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Beet soil-borne virus RNA 1: genetic analysis enabled by a starting sequence generated with primers to highly conserved helicase-encoding domains

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The complete sequence of the 5834 nucleotides of RNA 1 of beet soil-borne furovirus (BSBV, Ahlum isolate) was determined using a PCR product obtained with primers to highly conserved coding regions for helicase-like proteins in RNA 1 of furo-, hordei- and tobraviruses as a starting sequence. Unknown parts of the sequence upstream and downstream of this starting sequence were amplified by means of RT–PCR techniques using combinations of specific and random primers. BSBV RNA 1 contains one large ORF for a readthrough protein with a molecular mass of 204 kDa (204K protein) which is interrupted internally by a UAA stop codon terminating the coding region for a protein of 145 kDa (145K protein). The N- and C-terminal parts of the 145K protein and the readthrough domain of the 204K protein contain methyltransferase, helicase and RNA-dependent RNA polymerase motifs, respectively. Unlike other furo- and tobraviruses BSBV contains no further genes on its RNA 1.

Beet soil-borne furovirus (BSBV) is one of many plant viruses which have never been obtained in a highly purified form due to the fragility of their particles, their tendency to aggregate and/or their low concentration in plant sap. For its RNAs 2 and 3 we had previously obtained a few short pieces of cloned cDNAs (average size 200–300 nucleotides) (Kaufmann *et al*., 1992). Their sequences were used for designing specific primers which, together with random-primed cDNAs prepared for RNA from either immuno-captured virus particles or denatured dsRNA, allowed us to amplify and to analyse almost the complete nucleotide sequences of the two smaller BSBV RNA species upstream and downstream of the known sequences (Koenig *et al*., 1996, 1997). For determining the 3′- and 5′-terminal sequences of these RNAs, the small amounts of cDNAs available were trapped on streptavidin-coated magnetic beads via 3′-biotinylated oligonucleotides. By this means they could easily be washed and (dG) homopolymer-tailed. Their terminal sequences were then amplified by means of PCR using oligo(dC) and a specific primer derived from the known part of the sequence (Koenig, 1997).

For the determination of the nucleotide sequence of RNA 1 of BSBV we had no cloned cDNAs which could be used as a starting sequence for designing the specific primers needed for the amplification of unknown parts of the sequence. It is known, however, that the replicase-encoding RNAs of plus-strand RNA plant viruses contain highly conserved methyltransferase (MetT), helicase (Hel) and RNA-dependent RNA polymerase (RdRP) motifs (Koonin & Dolja, 1993). We used part of the coding sequences for Hel motifs I and II which are highly conserved in soil-borne wheat mosaic (SBWMV) (Shirako & Wilson, 1993), peanut clump (PCV) (Herzog *et al*., 1994) and potato mop-top (PMTV) (B. Reavy, personal communication) furoviruses and barley stripe mosaic hordeivirus (BSMV) (Gustafson *et al*., 1989) for designing primers UF8 (5′ dGATGGTGTACCCGGATGTGGAAAGTC 3′) and UF9 (5′ dATGCGCCATCAACGCTTCATCAAAATG 3′) for sense and antisense DNA synthesis, respectively. Despite the fact that BSBV RNA 1 occurs in only very low amounts in infected tissues (the band for its dsRNA form is barely visible on ethidium bromide-stained agarose gels; Kaufmann *et al*., 1992), we succeeded in obtaining a PCR product of the expected size of 239 nucleotides when we used the primer pair UF8/UF9 with cDNAs to RNA from either immuno-captured BSBV particles or denatured dsRNA from BSBV-infected *Chenopodium quinoa*. This PCR product provided the starting sequence by means of which we eventually obtained the complete nucleotide sequence of BSBV RNA 1 by the approaches described previously (Koenig *et al*., 1997; Koenig, 1997). The primer pair UF8/UF9 was also successfully used to generate a starting sequence for the genomic analysis of RNA 1 of a new furovirus from sugarbeet (R. Koenig and others, unpublished). By chance, we found that it could also be

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The EMBL accession number of the sequence reported in this paper is Z97873.

