Arch Virol (1999) 144: 479–490

Archives of Virology
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Printed in Austria

Molecular characterization and interviral relationships of a flexuous filamentous virus causing mosaic disease of sugarcane (*Saccharum officinarum* L.) in India*

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Accepted October 12, 1998

Summary. A virus isolate causing mosaic disease of commercial sugarcane was purified to homogeneity. Electron microscopy revealed flexuous filamentous virus particles of ca 890 × 15 nm. The virus isolate reacted positively with heterologous antiserum to narcissus latent virus form UK, but failed to react with potyvirus group specific antiserum. N-terminal sequencing of the intact coat protein (CP) and the tryptic peptides indicated that the virus was probably a potyvirus but distinct from several reported potyviruses. Comparison of the 3′-terminal 1084 nucleotide sequence of the RNA genome of this virus revealed 93.6% sequence identity in the coat protein coding region with the recently described sugarcane streak mosaic virus (Pakistani isolate). The molecular weight of the coat protein (40 kDa) was higher than that deduced from the amino acid sequence (34 kDa). The apparent increase in size was shown to be due to glycosylation of the coat protein which has not been reported thus far in the family, *Potyviridae*. This is the first report on the molecular characterization of a virus causing mosaic disease of sugarcane in India and the results demonstrate that the virus is a strain of sugarcane streak mosaic virus, a member of the *Tritimovirus* genus of the *Potyviridae*. We have named it sugarcane streak mosaic virus – Andhra Pradesh isolate (SCSMV-AP).

*The nucleotide sequence reported in this paper has been submitted to GenBank and assigned the accession number Y17738.

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Introduction

Sugarcane (*Saccharum officinarum* L.) is an important industrial cash crop accounting for about 60% sugar production in the world, and India ranks as one of the top producers of cane sugar [4]. The viruses reported to infect sugarcane naturally are *Badnavirus* (*Caulimoviridae*) sugarcane bacilliform badna virus (SCBV), *Fijivirus* (*Reoviridae*) Fiji disease virus (FDV), *Monogeminivirus* (*Geminiviridae*) sugarcane streak virus (SSV), *Potyvirus* (*Potyviridae*) sugarcane mosaic virus (SCMV), sorghum mosaic virus (SrMV) [3, 12, 17].

The main characteristics considered to define a potyvirus are particle morphology, cytopathology, mode of transmission and molecular characteristics such as genome structure and organization, coat protein sequence and serology [27]. When the complete sequence of the potyvirus genome is difficult to generate, partial nucleotide sequences covering 3′-untranslated region (UTR) and coat protein coding region are useful in distinguishing strains of a virus from distinct potyviruses [5]. Immunological studies using antibodies to N-terminal and core region of coat proteins and high performance liquid chromatography (HPLC) peptide profiling of coat proteins have lead to delineation of 17 SCMV/MDMV (maize dwarf mosaic virus) strains from Australia and the United States into four distinct potyviruses namely SCMV, SrMV, MDMV and johnsongrass mosaic virus (JGMV). It has been suggested that all these viruses should be grouped as a sugarcane mosic virus subgroup in the genus *Potyvirus* [24]. Recently, reverse transcription-polymerase chain reaction (RT-PCR) based restriction fragment length polymorphism (RFLP) analysis was developed for rapid discrimination between strains of SCMV and SrMV occurring in the United States [28]. The status of the different strains of these viruses reported from other countries is uncertain and needs to be thoroughly investigated.

Several isolates of sugarcane mosaic virus subgroup causing mosaic disease of sugarcane, maize, sorghum, pearl millet and finger millet have been reported from different parts of India [8, 11, 15, 19]. The virus isolates have been identified based on differences in physical properties, host range, particle morphology and limited serological data. None of these isolates have been characterized at molecular level. Recently, immunological characterization of a virus causing mosaic disease of sugarcane in Andhra Pradesh, India revealed that it is probably distinct from SCMV [8].

