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Sequence comparison of the 3′-terminal parts of the RNA of four German isolates of sugarcane mosaic potyvirus (SCMV)*

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Summary. The 3′-termini of the genome of four German isolates of sugarcane mosaic potyvirus (SCMV) were cloned and sequenced. The sequence data covered the 3′ non-coding region (3′NCR), coat protein and part of the nuclear inclusion b (NIb) genes of the isolates. Comparisons of the sequences revealed that the investigated isolates are very closely related. An alignment of the predicted coat protein amino acid sequences of the German isolates with sequence data for other members of the SCMV subgroup, in particular the two SCMV strains, SCMV-SC and SCMV-MDB, showed a limited degree of homology indicating that the German isolates may represent a distinct virus. However, this is mainly due to the previously reported unexpected sequence diversity in the surface exposed N-terminal region of coat protein of SCMV isolates. Comparisons of the amino acid sequences of the core region of the coat proteins and the nucleotide sequences of the 3′ NCR clearly show that the German isolates are strains of SCMV.

Introduction

Sugarcane mosaic potyvirus (SCMV) is the most prevalent virus infecting maize crops in Germany in contrast to other European countries where maize dwarf mosaic potyvirus (MDMV) is dominant [2, 23, 43]. Both viruses cause systemic mosaic pattern and occasional dwarfing and they are not distinguishable visually on maize plants because of their similar symptomatology.

In Germany diseased maize plants were found for the first time near Halle (Saxony-Anhalt) in 1982 [13] and in south-west Germany (upper Rhine valley) in

* EMBL accession numbers: SCMV-Seehausen type S26: X98165; SCMV-Seehausen type S288: X98166; SCMV-Borsdorf: X98167; SCMV-Boetzingen: X98168; SCMV-Hoehnstedt: X98169.

1983 [22]. Two potyviruses were isolated from the infected plants and were identified as SCMV and MDMV by serological investigations. Since then both these viruses have occurred regularly in maize crops in Germany [16]. The percentage of SCMV and MDMV in relation to the total attack differs from year to year, although SCMV has been prevalent in the last few years [16]. Field experiments revealed that an early infection of both SCMV and MDMV may reduce the plant height by 16.9%, the total plant weight by 37.1% and the corn-cob weight by 27.8% under moderate climatic conditions [14]. Other authors have reported grain yield losses of up to 54% in the case of susceptible varieties after mechanical inoculation with MDMV [27].

SCMV was first described in the USA by Brandes [6]. A large number of SCMV strains have since been reported from different parts of the world [46]. Particles of SCMV are flexuous filaments, about 750 nm long and 13 nm wide [45], containing a positive-sense single-stranded RNA genome of an apparent molecular weight of 3.4×10^6 Da [5]. The virus induces cytoplasmic inclusion bodies of the pinwheel, scroll and laminated aggregate type (subdivision III) in infected cells of maize [8, 29]. Like typical potyviruses, SCMV is aphid-transmitted in a nonpersistent manner. Experimentally the virus can be transmitted by 20 aphid species in 15 genera [9]. In Germany *Rhopalosiphum padi* L. seems to play a major role in transmitting and spreading the virus [15].

To date, a lot of serological data have been presented on strains of SCMV and MDMV [21, 24–26, 44, 47]. Since most strains are indistinguishable serologically [24, 47], there has been much confusion about the taxonomic status of the virus in the past. When Shukla et al. [40] compared 11 strains of SCMV and six strains of MDMV from the USA and Australia on the basis of their reactivities with cross-absorbed polyclonal antibodies directed to the N-termini of the coat proteins in Western blotting they found that these 17 strains did not represent only two potyviruses but, in fact, belonged to four different viruses named SCMV, MDMV, Johnson grass mosaic potyvirus (JGMV) and sorghum mosaic potyvirus (SrMV). These viruses were grouped together as the SCMV subgroup of potyviruses [41]. This assignment of former SCMV and MDMV strains into four distinct potyviruses based on N-terminal coat protein (CP) serology has been supported by several other biological and biochemical properties including: (1) reactions on selected sorghum and oat cultivars [48], (2) CP HPLC peptide profiling [31, 32], (3) CP sequence homology [11, 38], (4) 3′non-coding region (3′ NCR) sequence identity [10], and (5) nucleic acid (na) hybridisation [12].