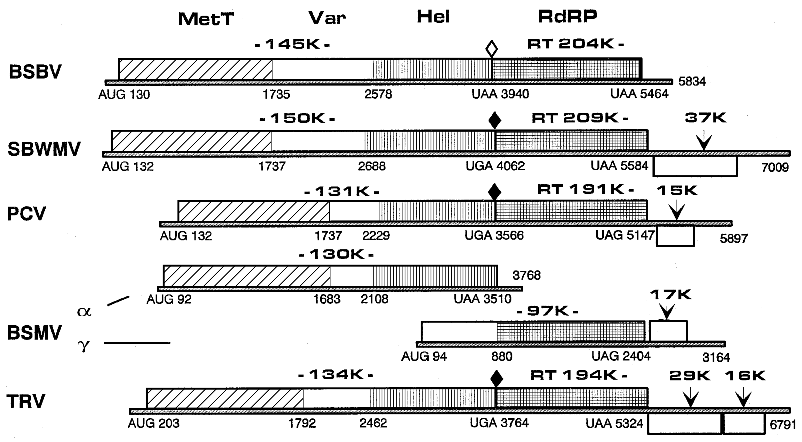

Fig. 1. Genetic organization of RNA 1 of BSBV and of the corresponding RNAs of other furoviruses (SBWMV and PCV), a hordeivirus (BSMV) and a tobravirus (TRV). The N- and C-terminal parts of the putative BSBV 145K protein and the readthrough (RT) domain of its putative 204K protein, which show considerable sequence identity with the corresponding proteins of the other viruses and which include the methyltransferase (MetT), helicase (Hel) and RNA polymerase (RdRP) motifs (Koonin & Dolja, 1993) are indicated by shading. A more detailed analysis of these parts of the proteins is shown in Fig. 3. The variable region (Var) is unique for each of the viruses shown. White and black diamonds indicate presumably leaky UAA (in BSBV RNA 1 only) and UGA stop codons, respectively. Only BSBV RNA 1 contains no further genes downstream of the RdRP domain. References: Shirako & Wilson (1993), Herzog *et al.* (1994), Gustafson *et al.* (1987, 1989) and Hamilton *et al.* (1987).

Fig. 2. Sequence alignment of the 3′-terminal 82 nucleotides of BSBV RNAs 1, 2 and 3 generated by means of the UWGCG programs LINEUP and PILEUP (Devereux *et al.*, 1984). The potential tRNA-like structures with pseudoknots (PS) and hairpins (SII), which are presumably involved in the aminoacyl acceptor arm, and two upstream hairpins (SIII and SIV), are shown. The 3′ terminus of RNA 1 is more similar to that of RNA 2 than to that of RNA 3. Nucleotide changes between the three RNAs are indicated by a black background. Asterisks and grey shading mark the anticodon for valine with is CAC for RNAs 2 and 3, but GAC for RNA 1.

employed for the amplification of the corresponding part of the Hel domain of RNA1 of tobacco rattle virus (TRV) (K. Uhde & R. Koenig, unpublished).

The genome of BSBV – like those of other plant viruses with a triple gene block-encoded transport function (Koonin & Dolja, 1993) – contains two domains encoding helicase-like proteins. The primer pair UF8/UF9 amplified only the corresponding coding region on BSBV RNA 1, not on RNA 3. This is apparently due to the fact that UF9 (5′ d *a*TGC*gcc*A*t*C*aacgc*TTCATC*a*A*aatg* 3′) proved to be complementary to nucleotides 3303–3277 of BSBV RNA1 with only 4 mismatches (underlined), but with nucleotides 1140–1114 of BSBV RNA 3 there are 15 mismatches (italicized lower case letters). UF8 (5′ d *gat*GG*t*GTACCCGG*at*GTGG*a*AAG*t*C 3′) corresponds to nucleotides 3067–3092 of BSBV RNA 1 with 4 mismatches (underlined), but with nucleotides 934–959 of BSBV RNA 3 there are 8 mismatches (italicized lower case letters).

