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TM Hemalatha
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

BV Bhaskara Reddy
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

R Sarada Jayalakshmi
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

SR Koteswara Rao
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

M Hemanth Kumar
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

G Mohan Naidu
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

Corresponding Author:
TM Hemalatha
Agricultural Research Station, Perumallapalle, Andhra Pradesh, India

Molecular characterization and phylogentic analysis of isolates of sugarcane yellow leaf virus infecting sugarcane from Andhra Pradesh

TM Hemalatha, BV Bhaskara Reddy, R Sarada Jayalakshmi, SR Koteswara Rao, M Hemanth Kumar and G Mohan Naidu

Abstract
Sugarcane Yellow Leaf Disease caused by Sugarcane yellow leaf virus (ScYLV) is an emerging disease affecting the production and productivity in many ruling sugarcane varieties in India. The losses due to the disease range from 60% in the main crop to 100% in the ratoon crop. Infected plants shows typical symptoms of midrib yellowing on adaxial surface with lateral spread of yellow discolouration to the leaf lamina. Later followed by tissue necrosis from the leaf tip spreading downwards along the midrib and a bushy appearance of the top of the plant due to internode shortening in maturing plants. In the present study, twenty five SCYLV infected leaf samples were collected from major sugarcane growing regions and amplified the ORF 3& 4 regions of the genome of the virus coding for coat protein and movement protein. RT-PCR was performed using specific primers (ScYLV-613F & ScYLV 613R) amplifying the coat protein and movement protein of the virus. Amplification of band of size 613 bp was observed in twelve of the samples. These amplicons were sequenced and were analyzed to determine the sequence identities, sequence variations and phylogenetic relationships with the SCYLV isolates reported earlier from India and abroad with the view to determine the variability among the SCYLV isolates reported from india and abroad. The nucleotide (nt) and amino acid (aa) sequence comparison in the CP and MP coding regions showed a significant variation between Indian isolates (SCYLV-IND) and other SCYLV isolates reported worldwide. The analysis of the nucleotide sequence data of 12 isolates under study revealed 95–100% identities among them and also with isolates of CUB ans COL isolates. The identities were 94-97% with other SCYLV isolates reported. The nucleotide (nt) and amino acid (aa) sequence comparison in the CP and MP coding regions showed a significant variation between Indian isolates (SCYLV-IND) and other SCYLV isolates reported worldwide There was greater similarity in aminoacid sequence ie., upto 94.2 - 99.2% between the Indian isolates and the CUB, COL isolates while the similarity was about 90.0-96.1% with other genotypes. This was further confirmed from the phylogenetic analysis where the CUB and COL isolates clustered with the isolates reported from India while the other genotypes (HAW, CHN, REU, PER, BRA) clustered into a separate group. The Indian isolates showed close relationship with the CUB and COL isolates. Hence it was confirmed from the study that SCYLV isolates collected from major sugarcane growing regions of Andhra Pradesh closely related to SCYLV-CUB and SCYLV-COL genotypes.

Keywords: Sugarcane, sugarcane yellow leaf virus, molecular characterization, coat protein, movement protein, phylogenetic analysis

1. Introduction
Sugarcane (*Saccharium officinarum*) is an important commercial crop of the world and is principal sources of sugar, ethanol, and jaggery globally. It is cultivated in more than 100 countries viz., Brazil, India, China, Thailand and Pakistan. India is the largest consumer of sugar and second largest producer of sugarcane after Brazil. The byproducts of it are also used as a fodder to feed livestock in many countries. Among different factors that limit the sugarcane production, diseases play a major role in reducing the sugarcane yields. Sugarcane is infected by more than 200 diseases caused by various fungi, bacteria, viruses, phytoplasma and nematodes. Among the major pathogens, Sugarcane yellow leaf virus causing Sugarcane yellow leaf Disease (SCYLD) is an emerging disease causing epidemic in the sugarcane growing countries. The virus belongs to genus *Polerovirus,* family *Luteoviridae*. It was first reported in Hawaii in the late 1988 and was subsequently observed in almost all sugarcane growing countries (Comstock *et al*., 2002b; Izaguirre-Mayoral *et al*., 2002; Lockhart *et al*., 1996; Lockhart and Cronje, 2000; Vega *et al*., 1997; Viswanathan, 2002) [9, 3, 16, 17, 25, 27].

