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Detection and partial molecular characterization of sugarcane mosaic virus infecting sugarcane genotypes

Authors Info

S. K. Holkar[1]*, A. Kumar[1], M. R. Meena[2] and R. J. Lal[1]

[1]Division of Crop Protection, ICAR-Indian Institute of Sugarcane Research, Lucknow–226 002, India

[2]Division of Plant Breeding, ICAR-Sugarcane Breeding Institute, Regional Station, Karnal, Haryana-132 001, India

*Corresponding Author Email : somnathbhu@gmail.com

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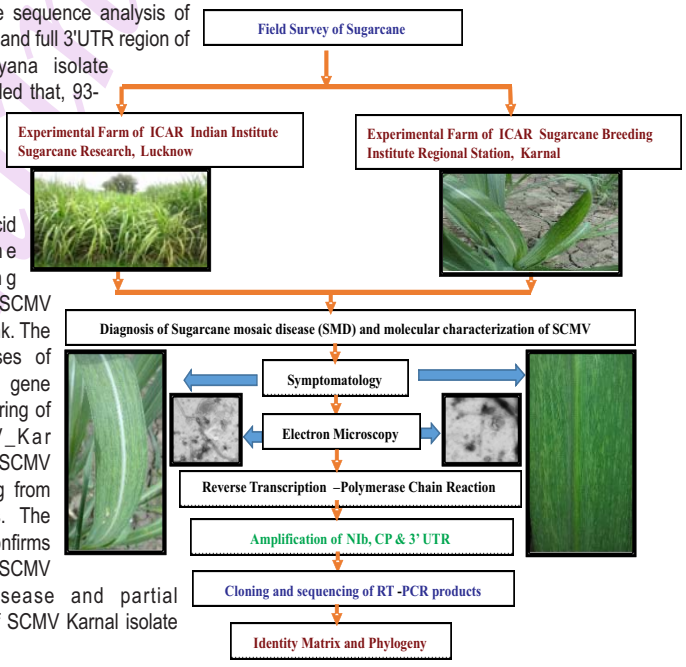
Abstract

Aim: *Sugarcane mosaic virus* (SCMV) is one of the causal agents of mosaic disease of sugarcane in India. Present investigation was carried out to detect association of specific virus with SMD and its molecular characterization.

Methodology: Thirty eight sugarcane genotypes showing mild, severe and necrotic mosaic symptoms were confirmed with the association of flexuous filamentous virion particles by electron microscopy. Of 38 symptomatic sugarcane samples only four samples were subjected to RT-PCR using *Potyvirus* universal primers which covers amplification of genomic regions *viz*., partial NIb, full CP and 3'UTR of approximately 1800 bp products. PCR amplicons were cloned in pGEMT Easy vector system. Seven recombinant clones were confirmed by colony PCR, restriction digestion with EcoRI and sequencing.

Results: Of these seven recombinant clones, restricted with EcoRI restriction enzyme, in two clones two fragments of approximately 1600 and 200 bp sizes were obtained, whereas rest of the five recombinant clones showed three restricted fragments of approximately 950, 550 and 300 bp which confirmed the variability in the genome of SCMV isolates infecting sugarcane genotypes.

Interpretation: The sequence analysis of partial NIb, full CP and full 3'UTR region of the Karnal, Haryana isolate (KF531632) revealed that, 93-96% sequence identity was found at nucleotide level and 97-98% identity at amino acid level with the corresponding regions of other SCMV isolates in GenBank. The phylogenic analyses of coat protein (CP) gene showed the clustering of present SCMV_Kar isolate with other SCMV isolates originating from different locations. The present study confirms the association of SCMV with mosaic disease and partial characterization of SCMV Karnal isolate in India.



Introduction

Sugarcane (*Saccharum* spp.) is one of the most important cash crops in India. The productivity of sugarcane is being hampered by several fungal, bacterial, viral and phytoplasmal diseases across the globe. Among the various viral diseases, sugarcane mosaic disease (SMD) caused by *Sugarcane mosaic virus* (SCMV) is one of the most important diseases in sugarcane which leads to yield loss up to 10-90% to the sugar industry (Rao *et al.,* 1998a, b; Viswanathan and Balmuralikrishnan, 2005). SCMV belongs to genus *Potyvirus* (Family: *Potyviridae*), infects maize, sorghum and some other graminaceous species (Alegria *et al.,* 2003; Achon *et al.,* 2007). SCMV is characterized by the presence of flexuous filamentous virions which contain a monopartite genome. It is a positive-sense single-stranded RNA virus with a genome size of approximately 10 kb, and is characterized by an un-translated region (5'UTR), a large open reading frame (ORF), and a 3' UTR region that has a poly-adenylated (poly A) tail. The ORF comprises 10 functional proteins including protein 1 (P1), helper component proteinase (HC-Pro), protein 3 (P3), 6K1, cylindrical inclusion protein (CI), 6K2, viral protein genome linked (VPg), major protease of small nuclear inclusion protein (NIa-Pro), large nuclear inclusion protein (NIb), coat protein (CP) (Shukla *et al.,* 1988; Padhi and Ramu, 2011), and PIPO protein (ORF2) (Chung *et al.,* 2008; Wei *et al.,* 2010).