RNA 1 of BSBV was found to consist of 5834 nucleotides (Fig. 1). For its 5′ terminus two independent cDNA clones yielded the sequences GGUAUUAUUUC … and GUAUUAUUUC …, respectively. These sequences resemble those at the 5′ termini of the RNAs of other definitive furoviruses which all start with GUA(U)$_n$ … which is followed by a C or an A residue (see Koenig *et al.*, 1996, 1997). The additonal 5′-terminal G residue in one of the clones is probably an artefact arising from the incorporation of a C residue in the cDNA in response to a 5′-cap structure during reverse transcription (see, for example, Bouzoubaa *et al.*, 1987).

The 5′-UTR of BSBV RNA 1 resembles those of the other

Fig. 3. Alignment of the N-terminal (*a*) and C-terminal (*b*) parts of the putative 145K protein of BSBV and the readthrough domain (*c*) of its putative 204K protein with the corresponding proteins of other furoviruses (SBWMV, PCV), a hordeivirus (BSMV) and a tobravirus (TRV). For the other viruses, only those amino acid residues which are identical to those in the BSBV proteins are shown. The methyltransferase motifs I–III, the helicase motifs I, Ia and II–VI and the RNA-dependent RNA polymerase motifs I–VIII (Koonin & Dolja, 1993) are boxed in (*a*), (*b*) and (*c*), respectively. Alignments were done by means of the UWGCG programs LINEUP and PILEUP (Devereux *et al.*, 1984). References are as in Fig. 1.

(a)

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BsBv      1 ..MESTNI.LTQI.....NRDDIIQAVLTTSASRTQSALHETLSRVIDDNIKNALKANTKKKTVDVKRNLSEEQVQYLCELYPERKIITSNSERGTHSMA
Sbwmv     1     S  I L  I        D I A   TSA    S LH T           A      KK  DV R LS EQ Q L  LYPER I  SN ERGTHS A
Pcv       1   M   NI          N     Q    T  S T S LH           K   K    K  VDV R LS E    LC LYPE   ITSN ERG H MA
Bsmv      1   M S  I                     T  S   S LH  L            K     K  DV RN S E  Q L   YPE     S  E GTHSMA
Trv       1   M   N  L Q                                RV  D           K  V V   LS      L E YPE  I         H  A

BsBv     93 AAMRKIETDLVLSLFP.KNSVIYDIGGNWATHAKCNDGRKVHCCCPILDYRDAQRKMTRMLNFHKFTSDSSEIPPDIREKAEMIAEDNAIITANVREGDL
Sbwmv    95 AA RKIETDL LS  P K S  YDIGGNWATH K NDGRKVHCCCP  D RD  RK  R     K      EIPP I E       D   I AN
Pcv      98 A  R  ET    SL P K  V  D GGNW TH K      VHCCCP LDYRDA RK  R L    F     E      E    I        A    G
Bsmv     94 A  R  ET   L   P K    YDIGGNW  H K    R  HCCCPIL  RD  R  TRM    K    S   P         I    A  T     G
Trv      99 AA RK    L L   P       DIGG W            H CCP LD RD QR   R                   E                R

BsBv    192 NSSALNGRWFCQNKFEDCVFDPRDILTGKTAKDNVMVYAMAIHSIYDINVYELATALQRKGIKRMVGTFLFSVDMLLGRKSGELPSVNGFYKLEGEYIKY
Sbwmv   194     L G W C N FEDCVF                 YAMAIHSIYDI    LA AL  K IK M GTFLFSVD LLG KSG LP   GF   E  Y KY
Pcv     198  S        C   F DC   P             M  AM  HS YD     L   L RKGI    G FLFS   LLG   GELP VNG  K     I
Bsmv    194 N   L G  FC N F DCV                   A A HSIYDI V E A AL RKGI    G FLF    L G K G LPSV G Y  E   IK
Trv     190    A      C N    C             K     YA A HS YD     L      K  K      LF    L     G LPSV G Y      I