In India, it was reported during 1999 (Viswanathan *et al*, 2002) [27]. The disease was reported from almost all the sugarcane growing regions in India (Maharashtra, Bihar, Uttar Pradesh, Punjab, Kerala, Tamil Nadu, Madhya Pradesh, Haryana and Andhra Pradesh). Disease incidence up to 100% in commercial fields in susceptible cultivars was reported in Florida (Comstock *et al.* 1999, 2001) [8, 7], India (Viswanathan 2002) [27], Island of Reunion (Rassaby *et al*. 2004) [19] and in Thailand (Lehrer *et al*. 2008) [14].

First association of RNA with yellow leaf syndrome was reported in Hawaii in 1994 (Borth *et al*., 1994) [5], and a luteovirus was found in diseased sugarcane from Florida and Brazil in 1995 (Lockhart *et al*., 1996; Vega *et al*., 1997) [16, 25]. SCYLV is an emerging virus that has evolved by recombination between ancestors of the three genera (Luteovirus, Polerovirus, and Enamovirus) forming the family Luteoviridae. The genome of sugarcane yellow leaf virus encodes at least six open reading frames (ORFs 0–5) and shows a genome organization typical of poleroviruses.

In Andhra Pradesh, sugarcane is grown in an area of 1.2 lakh hectares. At present the disease is spreading at an alarming stage infecting almost all the varieties grown by the farmers in A.P. The disease has spread to number of ruling varieties like. 2003V46, Co 86032, 83V15, 87A298, 86V96, Co 62175, 2002V48, 2005T16. In Andhra Pradesh, YLD symptoms were observed since 2004 in the farmers' fields of Nizambad (Bharathi and Kishan reddy, 2007) [4] and later observed in the coordinated trails at Regional Agricultural Research Station and Anakapalle. In chittoor district, the disease was first observed in the experimental plots of Agricultural Research Station Perumallapalle during 2009-10 in the entries Co 7219 and 87A298 and a number of varieties got affected in the AICRP trials. During 2010-11, the disease soon appeared in the farmer fields in almost all the sugarcane growing areas of the District.

SCYLV is evolved from recombination of three different viruses belonging to genera of Luteovirus, Polerovirus and Enamovirus. The genome of SCYLV has a positive- sense single stranded RNA containing six overlapping open reading frames (ORF 0– ORF 5) which is devoid of a poly(A) tail and three untranslated regions (UTRs) consisting of ~5.8 kb nucleotides.

Studies regarding phylogenetic origin of SCYLV revealed that 10 different genetic groups have determined *viz*., BRA (Brazil), CHN1, CHN2, CHN3 (China), COL (Colombia), CUB (Cuba), HAW (Hawaii), IND (India), PER (Peru) and REU (Reunion). These genotypes were determined based on analysis of the genetic diversity of their genome using partial sequences and complete genomes (Moonan and Mirkov, 2002; Abu Ahmed *et al*., 2006a; Elsayed *et al*., 2010; Gao *et al*., 2012, Chinnaraja *et al*, 2013; Lin *et al.* 2015) [18, 1, 10, 12, 6].

The main objective of the present study was to detect the prevalence and distribution of Sugarcane yellow leaf virus in sugarcane growing regions of Andhra Pradesh and to determine the diversity of SCYLV isolates in India and their phylogenetic origin.

2. Materials and Methods

During 2017, twenty five sugarcane leaf samples exhibiting typical midrib yellowing symptoms were collected from major sugarcane growing regions of Andhra Pradesh (Chittoor, Nellore, Vishakapatnam, Krishna, Prakasham and Vijayanagaram). The collected leaf samples were stored at −20°C until further processing. The detailsof the SCYLV isolates used in the study are given in table.1.

Table 1: Details of SCYLV isolates used in the current study

S. NO	Scylv Isolate	Location	District	Variety	Accession Number
1	SCYLV-SKHT1	Srikalahasti	Chittoor	2003V46	MN814252
2.	SCYLV-YPD	Yerpedu	Chittoor	83V15	MN850408
3	SCYLV-PTR	Puttur	Chittoor	2005T16	MN922287
4	SCYLV-YPD1	Yerpedu	Chittoor	CoT 8201	MN962641
5	SCYLV-SKHT2	Srikalahasti	Chittoor	86V96	MT060295
6	SCYLV-ANK1	Anakapalle	Vishakapatnam	87A298	MT124724
7	SCYLV-ANK2	Anakapalle	Vishakapatnam	93A145	MT882328
8	SCYLV-PPL1	Perumallapalle	Chittoor	92A30	MT882329
9	SCYLV-PPL2	Perumallapalle	Chittoor	Co 86032	MT913601
10	SCYLV-PPL3	Perumallapalle	Chittoor	CoC671	MT929272
11	SCYLV-ANK3	Anakapalle	Vishakapatnam	2000A240	MW309845
12	SCYLV-VYV	Vuyyur	Krishna	2009V89	MW118673