SCMV is transmitted by infected setts, mechanical inoculation and by several aphid species *viz*., *Longiunuis sacchari, Myzus persicae* and *Rhopalosiphum maidis* (Rao and Ford, 2001; Singh *et al.,* 2005). *Sugarcane mosaic disease* is suppose to cause by more strains of Sugarcane mosaic virus (Jain *et al.,* 1998; Viswanathan and Balmuralikrishnan, 2005). In spite of the fact that, a new causal pathogen of SMD has been reported and named as *Sugarcane streak mosaic virus* (SCSMV) (Hema *et al.,* 2002, 2003). A mixed infection of SCMV and SCSMV has also been reported in sugarcane in India (Viswanathan *et al.,* 2007; Singh *et al.,* 2009). Although, detection of SCMV and wide spread of disease incidence has been reported from several states of India based on the full and partial coat protein gene, but the studies on partial genome characterization is meagre. Hence, the present study was designed to detect the association of specific *Potyvirus* species with SMD and partial genome characterization of the SCMV-Kar isolate.

Materials and Methods

Collection of field samples and storage : Mosaic diseased and healthy samples from 24 sugarcane genotypes *viz*., Co 94008, Co 1148-242-2, CP 63-369, CYMA 08-502, CYMA 08-828, CYMA 09-836, CYM 10-25, GU 07-3704, GU 07-3785, GU 07-3803, ISH 99-430, ISH 99-97, ISH 99-92, ISH 99-81, ISH 99-438, KGS 200-48, PoJ 279, PoJ 679, PIO 88-1703, WL 1120, WL 00-331, WL 00-221, WL 00-224 and WL 002248 were collected from the experimental fields at Sugarcane Breeding Institute (SBI), Regional Station, Karnal, Haryana. Similarly, diseased and healthy samples from 14 sugarcane genotypes including, Baragua, Bo 91, Co 0238, CoJ 64, CoLk 94184, CoLk 8102, CoLk 9709, CoLk 7201, CoSe 92423, Co 1148, CoS 767, Co 05011, CoPant 97222 and Khakai were collected from the experimental farm at Indian Institute of Sugarcane Research (IISR), Lucknow, Uttar Pradesh. The collected samples were brought under cold condition and immediately stored in -80°C after freezing in liquid nitrogen till further use in various experiments.

Leaf-dip electron microscopy : The association of virus particles with infected sugarcane samples was ascertained by leaf-dip electron microscopy (Gibbs *et al.,* 1966). Diseased leaf bits (3-5 mm) were cut with help of cork borer macerated on a clean glass slide with a flat ended glass rod in 40-50 µl phosphate buffer (0.078 M, pH 6.5) and left the finally homogenized material for few seconds. A drop of supernatant (15-20 µl) from the homogenized virus material was put on the carbon coated grid (3 mm diameter, 400 mesh). Excess of supernatant was then washed off with 180-200 µl distilled water. A carbon coated grid was treated with 40-50 µl uranyl acetate (aqueous 2%, pH 4.2) for few seconds. Excess stain was removed and blotted dry by touching the edge of the grid with a strip of filter paper. The grid was air–dried for 1-2 min. The negatively stained grid was finally examined under electron microscope (JEM-1011) at the Advanced Centre for Plant Virology, IARI, New Delhi.

Reverse transcription polymerase chain reaction (RT-PCR) : The total RNA was isolated from the SMD affected sugarcane tissues with RNeasy plant mini kit (Qiagen, Chatsworth, CA, USA) according to manufacturer's instructions and the first strand cDNA was synthesized using *Potyvirus* universal reverse primer by ImProm-II™ reverse transcriptase kit (Promega, Madison, WI, USA). A 20 µl of cDNA reaction mixture contained 10 µl of template RNA (~2.5 µg), 1 µl (~200 ng) of reverse primer, 1 µl of 10 mM dNTP, 4 µl of 5x buffer, 0.5 µl of RNase inhibitor (40 Ul^{-1}) (Promega Madison, WI, USA) and 2.5 µl of sterile distilled water and 1 µlof reverse transcriptase (Uml^{-1}) was incubated at 42°C for 60 min. A first strand cDNA was used as a template for amplification in polymerase chain reaction (PCR) in 50 µl of reaction mixture containing 10 µl of cDNA, 5 µl of 10x PCR buffer, 2 µl of 10 mM dNTP, 1 µl (~100 ng) each of reverse and forward primers (PotyF 5'ACCACAGGATCCGGBAAYAAYAGYGG DCARCC3'and PotyR 5'CACGGATCCCG GG $(T)_{17}$3'), 1 µl 2.5 units of *Taq* DNA polymerase and 30 µl of sterile distilled water. The PCR was conducted in a thermo cycler (Biometra, Germany) with the following temperature conditions: 2 min hot start at 94ºC followed by 30 cycles of denaturation at 94 ºC for 1 min, annealing for 2 min at 57 ºC, synthesis at 72 ºC for 2 min, and a cycle of final extension at 72 ºC for 10 min.