Virus isolate(s) causing mosaic disease of sugarcane in India continue to be a potential threat to the sugarcane industry as it is a very common disease in almost all sugarcane growing regions because of the perpetuation of the disease causing virus through vegetative propagules (setts) [1].

In the present study, we report the molecular characterization of a virus causing mosaic disease of sugarcane in Andhra Pradesh, India. The 3′-terminal 1084 nucleotide sequence of the viral RNA genome showed 85.7% identity with a recently reported *Tritimovirus* (*Potyviridae*) sugarcane streak mosaic virus originating from Pakistan [7]. Molecular data on this viral genome is essential to develop primers necessary for RT-PCR and RFLP based discrimination of virus

isolates and to develop molecular diagnostic methods for detection of the virus, especially in the planting sugarcane material.

Materials and methods

Virus propagation

The virus isolate used in this study was collected from commercial sugarcane fields in Chittoor district of Andhra Pradesh (A.P.), India [13]. This was subsequently propagated on *Sorghum bicolor* cv. Rio plants grown in a glass house by periodical sap inoculation [8].

Virus purification

Infected sorghum leaves harvested 15 days post-inoculation were homogenized in three volumes of 0.02 M N-(2-Hydroxyethyl) piperizine-N′-(2-ethanesulfonic acid) (HEPES) buffer, pH 8.0 containing 0.01 M ethylenediamine tetraacetic acid (EDTA), 0.1 M urea and 0.2% 2-mercaptoethanol (v/v) and the extract was filtered through muslin cloth. The filtrate was clarified by the addition of chloroform and carbon tetrachloride (1:1 v/v, 30%) and stirred for 30 min. This was followed by low speed centrifugation at 7,800 **g** for 10 min. Triton X-100 was added to the supernatant to a final concentration of 2% and stirred for 1 h. The virus in the aqueous phase was pelleted by centrifugation at 140,000 **g** for 3 h using Sorvall AH-629 rotor. The virus pellet was resuspended in 0.02 M HEPES buffer, pH 7.2 (resuspension buffer) overnight at 4 °C. It was centrifuged at 4,400 **g** for 5 min, supernatant layered on preformed 20–50% sucrose density gradients prepared in resuspension buffer, and centrifuged at 120,000 **g** for 2 h using Sorvall AH-629 rotor. The light scattering zone was collected, diluted three times with resuspension buffer and pelleted at 145,000 **g** for 2 h using Sorvall T 1250 rotor. The final purified virus pellet was resuspended in minimal volume of resuspension buffer and a fraction of it was scanned in UV-visible spectrophotometer from 200–300 nm [10]. Infectivity of purified virus was checked by inoculating it on healthy *S. bicolor* cv. Rio plants.

Electron microscopy

Carbon shadowed formvar coated grids applied with purified virus particles were negatively stained with 1% phosphotungstic acid (w/v) in water and observed under Philips 301 electron microscope (50,000 ×).

Serology

Serological relationships of this virus isolate using antisera against narcissus latent virus and potyvirus group specific antiserum [16] were investigated by employing direct antigen coating enzyme-linked immunosorbant assay (DAC-ELISA) [9]. In DAC-ELISA, leaf antigens were extracted in coating buffer (1:10 w/v; 200 µl/well) and purified virus (200 ng/well) were used. The primary antibodies were used at a dilution of 1:500 and goat anti-rabbit IgG conjugated to alkaline phosphatase at 1:1000 dilution was used as the secondary antibody. The reaction was developed using p-nitrophenyl phosphate (PNP) and the absorbance was measured at 405 nm in a Biotek Ceres 900 ELISA Reader.

Glycoprotein staining

Glycoprotein staining of virus coat protein in polyacrylamide gel was performed as described earlier [29]. Physalis mottle virus coat protein (20 kDa) and trehalase from *Thermomyces lanuginosis* RM-B strain (145 kDa) were used as negative and positive controls, respectively.