Little information, however, exists about the relationship of European isolates. In this paper sequence data are presented for the 3′-terminal region of the genome of five German isolates of SCMV and compared with those of SCMV and MDMV strains reported previously [1, 11, 49].

Materials and methods

The isolates involved in this investigation were chosen from an available pool of 11 German isolates of SCMV from maize received from different geographical regions as well as collected

in different years. The isolates SCMV-Seehausen (SCMV-Se) and SCMV-Borsdorf (SCMV-Bor) were obtained from fields near Leipzig (Saxony) in 1984 and 1985, respectively. Isolate SCMV-Hoehnstedt (SCMV-Hoe) was isolated in 1995 in Saxony-Anhalt. SCMV-Boetzingen (SCMV-Boe) is an isolate collected in south-west Germany (Kaiserstuhl, Baden-Wuerttemberg) in 1994.

All isolates were propagated in mechanically infected maize plants cv. Bermasil. Virus purification was carried out according to a modified method of Langenberg [28]. RNA-extraction followed the method of Schubert and Rabenstein [37]. The cDNA was made using 1 µl oligo $d(T)_{15}$ primer according to the method of Gubler and Hoffmann [19]. For the first strand synthesis M-MuLV reverse transcriptase (Superscript II, Gibco) was used. The ends of the ds DNA were blunted with T_4 DNA polymerase (USB). The success of the reactions was monitored by addition of ^{32}P dATP (Amersham). The blunted ds DNA was fractionated on G50 columns (Pharmacia). The largest fragments were collected, precipitated with ethanol and ligated into the EcoR V site of the polylinker in the vector pBluescript SK II+ (Stratagene) using T_4 DNA ligase (Amersham). Recombinant plasmids were used to transform highly competent *Escherichia coli* XL-2 Blue cells (Stratagene). White colonies were proved on nylon filters (Hybond N, Amersham) with ^{32}P dATP labelled cDNA after alkali lysis of bacteria [18]. All positively reacting clones were tested by restriction analysis of small-scale preparations of plasmid DNA [36].

In order to obtain clones of the isolate SCMV-Boe an IC-RT-PCR was carried out. Plant material was homogenised 1:1 (w/v) with PBS-buffer containing 5% non-fat dry milk. After centrifugation 100 µl of the supernatant was loaded in the wells of ELISA plates (Covalink, Nunc) coated with polyclonal SCMV antiserum (1:25). After overnight incubation at 4 °C the wells were washed with PBS (4x) and water (2x). For first strand synthesis the primer 5′-(T_{20})GTCTC-3′ synthesised according to the sequence data obtained in advance for the isolate SCMV-Se was used. The mixture, 30 µl per well, contained 50 nmol primer DNA, 0.3 mM dNTP (Pharmacia) and 2.5 mM $MgCl_2$ in Ampli-Taq buffer (Perkin-Elmer). From different enzyme-buffer combinations tested the M-MuLV reverse transcriptase of USB (200 U per reaction) gave the best results. After 2.5 h incubation at 37 °C the reaction mixture was transferred to PCR tubes and 70 µl of a mixture containing 50 nmol second strand primer (5′-GAAGCATGGGGATATCCAGACCTTG-3′), 1.25 mM $MgCl_2$, 0.07 mM dNTP, 0.2 U RNase-H (Amersham) and 2.5 U Ampli-Taq (Perkin-Elmer) in Ampli-Taq buffer were added. PCR was carried out after 10 min pre-incubation at 37 °C and 2 min at 94 °C as follows: 35 cycles 30 sec at 94 °C/1 min at 58 °C/1 min at 72 °C, and finally 10 min at 72 °C. The PCR fragment was isolated from the LMA gel, ligated into the pGEM-T vector (Stratagene) and transformed into highly competent *E. coli* XL-2 Blue.

Nucleotide sequencing was performed with purified plasmids as templates ("Wizard" purification kit, Promega) by means of dideoxy-sequencing (AmpliCycle Sequencing Kit, Perkin Elmer) using ^{33}P dATP (Hartmann Analytics or ICI) for labelling. Sequencing was carried out in both directions using a series of sequential primers obtained successively from sequencing data (bootstrap method). From the clones obtained by IC-RT-PCR two independent clones were sequenced.