Cloning, sequencing and sequence analyses : The RT-PCR products were analyzed in 1% agarose gel electrophoresis at 60V

and purified using SV-gel and PCR clean-up system (Promega Corporation, Madison, WI, USA). Further, these amplicons of *Potyvirus*, approximately 1800 bp, were cloned in pGEM-T Easy vector (Promega Corporation, Madison, WI, USA) and the recombinant clones were screened by colony PCR and restriction digestion with EcoRI enzyme and the selected recombinant clones were sequenced (primer walking) by automated sequencing facility at Department of Biochemistry, University of Delhi South Campus, New Delhi. The nucleotide (nt) and amino acid (aa) sequence analyses were conducted by clustal W version 1.7 (Thompson *et al*., 1994) using Bioedit software (www.boiedit.software.informer .com). The phylogenetic tree was constructed by MEGA 5.0 software (http://www.megasoftware.net) and the evolutionary history was inferred using Neighbour-Joining method.

Results and Discussion

Symptomatology and leaf-dip electron microscopy : The collected sugarcane samples from SBI, Regional Station, Karnal, Haryana and IISR, Lucknow, Uttar Pradesh, exhibited mild, severe systemic mosaic and necrotic spots all over the leaf lamina (Fig. 1A-D). SMD disease was primarily characterized by symptoms observed on cane leaves. Moreover, the symptoms varied with genotype to genotype, but the most distinct symptoms included different shades of light/pale green and dark green along with boat shaped yellowish interveinal chlorotic areas. Subsequently, these interveinal chlorotic spots turned necrotic and reddening of leaf lamina was observed. Symptoms were more prominent on the younger leaves with broad leaf lamina than the older leaves. Total six random samples subjected to EM studies revealed the presence of slightly flexuous rod shaped particles in all the tested genotypes *viz*., PoJ 279, WL 00-221, Co 94008, Co 1148, PoJ 679 and WL 00-224 (Fig. 1E). During the month of October-November, 2015 the aphid colonies were spotted on mosaic affected Co 0238 and other sugarcane genotypes at IISR farm (Fig. 1F). In order to confirm the association of specific *Potyvirus* species with SMD, these samples were further subjected to reverse transcription polymerase chain reaction (RT-PCR) using universal *Potyvirus* primers followed by sequencing.

Detection and molecular characterization of SCMV in sugarcane genotypes : Of six EM positive samples, only four were subjected to RT-PCR. The RT-PCR from four random symptomatic sugarcane samples resulted in the amplification of approximately 1800 bp size products and apparently asymptomatic samples showed no amplification (Fig. 2A). The resulting amplicons were cloned and confirmed by colony PCR and restriction digestion using EcoRI. Restriction digestion using EcoRI was carried out for seven recombinant clones, the two clones (Fig. 2B; Lanes: 6 & 7) were restricted into two bands of approximately 1600 bp and 200 bp in contrast to the remaining five recombinant clones resulting in restriction of three fragment sizes of approximately 950 bp, 550 bp and 300 bp (Fig. 2B; Lanes 1, 2, 3, 4 & 5) along with the TA cloning vector, which might be either due to the presence of other closely related *Potyvirus* or the same *Potyvirus* having two restriction sites of EcoRI in the genome since, primers used in this study were universal *Potyvirus* which detects all the species of *Potyviruses*. Of these, single clone was sequenced (Fig. 2B; Lane: 6); the sequence of 1828 nucleotides shared maximum 96% sequence identity with those of *Sugarcane mosaic virus* isolates at nucleotide level after performing BLAST in GenBank. The sequence of SCMV_Karnal, Haryana (SCMV_Kar) isolate from the present study was submitted to the GenBank (accession no: KF531632). The sequence of SCMV_Lucknow isolate needs further characterization.