2.1 RNA extraction and RT-PCR

All the 25 samples were processed for total RNA extraction and RT-PCR analysis. One hundred mg of fresh sample powdered using liquid nitrogen was transferred to a 1.5 ml DEPC treated microfuge tube and to that 1 ml TRI reagent (Sigma, USA) was added. The tubes were vigorously shaken for homogenous mixing of TRI reagent with the sample and were kept at 4°C until all the samples were homogenized. The samples were allowed to complete dissociation of nucleoprotein complexes and release of RNA by incubating at room temperature for 5 min. All the insoluble materials such as cellular membranes, high molecular weight DNA and polysaccharides were precipitated at the bottom of the tubes by centrifugation at 12,000 rpm for 10 min at 4°C. The supernatant containing the RNA was transferred to a fresh tube and to that 200 µl of the chloroform for every 1 ml of TRI reagent was added. The tubes were shaken vigorously for 15 sec and again incubated at room temperature for 5–10 min and later centrifuged at 12,000 rpm for 15 min at 4°C. After centrifugation, the mixture separated into three phases: a red organic phenolic phase contained protein, an inter-phase of DNA and a colourless upper aqueous phase which contained the RNA. The RNA containing upper aqueous phase was transferred into a fresh tube and added 500 µl of isopropanol to precipitate the RNA. After incubation for 5 min at room temperature, the tubes were centrifuged at 12,000 rpm for 15 min at 4°C for pelletizing the RNA. The pellet was air dried for 10 min and dissolved the RNA with 40 µl of RNA resuspension buffer (Ambion, USA) and stored at −80°C.

The quality of the RNA was checked by Nanospectrophotometer. The forward primer SCYLV-613 F: 5′-ATGAATACGGGCGCTAACCGYYCAC-3′ and reverse

primer SCYLV- 613 R: 5′-GTGTTGGGGRAGCGTCGCYTACC-3′ designed by Viswanathan *et al*. (2008) [26] were used to amplify ~610 bp of ORF3 &ORF4 regions of SCYLV genome. The cDNAs were synthesized from total RNA of all the 25 samples separately using RevertAid H Minus first stand cDNA synthesis kit (MBI Fermentas, USA) primed with 50 pmol of SCYLV-613R by following the manufacturer's protocol. The PCR reaction was performed in a total volume of 25 µl containing 2 µl cDNA, 2.5 µl of 10×PCR buffer containing 15 mM $MgCl_2$, 0.5 µl of 10 mM dNTP mix, 10 pmol each of forward and reverse primers (SCYLV-613F & SCYLV- 613R), 1.25 units of Taq polymerase (Bangalore Genei, Bangalore), and sterile miliQ water to a final volume. 30 PCR cycles were performed in a PCR thermocycler (Mastercycler gradient; Eppendorf, Germany) with the conditions: initial denaturation at 94 °C for 4 min, denaturationat 94°C for 1 min, primer annealing at 65 °C for 1 min, extension at 72°C for 45 sec with a final 72 °C extension for 10 min. A 10 µl aliquot of each amplified product was analyzed by electrophoresis on 1.5% agarose gels stained with eithidium bromide.

2.2 Sequencing of PCR products, sequence analysis and phylogenetic relationship

cDNA from 12 positive samples were amplified with SCYLV-613F and SCYLV-613R by RT-PCR. The amplicon of 613 bp from 12 samples were eluted using GenElute Gel Extraction Kit (Sigma, USA). The nuleotide sequence of each virus isolate was sequenced with virus specific primers for ORF3 &ORF4 regions. The nucleotide sequence data of 12 SCYLV isolates were analyzed using Bioedit and MEGA 7.0 (Tamura *et al.* 2007) [24] to study the sequence identities/similarities with the other SCYLV isolates available in GenBank database including partial sequence data of ORF3 (CP) and ORF4 (MP) of representative genotypes (BRA from Brazil, CUB from Cuba, PER from Peru, and REU from Reunion Island) as defined earlier (Abu Ahmad *et al.* 2006a, b) [1]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) were shown next to the branches and phylogenetic tree was generated with Neighbour-Joining method.

Table 2: List of SCYLV isolates used for sequence similarity studies.