Data for comparison with other sequences for viruses of the SCMV subgroup were obtained from EMBL library (release 43, accession numbers: SCMV-MDB, an American strain of SCMV, referred to as MDMV-B: D00949; SCMV-SC, an Australian strain isolated from sugarcane: D00948; potato virus Y, PVY: X12456) and for MDMV-Sp from the corresponding publication of Achon et al. [1]. In addition, amino acid (aa) sequences of the N-termini (Q11 to core region) of five different Australian isolates [49] were used for comparisons of the glycine and glycine/serine content of this CP region.

Computer analysis of sequence data, alignments of sequence data and compilation of aa were performed using DNASIS (Hitachi) or Generunner (Hastings) software. The CLUSTAL option of the DNASIS program was used for estimation of relatedness of the isolates expressed in %. The print outs of phylogenetic trees were adopted.

For size estimation of the CP, infected plant material was homogenised with water 1:2 (w/v). The homogenate was centrifuged immediately and the supernatant mixed 3:1 with 4X loading buffer and boiled for 2 min. For protein electrophoresis (13% SDS-polyacrylamide gels), 15μl of the samples were used and gels were run at 120–140 V for about 3 h. The proteins were blotted onto a PVDF membrane (Roth) and were then treated with IgG from a polyclonal SCMV-Se antiserum (1:500) and goat-anti-rabbit secondary antibodies (1:1 000) conjugated to alkaline phosphatase. The blot was developed with BCIP/NBT (Serva).

Results and discussion

Several cDNA clones of the four isolates were investigated. For complete sequencing, clones were chosen which contained the 3′ region of the genomic RNA including the poly-A tail, the 3′NCR, the CP gene and at least a part of the nuclear inclusion b (NIb) gene of the virus genome. Since cloning was very effective, some nearly full-length clones were obtained. This was confirmed by comparing the deduced aa sequence of the clones with that of other potyviruses and by primer extension experiments (results not shown). These clones were used for further investigations.

Western blots of the viral CPs of 11 German SCMV isolates including those sequenced gave sizes of approximately 34 kDa for all German isolates except the isolate SCMV-Se which showed two bands (34 and 37 kDa). Two other SCMV isolates from the USA and the Cape Verde Islands, which had CP of about 36 kDa and 34/37 kDa, respectively, were also included in the Western blot assay (Fig. 1).

Sequence analysis of clones of the investigated virus isolates revealed the expected single large open reading frame. The lengths of the 3′ NCR (245 nucleotides [nt] for Se288, 239 nt for Se26, 258 nt for Hoe, Boe and Bor) are

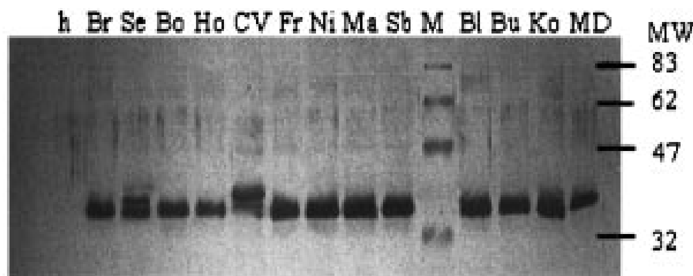

Fig. 1. Estimation of molecular weights of the cp of different SCMV isolates by Western blot. For detection a polyclonal antiserum was used. *Br* Borsdorf; *Se* Seehausen; *Bo* Boetzingen; *Ho* Hoehnstedt; *Fr* Friedrichshofen; *Ni* Ninburg; *Ma* Malling; *Sb* Schwabbach; *Bl* Blaustein; *Bu* Buehl; *Ko* Kossenblatt (all from Germany); *CV* SCMV isolate from the Cape Verde Islands; *MD* SCMV-MDB (USA); *h* healthy control; *M* marker, molecular weight markers (*MW*) in kDa on the right

within the reported potyvirus range of 169–475 nt [20]. Clones of the isolate SCMV-Se gave two different sequences, suggesting that it is a mixture of at least two different isolates. These are represented by the clones Se26 and Se288. The clones differ in length of the 3′NCR and size of the CP. Consequently, the sequences for five different isolates were obtained. According to the results of the Western blot analysis, SCMV-Se might contain still another isolate with a somewhat larger CP (37 kDa, Fig. 1).