The characterized partial polyprotein of SCMV_Kar isolate is composed of 1828 nucleotides of which 639, 1138 and 246 nucleotides corresponding to partial NIb region, complete coat protein (CP) gene and 3' UTR with poly (A) tail, respectively. Earlier, it was reported that the coat protein composed of three types of N terminal CP region sequences and similar findings suggested by Alegria *et al.* (2003) and Chen *et al.* (2002). Type one includes the isolates originating from South Africa (SCMV-SA, AF006738), Australia (SCMV-BRIS, AJ278405) and USA (SCMV-USLA, AF006736; SCMV-A, U57354; Handley *et al*., 1998). Whereas, isolates originating from isolates SCMV-MDB (A34976 and D00949) formed second type and designated as new *Potyvirus* species (Fan *et al*., 2003; Handley *et al*., 1998). Third type composed of isolates originating from China, Germany (SCMV-BOR, X98167) and Mexico (SCMV-Mx, AY195610), later Chinese isolates were divided further as maize and sugarcane-infecting. SCMV_Kar isolate clustered closely to the type one group isolates originating from USA (AJ278405 and U57354) and shared 97-98% sequence identity at amino acid level. Moreover, Zhong *et al.* (2005) identified naturally occurring recombinant isolate of SCMV causing maize dwarf mosaic disease and found that the CP sequences showed about 95% identities with the SCMV-Mx and SCMV-BOR, suggesting that the maize isolates classed as new strain but not the geographical isolates. Contrastingly, in a recent past association of SCMV with sorghum was reported in Tehran based on the results obtained by DAS-ELISA and RT-PCR with 900 bp amplicons (Mohammadi and Hajieghrai, 2009).

A complete CP gene sequence of SCMV_Kar isolate shared 89-98% identity with other SCMV isolates infecting maize and sugarcane both at nucleotide and amino acid levels, respectively. Whereas, it shared minimum 79% and 89% sequence identity with *Sorghum mosaic virus* (SrMV) at nucleotide and amino acid levels, respectively (Table 1). Similarly, CP sequence revealed maximum 96-98% sequence identity with SCMV originating from USA (accession no: U57355, AY953351, U57356 and U57357) and China (accession no: AY953351). In

Fig. 1 : Symptoms of *Sugarcane mosaic virus* (SCMV) on different sugarcane genotypes under field conditions. (A) shades of pale green along with boat shaped yellowish chlorotic areas all over the leaf surface, (B) chlorotic areas on the lower leaf surface, (C) severity of mosaic disease, (D) shades of dark green followed by necrotic spots symptoms varying with four different genotypes, (E) Electron micrographs of *Sugarcane mosaic virus* (SCMV) virion particles infecting sugarcane genotypes (WL 00221) showing flexuous rods and (F) aphid colonies observed on sugarcane leaves of Co 0238

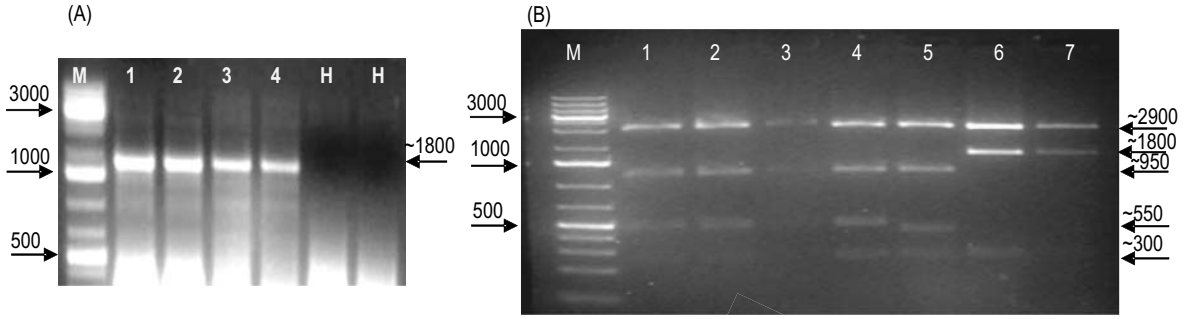


Fig. 2 : Agarose gel electrophoresis of RT-PCR based detection of *Sugarcane mosaic virus* (SCMV) infecting sugarcane. (A) PCR amplification of sugarcane samples infected by *potyvirus* using universal primers. Lane: M: 1 kb DNA ladder 1: POJ 679, 2: Co 94008, 3: WL 00-224, 4: Co 1148 H: healthy. (B) Restriction digestion of the recombinant pGEMT clones (1-7) by *Eco*RI restriction enzyme. The fragment sizes represented in base pairs