S. No	Origin	Genbank Accession no.	Genome portion
1	USA	MN097766	Complete genome
2	Columbia	MF622078	Complete genome
3	Mauritius	MF426270	Complete genome
4	Re-Union	KY052166	Complete genome
5	India-Lucknow	KF680098	Complete genome
6	Kenya	KR608791	Capsid protein gene
7	India-Tamil Nadu	JF925152	Complete Protein
8	India-Tamil Nadu	EU647735	Putative aphid transmission factor mRNA, partial cds; and capsid protein and movement protein mRNAs, complete cds.
9	India-Kerala	FJ430661	Aphid transmission factor gene, partial cds; and coat protein and movement protein genes, complete cds.
10	India-Tamil Nadu	FJ430663	Aphid transmission factor gene, partial cds; and coat protein and movement protein genes, complete cds.
11	India-Hyderabad	FJ430665	Aphid transmission factor gene, partial cds; and coat protein and movement protein genes, complete cds.
12	India-Pun	FJ430667	Aphid transmission factor gene, partial cds; and coat protein and movement protein genes, complete cds.
13	India-Uttar Pradesh	FJ430669	Aphid transmission factor gene, partial cds; and coat protein and movement protein genes, complete cds.
14	India-Madhya Pradesh	FJ430672	Aphid transmission factor gene, partial cds; and coat protein and movement protein genes, complete cds.
15	India- Coimbatore	EU647740	Truncated capsid protein/putative aphid transmission factor and truncated movement protein mRNAs, complete cds.
16	India-TN	EU089689	Read-through protein mRNA, partial cds; and coat protein and movement protein mRNAs, complete cds
17	India- AP	KX260957	Capsid protein gene, complete cds
18	India- Coimbatore	KF680098	Complete genome
19	India-Andhra Pradesh	JX287509	Coat protein and putative 17 kDa movement protein genes, complete cds
20	India- Coimbatore	EU6477439	Capsid protein/putative aphid transmission factor and truncated movement protein mRNAs, complete cds.
21	Cuba-CUB2	AJ582770	Capsid protein (partial) and gene for movement protein (partial), genomic RNA, isolate CUB2.
22	China-CHN-GX2	GU190162	Multifunctional protein and RNA-dependent RNA polymerase genes, partial cds; capsid protein and movement protein genes, complete cds; and putative aphid transmission factor gene, partial cds.
23	Peru-PER1	AJ582767	Capsid protein (partial) and gene for movement protein (partial),
24	China- CHN-YL1	AM072751	Complete genome
25	China- CHN-YN-ML1	HQ245346	Coat protein and movement protein mRNA,complete cds;putative aphid transmission factor mRNA,partial cds.
26	Hawaii-Haw87-4094	GU570006	Complete genome
27	Brazil-BRA1	AJ582772	Capsid protein (partial) and gene for movement protein (partial)
28	Re-Unio-REU-YL2	AM072756	Complete genome

3.1. Results

The typical symptoms of the disease observed were intense yellowing of midribs on the abaxial surface, lateral spread of yellow discoloration to the leaf lamina followed by tissue necrosis from the leaf tip spreading downwards along the midrib and a bushy appearance of the top of the plant due to internode shortening in maturing plants ((Fig.1a & 1b). In some sugarcane cultivars, leaves show a pinkish discoloration of the midrib on the adaxial surface. The disease incidence was significantly high (60–70%) at later stage (6–7 months of age) of the crop.

Fig 1A, B: A. Typical symptoms of midrib yellowing in Sugarcane plant infected with Sugarcane yellow leaf disease. B. Inter nodal shortening of the plant.

3.2. Detection of SCYLV by RT-PCR

The expected size (613 bp) fragment was successfully amplified with SCYLV-613F and SCYLV-613R primers by RT-PCR in 12 symptomatic samples collected from major sugarcane growing regions of Andhra Pradesh ie.,CO 86032, 93A145, 2003V46, 83V15, 87A298, 2005T16, 2009A240, 2009V89, CO T 8201, 92A30, CoC 671 (Fig.2).