BsBv    292 GFYDDPNCGYRHKFSSLKAYLTKTFVKAANGSVFYLELTDLRGDVMYFTMTDATEARASGI........MKDESFKCIPVDAKNK.VVFPLFVVDETTDT
Sbwmv   282 GF  D N GY H    L  YLTKTFV A  G   YLELT  RGDVM FTMTDATEAR  G           DESFKCIP D K   VVFP F VD  TD
Pcv     286  F DD   GY H       Y  K FV A  G V  LEL   RGD   F   D TE   S            D  FK  P       V  PL
Bsmv    285  F  DPN GY H       Y  KT V    G VF  EL   RGD M F  TD T                 DE FKCIP       VV PLF  D
Trv     280 GF  DP   Y H     K YL    V    G VFY E    RGD M F                        E    I        VV P F  E T

BsBv    383 LMFLERVLPKDFVHRAIEYVNRCKENQLTVESIVSYLSSTNNAVIIGGSARKVEEKVDPSLLPMIASTLLVYSEMQRAKQKTVIQKLRIHVKNTVTIGDL
Sbwmv   373 L F E  L  DFV RAIEY  R K  QLT      YL STNN  IIGGS  K   KVD  L   I  TL V  E     QK V  KLR   K  V    L
Pcv     383      R LPK  V R  E   R K N L  E    YL STN     G        K DP  L   A TLLV  E  R KQK     LR
Bsmv    377 L      LP   V          KE  L V     YLS  NN  I  GS      K  P L    A TL       R      I
Trv     371 L        K F      Y  R    QLT     SYLSS N    I G A K    VD   L   A TLLV     R              K      D

BsBv    483 LNHAFHKVF....G...AVGVGQLALQSFARWLKFFHGSGVIEMNDLFMYVEIEDRIRLW                       % identity   % similarity
Sbwmv   473     F K F    G    V   Q AL  FA W    HG   I       Y E  DRI LW                          51         71
Pcv     483     F K F    G    V      L   ARW     GS       L  YVEIED   LW                          38         59
Bsmv    477     F                                        D   YVEI DR                              26         59
Trv     471 L     K            G             K          N      E                                  28         53
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(b)

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BsBv    817 LPSKPDYLESDDFRMRAKKEFIWYLECKLVSDKSAMTDIVRDYVYNMYHNSLCEFPKNSCFLSYEGDDNGSWAWGRKPV.RLGHAYAVHFY..A.....A
Sbwmv   863 LP  PD          AK E  WYL CK VSD       I  D    M HN  CE PK S FL Y  D  G W  G KP  R GH Y V F
Pcv     700 LP  P Y   DD    A  E  WYL CK   D SAM   V D      H   CEFPKN  FL         WA  R      GH Y V F
Bsmv    672  P  P         M A  EF  Y  C              D   N         P N  F S      G W          GH YAVHF
Trv     754 LP  P           A  EF  YL    V          RDY            P        E                  HAY

BsBv    909 DWKTNCRLVSLSWNKDEEGNFVGDKPVISTNSGVYMLCDLTFLMNEMIILENLQFSLKTRFQKHIPHVTLIDGVPGCGKSTHIVKEARLENQYVLTMGRE
Sbwmv   956    T C LV L WN D  G     KPV         L DL F MNE I   NLQ   K          TL DGVPGCGKST I   A      VL  G E
Pcv     791        LV   W K                 G YM  DLT LMNE I    L         K    V LIDGVPGCGKST I     L  Q VL  GRE
Bsmv    769      CR V  SW K   G      P      G Y  CD T L N  I    L                LIDGVPGCGKST I          V   GR
Trv     841 DW                             S     CD T L N  IL  NL                L DGVPGCGKST IV  A      VL  GR

BsBv   1009 AAAELRERFK.TVRGSTEEQLKRVRTVDSFLMNDKDS..RAKILHFDEALMAHAGMVYFCADNLSARTIICQGDSQQIPFINRVESITLEYAKLEITNVV
Sbwmv  1055 A   L E F        E  LKR RTVDSFLM D D   RA   HFDEALMAHAG VYFCAD L A   ICQGDSQQIPFINRVESITL YAKL I
Pcv     884 A   LR RF             RVRTV SF         R    HFDEALMAHAGM Y C   L AR  ICQGDS QIPFINRVE I L Y
Bsmv    866 A   LRERFK            RVRT DS L         A   HFDEAL  H G   FCAD L A  I  QGD  Q P I RVE I L       T
Trv     932 A   L ERF             RV TVDSFLM   D       LHFDEALMAHAGMVYFCA    A   ICQGD  QI F  RV    L    L