Sequence Accession No.	U57354 strain A	AJ278405 strain A	U57355 strain B	AY953351 strain D	U57356 strain D	AY836523 strain E	U57357 strain E	KF531632*	AF494510	JN021933	EU091075	GU474635	JX188385	JX185303	AY569692	AY149118	AY042184	JX237863	JX237862	NC_003398	AJ297628	AY222743
U57354 strain A	100	95	94	95	94	95	94	93	79	77	81	78	79	80	79	79	80	88	94	80	80	78
AJ278405 strain A	95	100	95	97	96	97	96	96	80	78	82	79	79	82	80	80	80	90	97	80	80	79
U57355 strain B	94	95	100	95	98	95	95	93	80	77	81	79	79	81	80	80	80	89	95	80	80	79
AY953351 strain D	95	97	95	100	96	96	96	95	79	78	82	79	79	81	80	79	80	90	97	80	80	79
U57356 strain D	94	96	98	96	100	96	96	94	79	77	81	79	79	81	79	79	79	89	96	79	79	79
AY836523 strain E	95	97	95	96	96	100	98	95	80	77	81	79	78	81	80	80	80	90	97	80	80	79
U57357 strain E	94	96	95	96	96	98	100	95	80	77	81	78	79	81	80	80	80	90	97	80	80	79
KF531632*	93	96	93	95	94	95	95	100	80	77	82	79	79	81	80	80	80	90	96	80	80	80
AF494510	79	80	80	79	79	80	80	80	100	79	89	79	78	88	99	99	99	79	80	99	99	77
JN021933	77	78	77	78	77	77	77	77	79	100	80	78	80	80	79	79	79	78	78	79	79	77
EU091075	81	82	81	82	81	81	81	82	89	80	100	85	79	95	89	89	89	80	82	89	89	79
GU474635	78	79	79	79	79	79	78	79	79	78	85	100	91	84	79	79	80	78	79	80	80	80
JX188385	79	79	79	79	79	78	79	79	78	80	79	91	100	79	78	78	78	80	79	78	78	80
JX185303	80	82	81	81	81	81	81	81	88	80	95	84	79	100	88	88	88	80	82	88	88	79
AY569692	79	80	80	80	79	80	80	80	99	79	89	79	78	88	100	98	99	79	80	99	99	78
AY149118	79	80	80	79	79	80	80	80	99	79	89	79	78	88	98	100	98	79	80	99	99	77
AY042184	80	80	80	80	79	80	80	80	99	79	89	80	78	88	99	98	100	79	80	100	100	78
JX237863	88	90	89	90	89	90	90	90	79	78	80	78	80	80	79	79	79	100	90	79	79	80
JX237862	94	97	95	97	96	97	97	96	80	78	82	79	79	82	80	80	80	90	100	80	80	80
NC_003398	80	80	80	80	79	80	80	80	99	79	89	80	78	88	99	99	100	79	80	100	100	78
AJ297628	80	80	80	80	79	80	80	80	99	79	89	80	78	88	99	99	100	79	80	100	100	78
AY222743	78	79	79	79	79	79	79	80	77	77	79	80	80	79	78	77	78	80	80	78	78	100

5% 10% 15% 20% 25% 30% 35% 40% 45% 50% 55% 60% 65% 70% 75% 80% 85% 90% 95% 100%

Fig. 3 : Two-dimensional colour coded (Heat map) graphical representation of pair wise percent sequence identities of the *Sugarcane mosaic virus* (SCMV) isolates based on coat protein (CP), nuclear inclusion bodies (NIb) and three prime un-translated (3'UTR) region. *represents SCMV-Kar isolate sequence submitted to the GenBank from the present study

contrast to this, the SCMV Karnal isolate from the present study shared maximum 92% identity at amino acid level with *Maize dwarf mosaic virus* (MDMV) infecting sugarcane originating from Spain. Similar results been described by earlier studies Frenkel *et al.* (1991) suggesting that CP of SCMV originating from sugarcane (SCMV-SC) shared 92% identity with MDMV-B and hence these two viruses were considered the strains of the same *Potyvirus*. Whereas, Jiang and Zhou (2002) observed that the clustering of SCMV in a separate cluster in phylogenetic tree with only SCMV isolates while separate clusters of MDMV, SrMV and JGMV were clustered in separate clusters resulting in the formation of two groups. The isolates originating from China, Germany, Bulgaria and Spain (infecting maize: group I) and isolates originating from United States, Australia and South Africa (infecting sugarcane: group II) hence, a correlation of sequence pattern of CP with the respective hosts was drawn (Xiao *et al*., 1993). SCMV_Kar isolate shared maximum 95-96% identity with the Indian isolates originating from Tamil Nadu, Karnataka and Andhra Pradesh which revealed a maximum variation of 4-5% with Indian isolates and a maximum variation of 2-3% at amino

Table 1 : Sequence identity matrix of the complete coat protein (CP) gene of *Sugarcane mosaic virus* (SCMV) isolates. The nucleotide sequence similarity is represented on the upper side of the diagonal whereas deduced amino acid sequence similarity represented on the lower side of the diagonal. *sequence submitted to the GenBank from the present study