Partial sequence analysis of coat protein

Coat protein was isolated from purified virus suspended in 0.01 M HEPES buffer, pH 7.2 (10 mg/ml) [26]. The purity and homogeneity of coat protein was checked by 12% SDS-PAGE [14].

Purified virus was digested with trypsin (50 μg/mg purified virus) at 37 °C for 30 min. The tryptic peptides were separated by 12% tricine gel electrophoresis [22]. The gel was rinsed in tris-glycine buffer containing 20% methanol for 15 min and electroblotted onto Poly(vinylidene difluoride) (PVDF) membrane (Millipore) using Novablot apparatus (Pharmacia). After transfer, the PVDF membrane was stained for 5 min with Ponceau S, destained with water and air dried. The relevant bands were cut and loaded onto an automated gas phase protein sequenator PSQ-1 (Shimadzu).

Isolation of viral RNA

Infectious RNA was isolated by disruption method from freshly purified virus preparations as described [30]. The RNA pellet was resuspended in diethyl pyrocarbonate (DEPC) treated sterile water. The RNA was separated on 1% agarose gel using Tris-borate-EDTA buffer, pH 8.3 [20]. The infectivity of the RNA was checked by inoculating it to 10–15 days old healthy sorghum cv. Rio plants under sterile conditions.

cDNA synthesis and cloning

Isolated RNA was treated with RNase-free DNase (Boehringer Mannheim; 1 unit/reaction) at room temperature for 15 min, re-extracted with phenol-chloroform mixture and precipitated with isopropanol. The precipitated RNA was resuspended in 10 μl of DEPC treated sterile water and used immediately for cDNA synthesis using SuperScriptII reverse transcriptase and oligo dT primers (GIBCO-BRL) as per the manufacturer's instructions. The cDNA was fractionated on a Sephacryl S-1000 column (Sigma) and ligated to *Hinc* II cut pUC 19 vector at 16 °C for 12 h. Cloned cDNA was transformed into competent *E. coli* DH5α cells and recombinant clones were screened by blue/white colony selection [20]. Further screening of cDNA clones specific to viral RNA was carried out by colony hybridization [6] using labeled first strand cDNA as probe.

DNA sequencing and analysis

The cDNA clones were sequenced according to Sanger's method [21] using Sequenase version 2.0 (Amersham) and M13 forward and reverse primers manually as well as by automated ABI Prism DNA sequencer. The sequences were analyzed using the Wisconsin package (version 9.1) programs.

Results

Virus purification and particle morphology

The major problem encountered during purification was the aggregation of the virus particles and loss of the virus in low speed centrifugation. The procedure described in the methods helped to minimize these problems and lead to virus preparation relatively free of host contaminants. The purified virus particles had an $A_{260/280}$ ratio of 1.13 and the yields were approximately 10 mg/kg leaf tissue. Electron microscopy of the purified virus preparations (Fig. 1) showed that the particles were flexuous rods with an average dimension of 890 × 15 nm, similar to the members of the family *Potyviridae*.