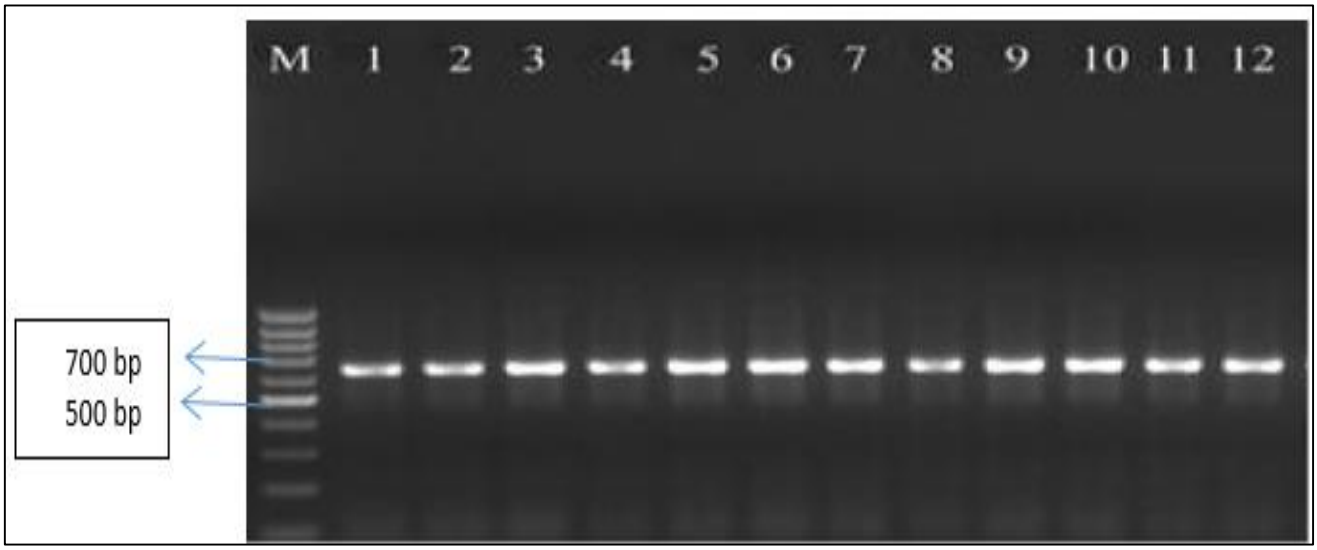

Fig 2: Amplification of ORF3&4 regions of 12 isolates of SCYLV using specific primers SCYLV-613F and SCYLV-613R.

3.3. Sequence and Phylogenetic analysis

The SCYLV amplicons of 613 bp with prominent intensity obtained were sequenced and the consensus sequence data obtained from two ends of the nucleotide sequence of each isolate were deposited in GenBank database. The analysis of the sequence data of each isolate revealed presence of nucleotides varying from 480 bp to 590 bp containing partial ORF 5 (aphid transmission protein) of SCYLV and two overlapping ORFs: ORF 3 (coat protein) and ORF 4 (movement protein). The sequence data of 12 isolates under study was deposited in GenBank database under accession numbers: MN814252, MN850408, MN922287, MN962641, MT060295, MT124724, MT882328, MT882329, MT913601, MT929272, MW118673, MW309845. All the newly identified SCYLV isolates were designated as IND isolates. The multiple sequence analysis of the isolates under the study and similar sequences of selected SYLCV isolates reported from India and abroad revealed 94.0 –100% identities (with REU, BRA, CHN, HAW, CUB, COL and PER genotypes) reported from other countries. The nucleotides (nt) and their deduced amino acid (aa) sequences of ORF 3& 4 of these isolates were also compared with similar sequences of SCYLV isolates available in GenBank database. The isolates shared 95.4–100% nucleotide identities among them and with other isolates SCYLV reported from India (Table 2). The 100% nucleotide identity was obtained between SCYLV-PPL3 (MN929272) and SCYLV- PTR (MN922287) and SSCYLV-PPL2 (MT913601); SCYLV-ANK2 (MT882328) and SCYLV-VYV (MW118673) isolates of Andhra Pradesh. The nucleotide identities were 97-99% with SCYLV-Cuba isolate (AJ582770) and SCYLV-COL isolate (MF622078) and 94–97% with SCYLV isolates (REU, BRA and PER genotypes) from other parts of the world.

The twelve Indian SCYLV isolates from this study were found to be very similar to each other and closely related to SCYLV-CUB (AJ582770) and SCYLV-COL isolate (MF622078) showing less than 3.6% sequence variation at nucleotide and amino acid levels) (Table.3). The isolates, SCYLV-YPD and SCYLV-ANK3 shared 5% variation at nucleotide level when compared with other 12 isolates under the study. The SCYLV isolates *viz*., SCYLV-ANK3 and CHN genotype showed variability up to 5.0% variation in nucleotide sequence and 10.0% in aminoacid sequence with Indian isolates under the study.