For further comparisons, sequence data were selected which start at the putative cleavage site between the NIb and CP genes (VxHQ/S,A; [7]) and cover the entire CP and 3′NCR up to the poly-A tail. The predicted aa sequences for the CPs are presented in Fig. 2. They result in a molecular weight of the CPs of 33.6 kDa what is in accordance with the electrophoretically estimated size (Fig. 1). Interestingly, the differences in sequences of the German isolates are not so striking although they had been isolated from distant places and in different years. All five German isolates revealed a putative Q/S cleavage site between NIb/CP in contrast to the published SCMV-SC and MDMV-Sp sequences which cleave at a Q/A site [1, 11]. The obtained aa sequences were aligned with those of other published sequences of SCMV-SC and SCMV-MDB [11] as well as MDMV [1]. The data for JGMV (EMBL accession no.:Z26920) [17] and SrMV (EMBL accession no.: U07219; Jilka JM and Clark JM Jr, unpublished) were not included in the comparison as these viruses were clearly distinct from SCMV and MDMV (results not shown).

In all cases the DAG motif thought to be involved in the aphid transmission [3, 4], which occurs downstream from the NIb/CP cleavage site, was present, although some of the investigated isolates (SCMV-Se and SCMV-Bor) have been propagated exclusively by mechanical transmission for more than ten years (i.e. 6–8 mechanical transmissions per year). All highly conserved long aa motifs in the CP, typical of viruses in the potyvirus genus, e.g. MVWCIENGTSP [37], were perfectly conserved, too (Fig. 2).

To answer the question whether a potyvirus isolate is a strain of an established virus or a distinct virus some criteria were established by Shukla and Ward [39] and Frenkel et al. [10]. They are based on the comparison of the aa/nt sequences of the CP, its N-terminus and core as well as the 3′NCR. In general, CPs of distinct members are 38 to 71% identical. They differ markedly in length and sequence of their N-termini. CPs of strains of individual viruses are more than 90% identical and have very similar N-terminal sequences [39]. The 3′NCR of strains are 80% or more identical, whereas those of distinct viruses are less than 50% identical [10]. Figures 3 and 4 show a cluster analysis of the sequenced isolates and other potyviruses of maize i.e. SCMV-SC, SCMV-MDB [11] and MDMV-Sp (a Spanish MDMV isolate [1]) for the entire CPs and for the 3′NCR, respectively. PVY [35] was used as an outgroup virus. Sequence data for the SCMV isolates investigated by Xiao et al. [49] were omitted in these comparisons since no complete CP and 3′NCR sequences were available.