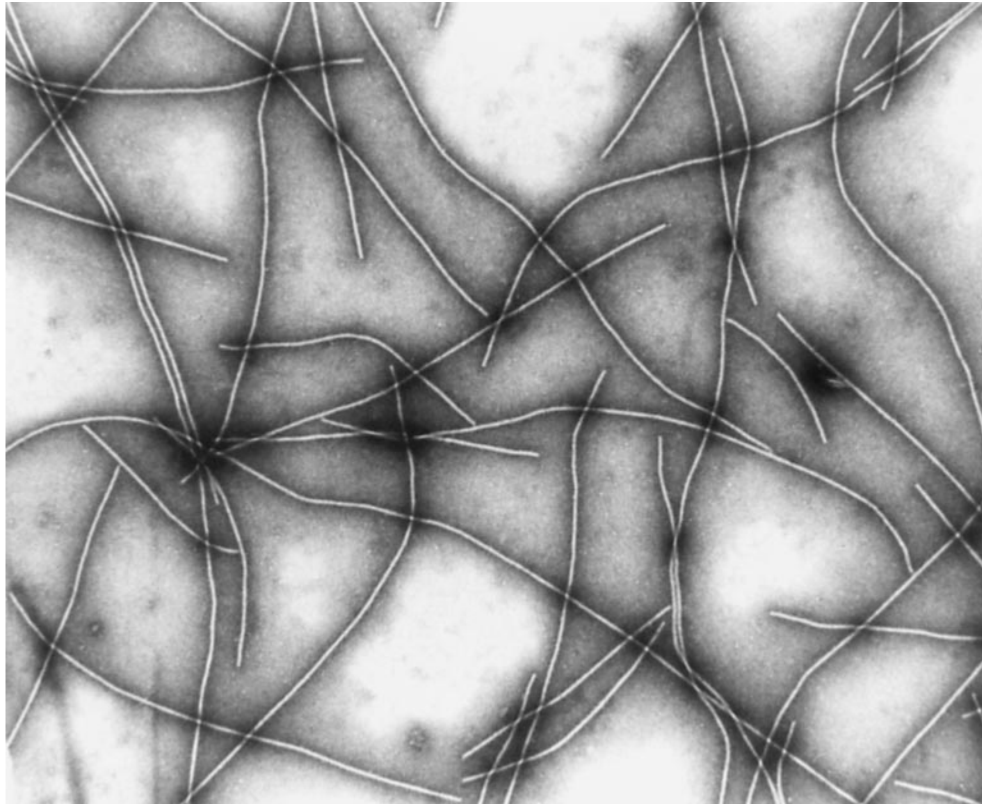

Fig. 1. Electron micrograph of purified virus particles causing mosaic disease on sugarcane were stained with 1% phosphotungstic acid. Magnification ×50,000

Serology

In an earlier study, we have shown that the virus does not cross react with many of the well characterized potyviruses including several SCMV strains [8]. When DAC-ELISA was carried out as described in the methods section, the virus did not cross react with potyvirus group specific antiserum but showed measurable reaction with narcissus latent virus, which has been suggested to be the member of *Macluravirus*, a new genus of the family *Potyviridae* [2] (Table 1). Further, these results also correlated well with EBIA analysis (data not shown).

Analysis of viral coat protein and the tryptic peptides

The purified virus resolved into two bands on 12% SDS-PAGE corresponding to molecular weights 40 kDa and 28.5 kDa (Fig. 2a). The lower band could be a degradation product of the coat protein as observed in many potyviruses. Western analysis with homologous antiserum confirmed that the lower band was indeed a degradation product (data not shown). However, in the isolated coat protein, there were other bands apart from the two major bands (40 kDa and 28.5 kDa) which could be the result of incomplete dissociation and degradation. The purified virus was subjected to mild trypsin treatment and transferred onto PVDF mambrane

Table 1. Reaction of sugarcane virus with different antisera in DAC-ELISA

Antisera used	Purified virus	Infected sorghum leaf tissue	Healthy sorghum leaf tisue
Homologous	3.69	1.38	0.22
Narcissus latent	1.21	0.73	0.16
Potyvirus group specific	0.02	0.08	0.04

The numbers represent A_{405} nm reading of two experiments (in triplicate)