Phylogenetic analysis revealed that the SCYLV isolates under the study clustered into two major groups. SCYLV isolates from India and from Colombia and Cuba clustered into one group and all other isolates from the other countries ie., China, Brazil, Peru, Reunion, Kenya, Hawaii, USA formed into a separate cluster. The 12 SCYLV isolates under study

showed close relationship with Cuba isolate (AJ582770) and Colombia (MF622078) isolate along with other 15 isolates from India. The 11 SCYLV isolates from other parts of the world formed into a separate cluster with independent clades: HAW, USA, MARITIUS, REU, KENYA, CHN, BRA, REU and PER. The SCYLV isolates under the study showed close relationship among them *viz*., (MN922287, MT929272), (MT882328, MW118673) while the isolate SCYLV-PPL2 (MN913601) from Perumallapalle showed close relationship with the Madyapradesh isolate (FJ430672).

The 12 isolates under study, 15 other Indian isolates, the Cuban (CUB) and Colombia (COL) isolates formed into one cluster while the isolates from other parts of the world formed another cluster which included CHN, HAW, PER, REU and BRA genotypes. The CUB isolate and Colombia isolate showed close homology in nucleotide sequence up to 99.4% forming into separate clade from Indian isolates.

4. Discussion

In the present study, the SCYLV was detected in all most all the commercial sugarcane varieties grown by the farmers in Andhra Pradesh. The typical symptoms of the disease was observed in all the twelve varieties. Similar symptoms of sugarcane yellow leaf disease was observed by Bharathi and Reddy (2007) [4] in sugarcane varieties Co 6907 and Co C 671 in Northern telangana zone of Andhra Pradesh. The symptoms were evident in mature leaves especially during the month of September. The incidence of disease varied from 3 to 36 per cent in different cultivars with maximum incidence in Co 6907 and Co C 671. Singh (2018) surveyed for YLD in sugarcane fields of Uttar Pradesh and revealed that the incidence of Yellow leaf disease was up to 30% and mostly appeared during 6-8 months age of the crop.

Successful PCR detection indicated that the SCYLV was wide spread among the most popular varieties grown in major sugarcane growing regions throughout India. Similar reports are available on the widespread occurrence of SCYLV in other parts of the world (Arocha *et al.* 2005; Fitch *et al*. 2001; Rott *et al*. 2008). The nucleotide sequence data presented here are in consistent with previous reports which include SCYLV, the causative agent of the yellow leaf in Brazil, USA, Australia, and Mauritius as a possible member Polerovirus of the family Luteoviridae (Rott *et al.* 2008; Scagliusi and Lockhart 2000) [17]. The sequence comparisons reported in this study contribute to a better understanding of the taxonomic status of SCYLV isolates throughout the world.

In the study, SCYLV was characterized based on the partial sequence data of ORF3 (CP) and ORF4 (MP) of an additional 15 isolates collected from different parts of India. A high level of homology was observed within the Indian isolates sequenced within this study and with most of the Indian isolates sequenced in prior studies. These Indian isolates showed close relationship with CUB isolate from Cuba and COL isolate from Colombia. These SCYLV isolates from India, Cuba and Colombia are genetically distinct from isolates sequenced from other regions of the world. Viswanathan *et al*. (2008) [26] studied the phylogentic analysis of SCYLV isolates based on partial ORF 1,2 and complete ORF 3 and 4 sequences of the virus genome. The sequence analysis suggested that the population that exists in India was significantly different from rest of the world. It was revealed from his study that CUB genotype was predominant among four genotypes (BRA, CUB, IND and PER) found in India.

It is remarkable to note that the isolates of India clustered with isolates of Cuba and Colombia and shared maximum sequence similarity upto 99.2%. Similarly, Moonan and Mirkov (2002) [18] revealed from their study that SCYLV was widespread in most of the sugarcane producing countries. The virus isolates collected from North, South and Central America were divided into two groups: one group contained only isolates from Colombia (C-population) and the other group contained the isolates from Argentina, Brazil, Guatemala, USA, Florida, Louisiana and Texas.

The SCYLV isolates from Cuba and Colombia showed closest homology with the indian isolates. These findings suggest that the mixed populations of SCYLV isolates that exist across India, may be due to the movement of the SCYLV isolates in the country through infected propagative material. In a study that included 18 geographical locations worldwide, the BRA-PER genotype occurred in most sugarcane producing areas wherever as genotypes CUB and REU were found in four geographical locations only. Afterwards, several isolates of SCYLV were detected in Brazil, Colombia, Guadeloupe, Mauritius and Reunion Island, suggesting different virus introductions and/or different evolution histories of the virus after its introduction into a new environment (Abu Ahmad *et al. 2*006 b). On the basis of SCYLV genotypes identified in Brazil, Colombia, Cuba and Peru or elsewhere, Abu Ahmad *et al.* (2006a, b) [1] suggested that they may have been introduced through infected planting material imported from elsewhere.