Interestingly, a comparison of the different regions of the genome gave different degrees of relatedness among the strains/viruses. Comparing the entire

```
                                      *
Bor        1 SG-SVDAGAQGGNSGSGASTPA-----------AGSGSGTRPPSTGSAAQ
Boe        1 SG-SVDAGAQGGNSGSGASASA-----------AGSGSGTRPPSTGSAAQ
Hoe        1 SG-SVDAGAQGGNSGSGASASA-----------AGSGSGTRPPSTGSAAQ
Se26       1 SG-SVDAGAQGGNSGSGASTPA-----------AGSGSGTRPPSTGSAAQ
Se288      1 SG-SVDAGAQGGNSGSGASTPA-----------AGSGSGTRPPSTGSAAQ
SCMV-MDB   1 SG-TVDAGAQGGSGSQGTTPPATGSGAKPATSGAGSGSGTGAGTGVTGGQ
SCMV-SC    1 AG-TVDAGAQGGGGNAGTQPPATGAAAQGGAQPPATGAAAQPPTT----Q
MD-Sp      1 AGENVDAGQKTDAQKEAEKKAAEEKKAKEAE----------AKQKETKDK
C

                                                  *     ⇨
Bor       51 GNTPTASGGSSGNNGGNQSGSNGTG----GQAGSSGTGGQRDKDVDAGST
Boe       51 GNTPPTSGGSSGNNGGGQSGSNGTG----GQAGSSGTGGQRDKDVDAGST
Hoe       51 GNTPPASGGSSGNNGGGQSGSNGTG----GQAGSSGTGGQRDKDVDAGST
Se26      51 GNTPTASGGSSGNNGSNQSGSNGTG----NQAGASGTGDQRDKDVDVGST
Se288     51 GNTPTASGGSSGNNGSNQSGSNGTG----NQAGASGTGDQRDKDVDVGST
SCMV-MDB  51 ARTGSGTGTGSGATGGQSGSGSGTEQVNTGSAGTNATGGQRDRDVDAGST
SCMV-SC   51 GSQLPQGGATGG--GGAQTGAGGTGSV---------TGGQRDKDVDAGTT
MD-Sp     51 ATEKADTGGSTG---------------------------KDKDVDAGTS
C                                                   DV

Bor      101 GKISVPKLKAMSKKMRLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEE
Boe      101 GKISVPKLKAMSKKMRLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEE
Hoe      101 GKISVPKLKAMSKKMRLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEE
Se26     101 GKISVPKLKAMSKKMRLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEE
Se288    101 GKISVPKLKAMSKKMRLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEE
SCMV-MDB 101 GKISVPKLKAMSKKMRLPKAKAKDVLHLDFLLTYKPQQQDISNTRATKEE
SCMV-SC  101 GKITVPKLKAMSKKMRLPKAKGQDVLHLDFLLTYKPQQQDISNTRATREE
MD-Sp    101 GSMSVPKLKAMSKKMRLPQAKGKNILHLDFLLKYKPQQQDLSNTRATRAE
C            G   P     K  P  G      HL  Y P Q   N RAT  Q

Bor      151 FDRWYDAIKKEYEIDDTQMTVVMSGLMVWCIENGCSPNINGNWTMMDGDE
Boe      151 FDRWYDAIKKEYEIDDTQMTVVMSGLMVWCIENGCSPNINGNWTMMDGDE
Hoe      151 FDRWYDAIKKEYEIDDTQMTVVMSGLMVWCIENGCSPNINGNWTMMDGDE
Se26     151 FDRWYDAIKKEYEIDDTQMTVVMSGLMVWCIENGCSPNINGNWTMMDGNE
Se288    151 FDRWYDAIKKEYEIDDTQIAVVMSGLMVWCIENGCSPNINGNWTMMDGNE
SCMV-MDB 151 FDRWYDAYKKEYEIDDTQMTVVMSGLMVWCIENGCSPNINGNWTMMDKDE
SCMV-SC  151 FDRWYEAIKKEYELDDTQMTVVMSGLMVWCIENGCSPNISGSWTMMDGDE
MD-Sp    151 FDRWYEAVQKEYELDDTQMTVVMSGLMVWCIENGCSPNINGVWTMMDGDE
C             F  W  V   Y     M    NG MVWCIENGTSP    G W MMDG

Bor      201 QRVFPLKPVIENASPTFRQVMHHFSDAAEAYIEYRNSTERYMPRYGLQRN
Boe      201 QRVFPLKPVIENASPTFRQVMHHFSDAAEAYIEYRNSTERYMPRYGLQRN
Hoe      201 QRVFPLKPVIENASPTFRQVMHHFSDAAEAYIEYRNSTERYMPRYGLQRN
Se26     201 QRVFPLKPVIEKASPTFRQIMHHFSDAAEAYIEYRNSTERYMPRYGLQRN
Se288    201 QRVFPLKPVIDNASPTFRQIMHHFT---EAYIEYRNSTERYMPRYGLQRN
SCMV-MDB 201 QRVFPLKPVIENASPTFRQIMHHFSDAAEAYIEYRNSTERYMPRYGLQRN
SCMV-SC  201 QTVFPLKPVIENASPTFRQIMHHFSDAAEAYIEYRNSTERYMPRYGLQRN
MD-Sp    201 QRTFPLKPVIENASPTFRQIMHHFSDAAEAYIEYRNSTEKYMPRYGLQRN
C             Q  YP  P    A P  RQIM HFS  AEAYIE RN    YMPRYGL RN

Bor      251 LTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
Boe      251 LTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
Hoe      251 LTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
Se26     251 LTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
Se288    251 LTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
SCMV-MDB 251 ISDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
SCMV-SC  251 LTDYSLARYAFDFYEMNSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNV
MD-Sp    251 LTDFSLARYAFDFYEISSRTPVRAKEAHMQMKAAAVRGSNTRLFGLDGNV
C             L D  LARYAFDFYE     TP RAREAH QMKAAA        FGLDG
```