BsBv   1106 EKRL..TYRSPLDVACFLTRKNFYW....HFHCYERKSNGRSISVVGPRDG..MTSNYSIPKKKGAQYLTFTQSEKEDMVRYL.GKGQ. WSVNTVHESQ
Sbwmv  1153   RL  TYRSP DVA  LT K  Y             S  RS  VVGPRD   MTS    P    AQYLTFTQSEK D    L  KG     VNTVHE Q
Pcv     981   R   TYR PLD    L  K  Y                 S    G       TS    P K    YLTF Q EKE     L G       V TVHE Q
Bsmv    966        YR P DVA  L  K FY             S  RS    G        S    P K    YLTF Q EKE     L  KG    S  T HE Q
Trv    1030       TYRSP DVA  L                       S  V          S      K GAQY TF QSEK   V  L         V TVHESQ

BsBv   1195 GKTYDDVILVRLKPTDNEIYPGGRKSKPYVVVGTTRHRRSLVYYTRAEDILYRDITEMMSVQEGKLHKHLFTESTQ       % identity   % similarity
Sbwmv  1245 GKT DDVI VRLK T NEIYPGGRK  PY  V TTRHRRSLVYYT  ED L  DI  M  V E KL K L  E T           54         71
Pcv    1070 GKT   V LVRLK TDNE YPGG KS PY  VG TRH RSLVYY   ED LY DI     V E  L K  F E T           45         61
Bsmv   1066 G TY  VILVRL  T NEIYPGG  S PY VVGT RH     Y     D L  DI                               37         57
Trv    1116 G T  DV LVR KPTD  I         Y  V   RH  SLVY T  ED     I E        L     TE             40         58
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(c)

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BsBv   1271 RCGSKYESITVSDRPVSVPDVGAVEDLQVMYDIAFPGNSVIETYFDGYDVATGGLEIDIANIKYYPNKQKRMWQEVRG.LTPALRTAMPEKRQSGLIESV
Sbwmv   863 R GSKYESI   DR V VPDVG     Q  YD   PGNS     FDGY V T    I   N    PNK    WQ  RG L P LRTAMP KRQ    E
Pcv     700 R GSK ESI V DR V  PDVG     Q  YD  FPGNS     FDGY VA   LE  I N K  PNK  R  QE      P LRTAMPEKRQ    ES
Bsmv    296 R     E  T   R V VP VG   D Q   D  F GNSV    FDGY VAT     D         K  R        L P LRTAMPEKR   LI SV
Trv    1189 R  S          P  VPD G   DL   YD  FPGNS      DGY VAT        N       K    E     L P  RTAMP KR     ES

BsBv   1370 LALNKRNMAAPRLQESVNEFEIIENVISKAKAVFFDEKFIDASPMNSVSNCQKWWDKQSVTAQKQMLADTRIISEIDLCTYNFMIKNDVKPKLDLSPQSE
Sbwmv   962 LAL KRNMAAP LQE VNEFE IE     AK  FFD   ID S          WW  QS TA  Q    TR   E DLC YN  IK D KPK D SPQ E
Pcv     799 LAL KRNMA PRLQESVNE  IIE  I KA    F   F D SP         WW KQS TA  QMLAD R    ID CTYNFMIKNDVKPKLDL PQSE
Bsmv    395 LA  KRN AAPRLQ   NE   IENV  KA  VFF E  ID            WWDKQSVTA  Q  AD R     D  TYNFMIKNDVKPKLDL PQ E
Trv    1289 LAL KRN AAP LQE V     IE    K K V  D   I A P         WW  QS   Q    AD R   EID   Y  MIK DVKPK DL PQ E