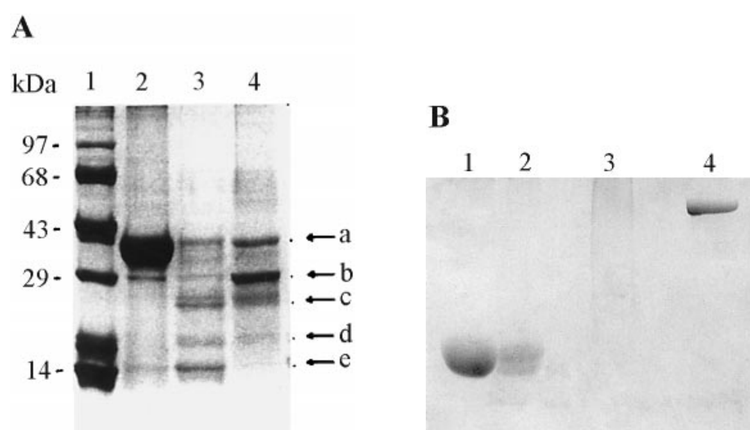

Fig. 2. SDS-PAGE analysis of sugarcane virus coat protein. **A** Tryptic peptide profile of the coat protein. Purified virus (100 μg) was treated with trypsin (50 μg/mg) as described in Materials and methods. The arrows (*a–e*) indicate the fragments used for sequencing after transferring a parallel gel onto PVDF membrane. (*a* intact coat protein, *b* degraded product of coat protein, *c* TP1, *d* TP2, *e* TP3). *1* Standard molecular weight markers; *2* intact purified virus; *3* purified virus treated with trypsin; *4* isolated coat protein. **B** Glycoprotein staining of the coat protein. The samples were separated on 12% SDS-PAGE were stained for the presence of carbohydrate using PAS (periodic acid sulfate) staining. Sugarcane viral intact (*1*) and degraded (*2*) coat protein, PhMV coat protein (*3*) trehalase (*4*)

after SDS-PAGE (Fig. 2a) as described in the methods section. The N-terminal sequence analysis of the isolated coat protein, its degradation product and tryptic peptides obtained is shown in Table 2. The analysis of the partial sequence data indicated that the sequence of TP 3 aligned well with the corresponding region in the trypsin resistant core of potyviruses (Table 3). The peptide TP 2 was impure and did not result in unambiguous sequence. When the sequences were compared with four strains of SCMV, it was found that the virus did not belong to this sub-group [24].

Table 2. Partial N-terminal amino acid sequence of the intact coat protein and the tryptic peptides of the virus isolated from sugarcane

	Amino acid sequence
Coat protein	GQGTQPPQ
Degradation product	NTVSQTMRSLYVPPLVK
TP 1	AVLNLDGAD
TP 3	NAKPGIRAIMRHFGELAYK

Table 3. Alignment of tryptic peptide (TP 3) of the sugarcane virus with other potyviruses

SCSMV-AP	NAKPGIRAIMRHFGELAYK
JGMV-JG	NAKPTLRQCMMHFSDAAEA
SCMV-B	NASPTFRQIMHHFSDAAEA
SrMV-SCI	YASPTFRQIMHHFSDAAEA
MDMV-A	NASPTFRQIMHHFSDAAEA
PVY	NAKPTLRQIMAHFSDVAEA
BaYMV	RMNGGLRRIMRNYSDETVL
RGMV	HAQPTLRSIMAHFSDAATA
SPMMV	NAQPTLRQVMRHFGEQAVA
NLV	VENGGLRKIMRHFSGITHE

The alignment was done by using already published sequences. JGMV-JG, SCMV-B, SrMV-SCI and MDMV-A [28]; potato virus Y (PVY), barley yellow mosaic virus (BaYMV), ryegrass mosaic virus (RGMV), sweet potato mild mottle virus (SPMMV) and narcissus latent virus (NLV) [2]

Characterization of viral genome

The isolated RNA was found to be infectious upon mechanical inoculation on healthy sorghum plants and was used for cDNA synthesis. The recombinant plasmids harboring cDNA inserts (0.3–1.5 kb) were sequenced as described in the methods section. The authenticity of these clones were confirmed by manual sequencing which showed the presence of poly dT tracts.