Seq->	KF53 1632*	JN02 1933	EU09 1075	GU47 4635	AF49 4510	JX18 8385	JX18 5303	AY56 9692	AY14 9118	AY04 2184	JX23 7863	JX23 7862	NC_0 03398	AJ29 7628	AM11 0759	AJ27 8405	AM11 0758	U57 355	AY95 3351	U57 356	AY83 6523	U5 7357	U0 7219	GQ38 6848	GQ38 6847	GQ38 6846	DQ86 6746	DQ86 6745
KF531632*	ID	89%	91%	91%	91%	92%	92%	91%	91%	91%	91%	97%	91%	91%	91%	98%	82%	96%	96%	97%	97%	98%	79%	95%	95%	95%	96%	96%
JN021933	97%	ID	93%	92%	92%	91%	92%	93%	92%	92%	89%	89%	92%	92%	93%	90%	79%	90%	90%	90%	89%	89%	79%	90%	90%	90%	91%	91%
EU091075	97%	99%	ID	94%	95%	94%	98%	95%	95%	92%	90%	91%	95%	95%	95%	91%	83%	91%	91%	91%	91%	91%	80%	91%	91%	91%	92%	92%
GU474635	96%	98%	99%	ID	93%	97%	94%	93%	93%	93%	89%	91%	93%	93%	93%	91%	82%	91%	90%	91%	91%	91%	80%	91%	90%	90%	91%	91%
AF494510	97%	99%	100%	99%	ID	100%	99%	100%	100%	100%	97%	98%	100%	100%	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
JX188385	97%	99%	100%	99%	100%	ID	99%	100%	100%	100%	97%	98%	100%	100%	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
JX185303	98%	99%	99%	98%	99%	99%	ID	99%	99%	99%	97%	97%	99%	99%	99%	97%	91%	96%	97%	97%	97%	97%	89%	97%	96%	97%	99%	99%
AY569692	97%	99%	100%	99%	100%	100%	99%	ID	100%	100%	97%	98%	100%	100%	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
AY149118	97%	99%	100%	99%	100%	100%	99%	100%	ID	100%	97%	98%	100%	100%	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
AY042184	97%	99%	100%	99%	100%	100%	99%	100%	100%	ID	97%	98%	100%	100%	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
JX237863	97%	97%	97%	96%	97%	97%	97%	97%	97%	97%	ID	97%	97%	97%	97%	99%	92%	98%	99%	99%	98%	97%	89%	96%	95%	96%	98%	98%
JX237862	97%	97%	98%	97%	98%	98%	97%	98%	98%	98%	97%	ID	98%	98%	97%	98%	92%	97%	97%	98%	99%	98%	89%	97%	96%	97%	98%	98%
NC_003398	97%	99%	100%	99%	100%	100%	99%	100%	100%	100%	97%	98%	ID	100%	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
AJ297628	97%	99%	100%	99%	100%	100%	99%	100%	100%	100%	97%	98%	100%	ID	99%	98%	91%	97%	97%	98%	98%	97%	90%	98%	97%	97%	99%	99%
AM110759	97%	100%	99%	98%	99%	99%	99%	99%	99%	99%	97%	97%	99%	99%	ID	97%	91%	96%	97%	97%	97%	97%	89%	97%	96%	97%	99%	99%
AJ278405	97%	97%	98%	97%	98%	98%	97%	98%	98%	98%	99%	98%	98%	98%	97%	ID	92%	99%	99%	100%	99%	97%	89%	97%	96%	96%	98%	98%
AM110758	92%	91%	91%	90%	91%	91%	91%	91%	91%	91%	92%	92%	91%	91%	91%	92%	ID	91%	92%	92%	92%	92%	89%	90%	89%	90%	92%	92%
U57355	96%	96%	97%	96%	97%	97%	96%	97%	97%	97%	98%	97%	97%	97%	96%	99%	91%	ID	98%	99%	98%	96%	88%	96%	94%	95%	97%	97%
AY953351	97%	97%	97%	96%	97%	97%	97%	97%	97%	97%	99%	97%	97%	97%	97%	99%	92%	98%	ID	99%	98%	97%	89%	96%	95%	96%	98%	98%
U57356	97%	97%	98%	97%	98%	98%	97%	98%	98%	98%	99%	98%	98%	98%	97%	100%	92%	99%	99%	ID	99%	97%	89%	97%	96%	96%	98%	98%
AY836523	97%	97%	98%	97%	98%	98%	97%	98%	98%	98%	98%	99%	98%	98%	97%	99%	92%	98%	98%	99%	ID	98%	90%	97%	96%	96%	98%	98%
U57357	98%	97%	97%	96%	97%	97%	97%	97%	97%	97%	97%	98%	97%	97%	97%	97%	92%	96%	97%	97%	98%	ID	90%	96%	95%	96%	98%	98%
U07219	89%	89%	90%	89%	90%	90%	89%	90%	90%	90%	89%	89%	90%	90%	89%	89%	89%	88%	89%	89%	90%	90%	ID	88%	88%	88%	89%	89%
GQ386848	96%	97%	98%	97%	98%	98%	97%	98%	98%	98%	96%	97%	98%	98%	97%	97%	90%	96%	96%	97%	97%	96%	88%	ID	98%	96%	98%	98%
GQ386847	95%	96%	97%	96%	97%	97%	96%	97%	97%	97%	95%	96%	97%	97%	96%	96%	89%	94%	95%	96%	96%	95%	88%	98%	ID	95%	97%	97%
GQ386846	96%	97%	97%	96%	97%	97%	97%	97%	97%	97%	96%	97%	97%	97%	97%	96%	90%	95%	96%	96%	96%	96%	88%	96%	95%	ID	98%	98%
DQ866746	98%	99%	99%	98%	99%	99%	99%	99%	99%	99%	98%	98%	99%	99%	99%	98%	92%	97%	98%	98%	98%	98%	89%	98%	97%	98%	ID	100%
DQ866745	98%	99%	99%	98%	99%	99%	99%	99%	99%	99%	98%	98%	99%	99%	99%	98%	92%	97%	98%	98%	98%	98%	89%	98%	97%	98%	100%	ID