The phylogenetic analyses of the entire genome of SCYLV described by Abu Ahmad *et al.* (2006a) [1] revealed the occurrence of three genotypes of SCYLV (BRA, PER and REU) based on the geographical location where it was first detected; Brazil, Peru and Reunion, respectively. Additionally, a virus isolate from Cuba (that was partially sequenced) showed only 77–80% amino acid sequence identities in ORF1 with isolates of genotypes BRA, PER and REU, which suggest that the Cuban isolate represent a new genotype (CUB).

Seven different genotypic groups of SCYLV were described based on phylogenetic analyses of partial or complete genome sequences ie., BRA (Brazil), CUB (Cuba), PER (Peru), REU (Reunion Island), IND (India), CHN1 (China) and HAW (Hawaii). Based on sequence analysis of different isolates of SCYLV reported from all around the world, sequence identity varied from 70.5 to 98.5% among isolates of genotypes BRA, REU, PER, CUB, HAW,CHN1 and and IND isolates. The amino acid sequence similarity varied from 60.6-99.0% among the reported isolates. The phylogram studies made by using the sequences of different ORF regions of the virus with the reported sequences revealed that the isolates of India are much similar to the CHN1 genotype sharing 95.2–95.6% similarities followed by 86.3–86.6% with REU and 86.1–86.7% with BRA genotypes (Chinnaraja *et al*., 2013) [6].

Singh *et al.*, (2011) characterized SCYLV based on sequence data of partial ORF5 and complete ORF3 (CP) and ORF4 (MP) of 13 isolates collected from nine locations from India. The results revealed that a high level of homology was observed within the Indian isolates and the CUB isolate from Cuba. The nucleotide similarity analysis of the 13 isolates under the study revealed 96–100% nucleotide identities among the Indian isolates and also with SCYLV-Cuban isolate. The nucleotide identities were 90–94% with other isolates of SCYLV reported from other countries.

Table 3: Percent identities of SCYLV isolates for ORF3&4 regions under studies with other reported SCYLV isolates at nucleotide level