The nucleotide sequence of pSV 7 was determined and it corresponded to 3′ terminal 495 nucleotides of the viral genome. The sequence of another clone pSV 5 overlapped with the 5′ sequence of pSV 7. Thus, 3′ terminal 1084 nucleotides of the viral genome could be compiled (Fig. 3). This sequence consisted of 193 nucleotides of 3′ untranslated region and a partial open reading frame. The partial ORF encompassed the complete coat protein sequence. The N-terminal amino

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                                                    ↓
     S  S  R  V  E  T  L  V  F  H  A  T  V  D  G  Q  G  T  Q  P  P  Q  N  Q  S  S  S  P  A  T  T  S  S  I
     UCCUCUAGAGUCGAAACUCUUGUGUUUCAUGCAACGGUCGAUGGACAAGGAACGCAGCCACCUCAGAAUCAGAGUAGUUCACCAGCGACUACAUCAAGCA
   1 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  100

      S  S  T  T  T  S  Q  V  D  S  Q  T  T  G  N  L  S  N  T  V  S  Q  T  M  R  S  L  Y  V  P  P  L  V
     UAUCAUCAACAACAACGAGUCAAGUUGAUAGUCAGACAACAGGGAAUUUAUCUAAUACAGUCUCUCAGACCAUGAGAUCCCUGUACGUUCCGCCAUUGGU
 101 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  200

      K  S  L  K  T  E  A  K  A  K  Q  M  M  R  Y  T  P  P  Q  A  L  I  S  S  S  A  A  S  I  R  Q  F  N
     GAAAUCGCUCAAAACAGAAGCCAAGGCGAAGCAGAUGAUGAGGUACACACCACCACAAGCUCUUAUCUCUUCAUCAGCCGCCUCAAUACGACAGUUCAAU
 201 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  300

     D  W  A  N  T  A  A  E  G  Y  G  K  T  I  Q  Q  F  T  D  E  L  L  P  F  W  I  Y  W  C  V  V  N  G  A
     GAUUGGGCGAAUACAGCAGCAGAAGGAUAUGGGAAGACUAUUCAACAAUUUACAGAUGAACUACUUCCAUUCUGGAUUUACUGGUGUGUUGUUAACGGAG
 301 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  400

      T  E  E  N  K  T  K  P  K  W  T  K  A  V  L  N  L  D  G  A  D  G  T  E  I  T  V  D  E  N  G  P  Q
     CGACAGAAGAGAACAAAACCAAGCCCAAGUGGACAAAAGCUGUGUUAAAUCUGGAUGGAGCUGAUGGGACAGAGAUAACUGUUGACGAAAAUGGGCCUCA
 401 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  500

      V  E  F  E  M  G  P  M  Y  R  N  A  K  P  G  I  R  A  I  M  R  H  F  G  E  L  A  Y  K  W  V  Q  F
     AGUGGAGUUCGAAAUGGGGCCAAUGUAUAGAAACGCCAAACCUGGUAUUCGCGCAAUCAUGAGACAUUUUGGCGAAUUGGCGUACAAGUGGGUUCAGUUC
 501 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  600

     S  V  R  S  G  K  P  I  I  P  H  N  A  V  E  A  G  L  T  T  P  E  F  Y  P  C  C  I  D  F  V  M  V  N
     UCGGUUCGUAGCGGGAAACCCAUAAUACCACACAAUGCAGUAGAGGCAGGAUUGACCACACCAGAGUUUUAUCCUUGCUGCAUUGAUUUUGUGAUGGUGA
 601 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  700

      I  L  S  P  A  E  I  D  V  R  N  Q  V  I  N  A  R  T  P  R  M  G  R  P  L  F  R  H  A  L  R  A  G
     ACAUCCUCUCACCAGCAGAAAUUGACGUGCGUAACCAGGUGAUCAACGCACGCACACCCCGGAUGGGAAGGCCUUUAUUCCGUCACGCGCUUAGAGCCGG
 701 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  800

      G  D  E  D  T  D  L  R  R  E  D  D  A  H  Y  G  R  T  Q  I  G  G  A  Q  F  G  R  A  Q  H  *
     GGGAGAUGAGGACACGGACCUGCGUAGAGAAGAUGAUGCUCAUUAUGGAAGAACGCAGAUCGGAGGCGCCCAGUUUGGGCGUGCUCAGCACUGAUUACCA
 801 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+  900