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                          ➔
Bor       301 GETQENTERHTAGDVSRNMHSLLGVQQHH
Boe       301 GETQENTERHTAGDVSRNMHSLLGVQQHH
Hoe       301 GETQENTERHTAGDVSRNMHSLLGVQQHH
Se26      301 GETQENTERHTAGDVSRNMHSLLGVQQHH
Se288     301 GETQENTERHTAGDVSRNMHSLLGVQQHH
SCMV-MDB  301 GETQENTERHTAGDVSRNMHSLLGVQQHH
SCMV-SC   301 GETQENTERHTAGDVSRNMHSLLGVQQHH
MD-Sp     301 GEAHENTERHTAGDVSRNMHSLLGVQQGH
C                  E TERHT  DV   MH L G
```

Fig. 2. Comparison of the aa sequences of the CPs of different maize potyviruses (SCMV-Bor,-Boe,-Hoe,-Se26,-Se288,-MDB [11], -SC[11] and MD-Sp, a Spanish MDMV isolate [1]. ⇨ Beginning of the core region; → beginning of the c-terminal region; *C* highly conserved aa of potyviral CPs (at least 26 out of 32 compared potyviral CPs show the corresponding aa, e.g. 80%), aa is underlined, if any aa of a SCMV-isolate is divergent;* start and end points of sequences omitted for better alignment of CP aa; boxed areas – aa repeats identified by Frenkel et al. [11]

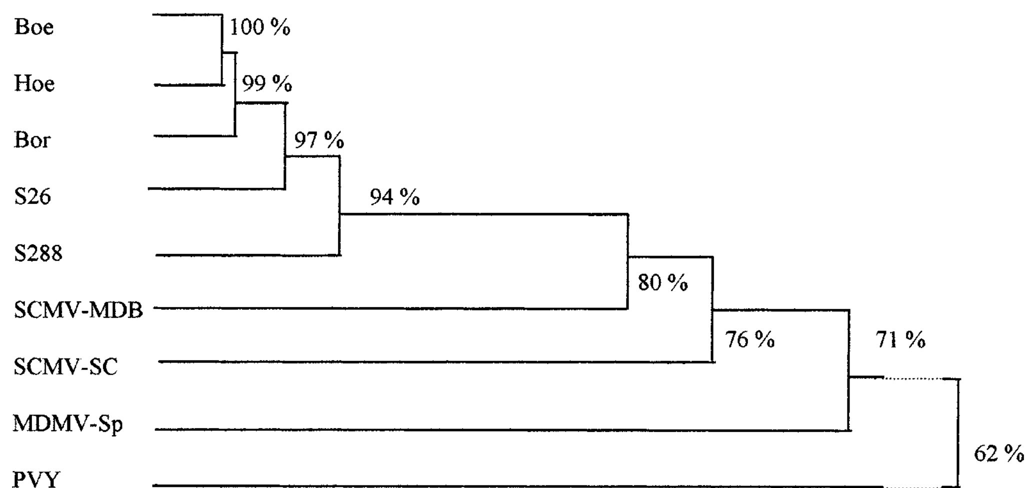

Fig. 3. Comparison of the aa sequences of CPs of different maize potyviruses and PVY as an outgroup virus (see legend to Fig. 2)

CPs (Fig. 3) the German isolates grouped separately from both other SCMV isolates. The same holds true for MDMV-Sp which is distinct from all SCMV isolates. The degree of homology between the CP aa sequences of the SCMV isolates appeared to lay between the range for distinct viruses (38 to 71% identity) and that for related strains (90 to 99% identity) [39]. A divergent picture was obtained when looking only at the 3′NCR sequences (Fig. 4). The degree of homology of the na in this part of the genome (80% identity) ranged in a region characteristic for strains of a virus [10]. The identity for MDMV-Sp was only 40%, clearly indicating that it is a different virus. When comparing only the core regions of the CP, a similar relationship could be obtained. The core regions of all German isolates and SCMV-SC as well as SCMV-MDB were very similar

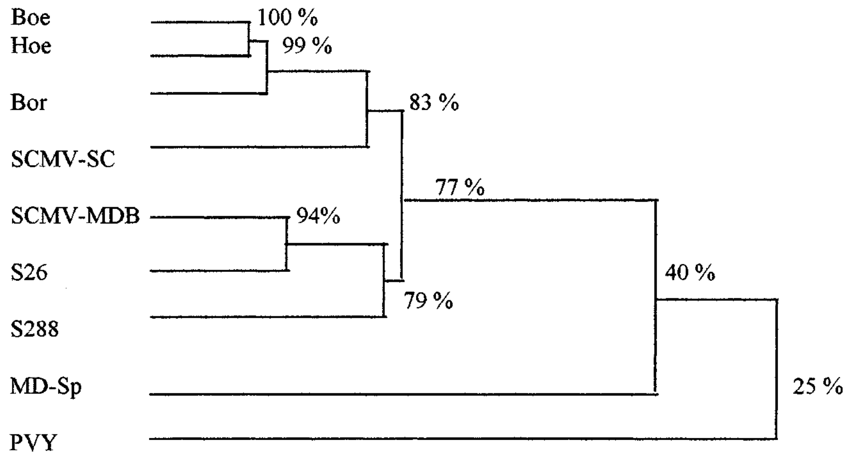

Fig. 4. Comparison of the 3′NCR na sequences of different strains/viruses of maize potyviruses and PVY as an outgroup virus (legend: see Fig. 2)

(Fig. 2, degree of homology more than 93%). The core region of MDMV-Sp differed from the SCMV isolates (86% homology). So, if only the core region of the CP, which is the best marker for sequence relationships between strains and distinct viruses in the genus *Potyvirus* [42] is used for comparing SCMV strains, the sequence identity very well matches with those observed between strains of the same potyvirus. In part this can be explained by striking differences in the aa sequences of the N-termini of the CPs. One of such differences consists in different sizes of this region, SCMV-MDB has 91 aa while SCMV-SC and the German isolates have only 76 aa. Frenkel et al. [11], who first described the unexpected diversity in the aa sequences of the N-terminal part of SCMV-SC and SCMV-MDB, identified two aa repeats which in part explain the different size and the resulting diversity (Fig. 2). Omitting these regions of diversity in their case the identity reached 92% what solved the problem of