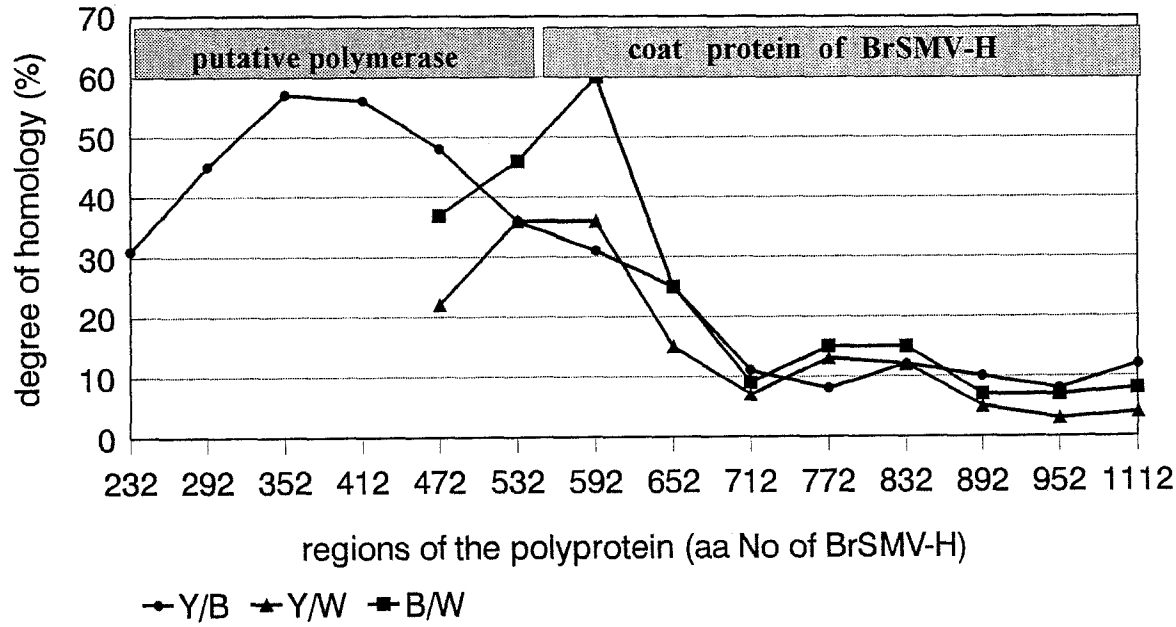

Fig. 4. Comparison of the degree of homology of aa in the different regions of the viral polyproteins. The compared regions include 60 aa (compare Fig. 2), the first region beginning with aa 172 of BrSMV and eating with aa 232.
Compared viruses: Y/B – PVY/BrSMV, Y/W – PVY/WSMV, B/W – BrSMV/WSMV.

Table 1. Detection of different members of the genus Rymovirus by DAS- and PTA-ELISA

	DAS-ELISA			PTA-ELISA			
				Antisera			
Viruses	AgMV	BrSMV-H	RgMV	WSMV-PV-91	TuMV-314	WSMV-PV-91	BrSMV-H
AgMV	+[1]	–	–	–	+	–	–
BrSMV (11–Cal)	–[2]	+	–	–	–	–	+
BrSMV-H	–	+	–	–	–	–	+
RgMV	–	–	+	–	+	–	–
WSMV-PV-91	–	–	–	+	–	+	–
WSMV-Cs	–	–	–	+	–	+	–

+[1] = positive reaction; A_{405} was > three times the negative control.
–[2] = no reaction.

An interesting observation can be made in Fig. 3. As visible in the case of the expression of the fusion protein, consisting of the viral cp, the putative NI_b and the T7 gene 10 protein, the protease cleavage site of the viral polyprotein is supposely recognised by bacterial proteases and the cp is released. Similar observations were made for PVY expression constructs (results not shown.) Such a phenomenon was previously observed by Eggenberger et al. [1989] for soybean mosaic potyvirus.

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