     GUGCAGUUAUCCAUAUAUUAUUGUAUCUACGUAUAAAAUGGUUCUCUUGGAUAGGCUGAUGGUAGUUGCCUGACCGCUUAGUCCAUCAUCAGGAGCUACU
 901 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 1000

     GGUGGAUCAUUCGGAGAUUGCUCACCCUGUUUUCAGACCAGUGAGAUUCUGCCGAACCCACAAUCAACCUGCCCCGUGAGGAGGA(n)
1001 ---------+---------+---------+---------+---------+---------+---------+---------+----  1084
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Fig. 3. Nucleotide and deduced amino acid sequence of the 3′ terminal region of the sugarcane viral RNA. The N-terminal amino acid sequence of the coat protein and tryptic peptides as determined by amino acid sequencing is underlined. The arrow indicates the putative cleavage site between NIb and coat protein for NIa protease

acid sequence determined from the coat protein and two of the tryptic peptides (TP 1 and TP 3) were identical to the deduced sequence at corresponding segments (underlined in Fig. 3). The N-terminal amino acid sequence of the coat protein is suggestive of a noval D-G cleavage site between the NIb and coat protein for the NIa protease. The size of the coat protein as deduced from the sequence data is 34 kDa in contrast to the molecular weight as determined by SDS-PAGE (40 kDa). In order to account for this discrepancy the protein was stained for glycoproteins. Interestingly, the coat protein of the present isolate is a glycoprotein (Fig. 2b).

Analysis of the viral genome

The 3′ terminal 1084 nucleotides were compared with all the members of the *Potyviridae* and was found to be closely related to the recently described *Tritimovirus*

(*Potyviridae*) sugarcane streak mosaic virus from Pakistan [7]. The overall identity with SCSMV at nucleotide level was 85.7%. The 3′ UTR was better conserved than coat protein nucleotide sequence (94.3% and 85.5% identity, respectively). However, the deduced amino acid sequence of the coat protein of the present isolate showed 93.6% identity with SCSMV. These results suggest that the virus isolated from Andhra Pradesh is a strain of SCSMV. Complete genomic sequence of two other tritimoviruses [18] are known namely WSMV (AFO57533) and BrSMV (Z48506). Interestingly the deduced coat protein amino acid sequence of SCSMV-AP reported here and SCSMV from Pakistan [7] showed only 32.5% and 31.3% identity with WSMV and 27.6% and 30.2% identity with BrSMV respectively. However, the two isolates did not show significant identity with any other members of the *Potyviridae*. The coat protein of the present isolate lacks the DAG present in aphid transmissible potyvirus coat protein. It also lacks the MVWCIENG motif present in the core region of the coat protein of most of the potyviruses. A heptapeptide RAIMRHF (also shown in Table 3) is the largest conserved stretch among the SCSMV, WSMV and BrSMV. Although the virus cross-reacted weakly with narcissus latent virus in DAC-ELISA and EBIA tests, no significant homology was observed between the coat protein sequences of the two viruses.

Discussion

The family *Potyviridae* is the largest and economically the most important group of plant viruses [25]. New strains and distinct viruses are constantly described and added to this ever growing taxon. There are now 6 genera of the family, they are *Potyvirus, Bymovirus, Rymovirus, Ipomavirus, Macluravirus* and *Tritimovirus* [18].