acid level with other SCMV isolates originating from different regions of the world (Table 1). However, based on the earlier reports the sequence data of the NIb coding region of SCMV from Australia, it was found that there was a maximum of 3.3% variation between isolates at the nucleotide level and a maximum of 0.8% at the amino acid level (Handley *et al.,* 1996). SCMV_Kar isolates showed a maximum variation of 4-6% of at nucleotide (nt) level and 2-3% variation at amino acid (aa) level with all the other

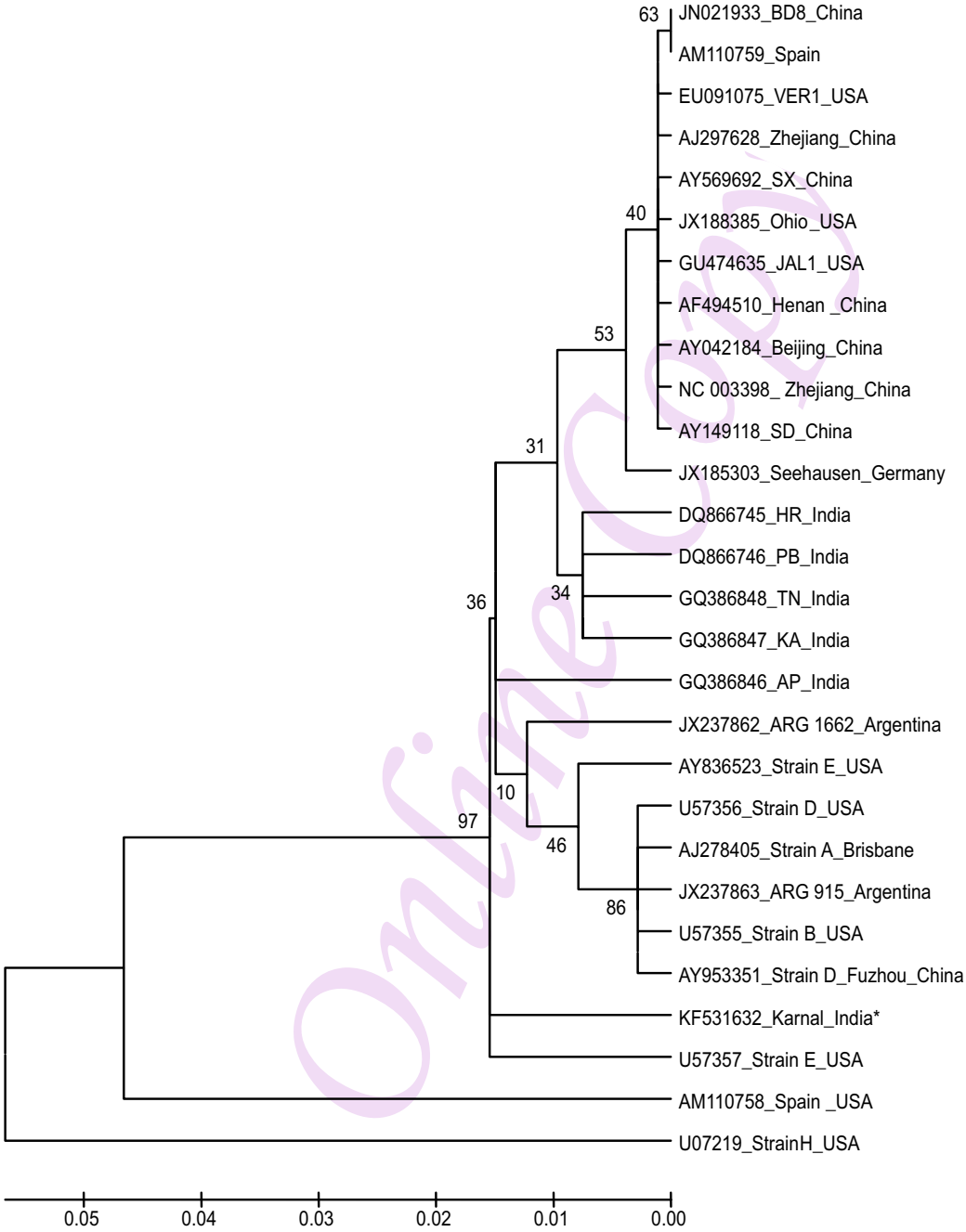


Fig. 4 : Phylogenetic tree (neighbor-joining method with bootstrapping 1000 replicates) constructed based on the comparison of the deduced amino acid sequences of the partial coat protein (CP) gene of *Sugarcane mosaic virus* (SCMV) isolates. SCMV_Kar isolate from present study showing clustering with SCMV *Sequence submitted to the GenBank from the present study

isolates of SCMV this forms the existence of maximum genetic variability in SCMV genome. A two-dimensional colour-coded graphical representation of pair wise percent sequence identities among the *Sugarcane mosaic virus* (SCMV) isolates based on the CP, NIb and 3' UTR showed maximum 95-97% sequence identity with SCMV_ARG 915 and SCMV_ARG 1962 isolates originating from Argentina, whereas it shared 82% minimum identity with the MDMV infecting maize from Spain (Fig. 3). The phylogenetic tree revealed close clustering of the KF531632 (SCMV_Kar) isolate with SCMV from USA and Australia, whereas a separate cluster of Indian SCMV isolates from Punjab, Haryana, Tamil Nadu, Karnataka and Andhra Pradesh infecting sugarcane was also observed (Fig. 4).

Antigenic and biological diversity among SCMV isolated from different geographical regions of India had been analysed by ELISA and found SCMV was detected using the SCSMV antiserum (Rao *et al.,* 2004). Subsequently, molecular characterization of SCMV isolated from North Eastern Region (NER) of India was carried out by immuno-based PCR and evidenced that the amino acid sequence showed closest homology with SCMV_SC isolated from Australia (99%), followed by 97% with SCMV-A (USA), 87% SCMV-BJ (China) and 80% SCMV –MDB (USA), respectively (Gaur *et al.,* 2003). Recently, Adams *et al.