BsBv   1470 YSALQTVVYPDKIVNAIFGPVMKEINERILLALRPHVVYNTRMTAEELDRSVEFLDVREQYDAVEIDFSKFDKSKTSLHIRAVIELYKIFGLDDMLAFLW
Sbwmv  1062 Y ALQTVV PDKIVNA FGP MKEINERI LAL PHVVYN RM AEEL R VEFLD  E   A EIDFSKFDKSKTSLHIRAVIELYK FGL D  A LW
Pcv     899 Y ALQTVVYP KIVN  FGPV KEINER L AL P V  NTRMT EEL R  E L        VEIDFSK DKSKTSLHIR VI LY  FGL      LW
Bsmv    495 Y ALQTVVYPDKIVNA FGP  K INERI  ALRPHVV N RMTA EL   V FL     Y A EIDFSKFDKSKT LHI AVI LYK FGLD  L  LW
Trv    1389 YSALQTVVY  K  N  FGP  KEINER L A  PH V NTRMT   L   V FL     YD VEID SKFDKS    H     E Y  FGLD   AFLW

BsBv   1570 EKSQCQTTIRDRQNGLIAHILYQQKSGNCDTYGSNTWSAALALLDALPLERAKFMVFGGDDSLVFFPKNMNLADPCGRLASLWNFDCKFFNFQNNMFCGKF
Sbwmv  1162 EKSQCQT IRD  NG  A  LYQQKSGNCDTYGSNTWSAALALL   PLE AKFM FGGDDSL  FPK     DPC RLASLWNFDCK F F  NMFCGKF
Pcv     999 EKSQ QT   DR  G  A ILYQQKSGNCDTYGSNT S   ALLD LP E A    FGGDDSL  FPK     DPCGRLASLWNFDCK   F    FCGKF
Bsmv    594 EKSQ QT   DR  GL A  LYQQKSGNCDTYGSNTWSAALALLD LPLE A F VFGGDDSL  F       DPC  LA  WN  CK F F    FCGKF
Trv    1489 E S  QTT RD QNG  AHI YQQKSG  DTY  N      ALL  LPLE A     GGDDSL  FP      DPC  LA  WNF CK F     MFCGKF

BsBv   1671 LLKIGENYKFAPDPFKLLTKLGRKHIK............NDILLSEIFISISDNYRSYHDYRVLEALNVAVVERYKLSQDVLFGLCALKKYLFNFDLFR
Sbwmv  1263 LLK G   KFAPDP KL TKLGRK I                LLSEIF S  DNYRSY DYR LE L  A  ERY    D    L ALKKY F F L
Pcv    1300 L   G   Y FPDP KL TKLG K I                LLSEI  SI DNY  Y D RVL AL  A V RYK        L  L K L  F  F
Bsmv    695 LL I    Y FPD  K  TKLGR                    LSEI ISI DNY SY D  VL AL  A V RY          L  L    F F  F
Trv    1589 LLK     Y FPDP K LTKLG K IK                L EI IS  D  R    Y V   L   V  RY    D    LCAL K    F

BsBv   1758 TLF....S..YKGK.....LHCTNVSRNFDW*                                                   % identity   % similarity
Sbwmv  1350   F       YKG           V RNF W*                                                      63         80
Pcv    1199  LF         G           V     W*                                                      59         74
Bsmv    782  LF        K                F W*                                                      56         74
Trv    1677 TLF                     VS   DW*                                                      47         65
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Fig. 3. For legend see facing page.