classification. Thus, Frenkel et al. [11] suggested that both isolates are strains of the same virus. Omitting the corresponding sequences of the German isolates (Q21 to Q70, i.e. 65% of the N-terminal sequences, Fig. 2) such a high degree of homology of the CPs of all compared SCMV isolates, 93%, can be obtained, too. However, in contrast to Frenkel et al. [11] and Xiao et al. [49], who found repeated sequence motifs in the N-terminal part of the CP, no such pattern could be detected in the German isolates.

Further evidence for supporting the hypothesis of identical viruses, which only differ in their extremely diverse N-termini of the CPs Frenkel et al. [11] obtained by examination of conserved aa of the CP core sequences. They detected for both SCMV isolates identical changes of aa previously found to be invariant. The same holds true, in most cases, for the German isolates of SCMV too, but even for MDMV-Sp (Fig. 2). Hence, this criterion cannot be used for verification of classification.

An interesting feature of some of the SCMV isolates (German isolates, SCMV-MDB, SCMV-BC) was the very high content of glycine and

Table 1. Comparison of the glycine and glycine/serine content in the N-terminus of the coat protein (CP) of SCMV isolates from Germany (-Bor, -Boe, -Hoe, -Se26 and Se288), the US (SCMV-MDB [11] and from Australia (-SC [11], -BC, -Brisbane, -Isis, -Sabi, -Bundaberg [49])

SCMV virus isolate	Content (%) in the N-terminal part of the coat protein	
	glycine	glycine/serine dipeptides
-SC	18	6
-MDB	34	22
-Bor	32	21
-Boe	33	21
-Hoe	33	21
-Se26	28	21
-Se288	28	21
-BC[a]	37	24
-Brisbane[a]	33	3
-Isis[a]	27	6
-Sabi[a]	28	0
-Bundaberg[a]	33	4

[a] Only available from Q11 of CP aa sequence [49]

glycine/serine dipeptides in the N-terminus of the CP (Table 1). All five investigated German isolates showed such high contents of these aa, 32% and 21%, respectively. In contrast, SCMV-SC revealed only 18% and 6%, respectively. No other potyvirus has such a high content of the mentioned aa in the N-terminus of the CP (results not shown). The Australian SCMV strains Brisbane, Isis, Sabi and Bundaberg exhibited only a high glycine content while glycine/serine dipeptides were found rarely (Table 1).

The nt sequences of the 3′NCR of SCMV-MDB and Se26 showed another interesting feature. Though rather different in their N-terminal aa sequences of the CP (Fig. 2), they have an extremely high degree of homology in this usually very variable region (Fig. 5). This can be explained in two ways. One possibility is that this sequence is the originally one of the ancestor virus of SCMV. Consequently, the 3′NCR of the other isolates were changed during evolution. The other possibility is that a recombination between both strains took place. For other potyviruses, e.g. PVY and bean common mosaic virus, recombinations were demonstrated by statistical methods especially in the N-terminal region of the CP and in the 3′NCR [34]. Revers et al. [34] could not detect interspecific recombinations between potyviruses. So, recombination between Se26 and SCMV-MDB could be a further evidence that both isolates belong to the same virus. This also let assume that both isolates occurred during a definite time in the same geographical region.