* (2013) identified the association of *Maize chlorotic mottle virus* (MCMV) and SCMV together with maize lethal necrosis disease, which was not reported so far from India. Moreover, genetic diversity and genomic evidence of intraspecific recombination in SCMV was detected and 6KI-VPg-NIaPro-NIb region to be recombination hotspot (Li *et al*., 2013; Padhi and Ramu, 2011) this region was partially characterized in the present study.

In the present study, it was evidenced that the SCMV was associated with SMD and symptoms varied with genotype to genotype at Karnal and Lucknow. However, in Uttar Pradesh, Maharashtra and Tamil Nadu the detection of SCMV and SCSMV was carried out using RT-PCR assays and revealed that SCSMV was found more commonly associated with SMD in India in comparison to the SCMV (Rao *et al.,* 2006). In India, SCMV and SCSMV are the only pathogens of SMD while, SrMV was reported to infect sorghum and maize but not the sugarcane (Rao *et al*., 2006). Although the mosaic disease caused by SCMV and SCSMV, induced similar symptoms and difficult to differentiate under field conditions, in the present study the association of SCMV with SMD was specifically detected based on the sequence information generated. Expression of symptoms and severity of SCMV varied with genotype to genotype (data not shown), earlier study has been done on the effect of symptom expression in SCMV infected plants on the sugarcane yield and detected the presence of the SCMV population in symptomless genotypes using ELISA (Cronje *et al.,* 1994). Till date from India, 74 total sequences of SCMV (including isolate from present study), SCSMV and SrMV were available at GenBank, of which 45 sequences of SCMV, 15 sequences of SCSMV and 13 sequences of SrMV (http://www.ncbi.nlm.nih.gov). Present study is the evidence of the association of SCMV with the SMD of sugarcane and its partial molecular characterisation (SCMV_Karnal isolate). The molecular characterization of SCMV and/ SCSMV infecting sugarcane in the sub-tropical India would be the future line of research work.

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