two BSBV RNAs in having a high content of U residues (44% versus 33·5% and 41·3% in RNAs 2 and 3, respectively). There are, however, no continuous stretches of more than seven identical nucleotides in the 5′-UTRs of the three BSBV RNAs. The RNA 1 5′-UTR is followed at nucleotide 130 by a single large ORF which is interrupted at nucleotide 3940 by a UAA stop codon and then continues in-frame for an additional 1521 nucleotides until it reaches another UAA stop codon at nucleotide 5464 (Fig. 1). The sequence from AUG (nucleotide 130) to the first UAA stop codon has the coding capacity for a protein with a molecular mass of 145 kDa (145K protein). The 5′- and 3′-context of this UAA stop codon (CAA<u>UAA</u> CGGUGUGGGUC, stop codon underlined) is very similar to that of the UGA stop codons (AAA<u>UGA</u>CGGUUUGGGUC) in the corresponding positions in RNAs 1 of SBWMV (Shirako & Wilson, 1993) and PCV (Herzog *et al*., 1994). Presumably, this context confers leakiness to UGA and possibly also UAA stop codons. Readthrough of the UAA stop codon would result in a protein with a molecular mass of 204 kDa (204K protein). No further ORFs for peptides of more than 4 kDa were detected on the plus strand of BSBV RNA 1. The 3′-UTR consists of 371 nucleotides. The sequence of the 3′-terminal 82 nucleotides of BSBV RNA 1 which, like those of the other two BSBV RNAs (Koenig *et al*., 1996, 1997), has the potential to fold into a tRNA-like structure is more similar to that of BSBV RNA 2 (only six nucleotide changes) than to that of RNA 3 (17 nucleotide changes) (Fig. 2). It differs from that of the other two RNAs in that its possible anticodon for valine is <u>G</u>AC rather than <u>C</u>AC. In RNA 1 of SBWMV, the anticodon for valine is also <u>G</u>AC, but in its RNA 2 it is <u>C</u>AC (Shirako & Wilson, 1993).

The N- and C-terminal parts of the putative BSBV 145K protein and the readthrough domain of its 204K protein show considerable amino acid sequence identity with those of the corresponding proteins of other rod-shaped viruses (Fig. 3). These areas contain the typical MetT, Hel and RdRP motifs, respectively (Koonin & Dolja, 1993). Also, outside these motifs, blocks of highly conserved sequences are found (Fig. 3). The relationship to SBWMV seems to be especially close. No appreciable sequence identity was detectable for the central parts of the smaller proteins of the viruses listed in Fig. 1. In the BSBV 145K protein this variable region (Var in Fig. 1) extends approximately from amino acids 585 to 815 corresponding to nucleotides 1735–2577 of BSBV RNA 1.

Although the MetT, Hel and RdRP regions of the putative BSBV proteins show high percentages of sequence identity with those of the other above-mentioned viruses, the genome organization of BSBV is different. For BSBV RNA 2 and 3, this has been discussed previously (Koenig *et al*., 1996, 1997). BSBV RNA 1 differs from those of the definitive furo-, hordei- and tobraviruses by not containing any further genes downstream of the RdRP region (Fig. 1). Genes for 37 kDa or 29 kDa movement proteins are found, in addition to the replicase genes, on RNAs 1 of SBWMV (Shirako & Wilson, 1993) and TRV (Hamilton *et al*., 1987), respectively. For the other viruses, including BSBV, there seems to be no need for such a gene because their movement functions are apparently encoded in a triple gene block which forms part of their smaller RNAs. Genes for structurally and possibly functionally related proteins with molecular masses of 15–17 kDa are found near the 3′-ends of PCV and TRV RNA 1 and on BSMV RNA (Herzog *et al*., 1994; Hamilton *et al*., 1987; Gustafson *et al*., 1987), whilst a gene for a possibly similar cysteine-rich protein with a molecular mass of 8 kDa is found near the 3′ terminus of RNA 3 of PMTV (Scott *et al*., 1994). The genome of BSBV apparently lacks such a gene. In all other properties, however, it corresponds to those of the recently proposed genus pomovirus which contains fungus-transmitted rod-shaped viruses with a tripartite genome (Torrance & Mayo, 1997).

We are greatly indebted to Dr Bryan Reavy for making a short piece of the sequence of PMTV RNA 1 available to us, which we used, in addition to those of SBWMV, PCV and BSMV, for designing the primers UF8 and UF9. The financial support given by the Deutsche Forschungsgemeinschaft (grant Ko 518/13-1) and the Gesellschaft zur Förderung der Privaten Deutschen Pflanzenzüchtung (GFP, grant AIF 103/50) for different aspects of this research is gratefully acknowledged.

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