```
MDB   TAGTCTCCTG GAAACCCTGT TTGCAGTACC AATAATATGT ACTAATATAT
Se26  TAGTCTCCTG GAAACCCTGT TTGCAGTACC TATAATATGT ACTAATATAT

MDB   AGTATTTTAG TGAGGTTTTA CCTCGTCTTT ACTGTTTTAT TACGTATGTA
Se26  AGTATCTCAG TGAGGTTTTA CCTCGTCCTT ACTATTCTAT TACGTATGTA

MDB   TTTAAAGCGT GAACCAGTCT GCAACATACA GGGTTGGACC CAGTGTGTTC
Se26  TTTAAAGCGT GAACCAGTCT GCAGCATACA GGGTTGGACC CAGTGTGTTC

MDB   TGGTGTAGCG TGTACTAGCG TCGAGCCATG AGATGGACTG CACTGGGTGT
Se26  TGGTGTAGCG TGTACTAGCG TCGAGCCATG AGACGGACTG CACTGGGTGT

MDB   GGTTTTGCCA CTTGTGTTGC GAGTCTC-TG GTAAGAG--A AA
Se26  GGCTTTGCCA CTTGTGTTGC GAGTCTCCTG GTAAGAGACA AA
```

Fig. 5. Alignment of na sequence of the 3′NCR of SCMV-MDB (MDB) and Se26. Double underlines denote differences in the sequences

Concerning the biological and serological properties of SCMV-MDB and the German isolates, all isolates are almost identical [16]. They have, as far as known, the same host range and they are transmitted by the same vectors. They form the same inclusion bodies [29]. The only known resistance gene in maize, *mdm-I* [33], acts against both SCMV and MDMV [30], and also German isolates cannot overcome *mdm-I* (L. Kuntze, pers. comm.).

The comparison of different features of the sequences of SCMV-SC/-MDB and the German SCMV isolates as well as their biological characteristics lead to the conclusion that the German isolates can be referred to as SCMV strains which are, however, distinct from those described previously. The results of the sequence comparisons let assume that the evolution of this virus is still continuing, leading to the observed differences in the N-terminal part of the CP and, less pronounced, in the 3′NCR. About the biological significance of the extreme differences in the N-terminus of SCMV isolates, about the significance of RNA recombination in the 3′NCR and about the duplications in the N-terminal parts of the CPs one can only speculate. It will be of interest to see whether these changes affect biological properties of the investigated isolates. One effect could be a different natural host range. For Central Europe no natural perennial hosts of SCMV, important for "overwintering" of this virus, are known so far. Possibly, the differences in the sequences reflect the adaptation of the virus to special vector/host plant combinations which are different in different geographical regions. The findings of Xiao et al. [49] concerning the correlation of the sequence diversity in the amino-terminal region of the CPs of Australian and American SCMV strains with their host range support this idea. Further experiments shall be conducted to answer this question. They must include the comparison of other parts of the viral genome, too.

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