The present virus isolate has flexuous filamentous particle morphology (Fig. 1), pinwheel inclusions (data not shown), monopartite RNA genome and single capsid protein with Mr 40 kDa which indicates its taxonomic affiliation to the family *Potyviridae*. The vectors involved in the spread of the virus are yet to be identified. Our initial attempts to transmit the virus by aphids (*Aphis craccivora*, *Rhopalsiphum maidis*) in non-persistent way were negative. The molecular weight of the coat protein was higher than the average molecular weight of well characterized potyviruses. The coat protein molecular weight of narcissus latent virus and maclura mosaic virus were also higher in the range of 39–40 kDa, although the deduced coat protein sequence of these viruses suggested a range of 32–34 kDa [2]. Interestingly, the molecular weight as suggested by coat protein sequence in the present study was also lower than the estimated Mr on SDS-PAGE (Fig. 2a). The apparent increase in molecular weight and the results from PAS staining shows that it is a glycoprotein (Fig. 2b). This is the first report of a glycosylated coat protein among potyviruses. The DAC-ELISA results presented in this paper clearly demonstrate that the virus causing mosaic disease on sugarcane in India is antigenically distinct from sugarcane mosaic virus subgroup [8]. The virus appeared to be distantly related to narcissus latent virus. Treatment of intact virus

with trypsin did not yield the trypsin resistant core as reported for potyviruses, but 4–5 distinct bands were obtained (Fig. 2a). N-terminal amino acid sequence of one of these peptides showed significant identity with the potyviral coat protein sequence (Table 3).

Amino acid sequence of coat proteins is being used to classify distinct potyviruses and its strains. It has been suggested that distinct potyviruses possess coat protein sequence identities less than 70% whereas strains of individual viruses possess identity greater than 90% [23]. The 3′-untranslated sequence of potyviral RNA has great value in the identification of potyviruses and can be used to distinguish viruses from strains [5]. The 3′-1084 nucleotide sequence reported here is closely related to the recently reported *Tritimovirus* (*Potyviridae*) sugarcane streak mosaic virus [7]. Hall et al. [7] after extensive sequence analysis of SCSMV have suggested that this virus could be grouped along with wheat streak mosaic virus and brome streak mosaic virus belonging to the genus *Rymovirus* of *Potyviridae*. However, SCSMV CP sequence has only 31.3% and 30.2% identity with that of WSMV and BrSMV, respectively. Recently in the AAB Descriptions of Plant Viruses No. 245 [18] the genus *Rymovirus* has been split into *Tritimovirus* and *Rymovirus*. WSMV and BrSMV have been grouped under *Tritimovirus* genus. Hence, SCSMV could be grouped under the same genus.

Based on the above observations we would like to name the isolate described here as SCSMV-AP. The uncharacterized virus isolates from various locations (Uttar Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu and Karnataka states) in India cross-reacted with the antiserum to this virus isolate in ELISA and EBIA (data not shown). It is possible that these isolates are further strains of SCSMV. The N-terminus of SCSMV Pakistani isolate is blocked [7]. However, the N-terminus is not blocked in SCSMV-AP and it reveals a novel D-G cleavage site between NIb/CP for NIa protease. Thus, the present study establishes that the mosaic disease in sugarcane in India is not caused by the strains of sugarcane mosaic virus subgroup but by the newly described *Tritimovirus*, sugarcane streak mosaic virus.

Acknowledgements

M. Hema and P. Sreenivasulu are greateful to USDA (PL 480 project No.IN-ARS-718) for financial assistance. This work was also supported by financial grant to H. S. Savithri from Department of Science and Technology and Department of Biotechnology, Government of India. We would like to thank Jeffrey Hall for making available the preprint of their paper. We thank Dr. K. S. Ravi, Center for Application of Molecular Biology to International Agriculture, Australia and Dr. D. J. Robinson, SCRI, Dundee for providing potyvirus group specific antiserum and antisera against narcissus latent virus. The assistance by Dr. Indi for the electron microscopic studies, Mr. G. S. Girish for glycoprotein staining, Mr. Srihari Reddy for amino acid sequencing, Ms. Savitha for automated DNA sequencing, and Mr. P. Elango for nucleotide sequence analysis, is gratefully acknowledged. We thank DBT, India for providing the facilities for amino acid and DNA sequencing and Bioinformatics at the Indian Institute of Science, Bangalore.

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Received August 7, 1998