SCYLV Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
MN097766-USA	100																																							
MF622078-COLM	96.9	100																																						
MF426270-MARITIUOS	100	96.9	100																																				
KY052166-REU	100	96.9	100	100																																			
KF680098-IND-LUNKW	97.2	99.2	97.2	97.2	100																																		
KR608791-KENYA	100	96.9	100	100	97.0	100																																	
JF925152-IND	97.4	98.9	97.4	97.4	99.0	97.0	100																																
EU647735-IND-TN	97.2	98.7	97.2	97.2	99.0	97.0	99.0	100																															
FJ430661-KER-IND	96.9	98.9	96.9	96.9	100	97.0	99.0	99.0	100																														
FJ430663 TN-IND-1	96.9	98.9	96.9	96.9	100	97.0	99.0	99.0	100	100																													
FJ430665 Hy-IND-1	96.7	98.7	96.7	96.7	99.0	97.0	99.0	98.0	99.0	99.2	100																												
FJ430667-Pun-IND-1	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100																											
FJ430669-UP-IND-1	96.9	98.9	96.9	96.9	100	97.0	99.0	99.0	99.0	99.4	99.2	99.7	100																										
FJ430672 MP-IND	96.7	98.7	96.7	96.7	99.0	97.0	99.0	98.0	99.0	99.2	98.9	99.4	99.2	100																									
MN814252 IND	96.7	98.7	96.7	96.7	99.0	97.0	99.0	98.0	99.0	99.2	98.9	99.4	99.2	98.9	100																								
MN850408 IND	95.4	96.4	95.4	95.4	97.0	95.0	97.0	97.0	97.0	96.9	96.7	97.2	96.9	96.7	96.9	100																							
MN962641 IND	96.4	98.4	96.4	96.4	99.0	96.0	98.0	98.0	99.0	98.9	98.7	99.2	98.9	98.7	99.7	96.7	100																						
MN922287 IND	95.9	97.9	95.9	95.9	99.0	96.0	98.0	98.0	98.0	98.4	98.2	98.7	98.4	98.2	98.2	96.2	98.0	100																					
MT929272 IND	96.2	98.2	96.2	96.2	99.0	96.0	98.0	98.0	99.0	98.7	98.4	98.9	98.7	98.4	98.4	96.4	98.0	99.7	100																				
MT124724 IND	96.9	98.9	96.9	96.9	100	97.0	99.0	99.0	99.0	99.4	99.2	99.7	99.4	99.2	99.2	96.9	99.0	98.4	98.7	100																			
MT060295 IND	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100	99.7	99.4	99.4	97.2	99.0	98.7	98.9	100	100																	
MT913601 IND	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100	99.7	99.4	99.4	97.2	99.0	98.7	98.9	100	100	100																
MT882328 IND	96.7	98.7	96.7	96.7	99.0	97.0	99.0	98.0	99.0	99.2	98.9	99.4	99.2	98.9	98.9	96.7	99.0	98.2	98.4	99.0	99.0	99.0	100															
MT882329 IND	97.7	99.2	97.7	97.7	99.0	98.0	100	99.0	99.0	99.2	98.9	99.4	99.2	98.9	98.9	97.2	99.0	98.2	98.4	99.0	99.0	99.0	99.0	100														
MW118673IND	96.7	98.7	96.7	96.7	99.0	97.0	99.0	98.0	99.0	99.2	98.9	99.4	99.2	98.9	98.9	96.7	99.0	98.2	98.4	99.0	99.0	99.0	100	99.0	100													
MW309845 IND	95.4	97.4	95.4	95.4	98.0	95.0	97.0	97.0	98.0	97.9	97.7	98.2	97.9	99.7	97.7	95.4	97.0	97.0	97.2	98.0	98.0	98.0	98.0	98.0	98.0	100												
EU64773 IND	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100	99.7	99.4	99.4	97.2	99.0	98.7	98.9	100	100	100	99.0	99.0	99.0	98.2	100											
EU089689 IND	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100	99.7	99.4	99.4	97.2	99.0	98.7	98.9	100	100	100	99.0	99.0	99.0	98.2	100	100										
KX260957 IND	96.2	97.7	96.2	96.2	98.0	96.0	98.0	98.0	98.0	98.2	97.9	98.4	98.2	97.9	97.9	96.2	98.0	97.2	97.4	98.0	98.0	98.0	98.0	98.0	98.0	96.7	100	98.0	100									
KF680098	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100	99.7	99.4	99.4	97.2	99.0	98.7	98.9	100	100	100	99.0	99.0	99.0	98.2	100	100	100	100								
JX287509	97.4	98.9	97.4	97.4	100	97.0	99.0	99.0	99.0	99.4	99.2	99.7	99.4	99.2	99.2	97.4	99.0	98.4	98.7	99.0	100	100	99.0	100	99.0	97.9	100	100	100	100	100							
AJ582770-CUBA	96.9	99.4	96.9	96.9	99.0	97.0	99.0	99.0	99.0	98.9	98.7	99.2	98.9	98.7	98.7	96.4	98.0	97.9	98.2	99.0	99.0	99.0	99.0	99.0	99.0	97.4	100	99.0	100	99.0	100	100						
EU647740-IND	97.2	99.2	97.2	97.2	100	97.0	99.0	99.0	100	99.7	99.4	100	99.7	99.4	99.4	97.2	99.0	98.7	98.9	100	100	100	99.0	99.0	99.0	98.2	100	100	100	100	100	99.2	100						
GU190162-CHN	100	96.9	100	100	97.0	100	97.0	97.0	97.0	96.9	96.7	97.2	96.9	96.7	96.7	95.4	96.0	95.9	96.2	97.0	97.0	97.0	97.0	98.0	97.0	95.4	100	97.0	100	97.0	100	96.9	97.0	100					
AJ582767-PER1	99.7	96.7	99.7	99.7	97.0	100	97.0	97.0	97.0	96.7	96.4	96.9	96.7	96.4	96.4	95.2	96.0	95.7	95.9	97.0	97.0	97.0	96.0	97.0	96.0	95.2	100	97.0	100	97.0	100	96.7	97.0	99.7	100				
AM072751-CHN-YL1	99.2	96.2	99.2	99.2	96.0	99.0	97.0	96.0	96.0	96.2	95.9	96.4	96.2	95.9	95.9	94.7	96.0	95.2	95.4	96.0	96.0	96.0	96.0	97.0	96.0	94.7	100	96.0	100	96.0	100	96.2	96.0	99.2	98.9	100			
HQ245346-CHN	99.7	96.7	99.7	99.7	97.0	100	97.0	97.0	97.0	96.7	96.4	96.9	96.7	96.4	96.4	95.2	96.0	95.7	95.9	97.0	97.0	97.0	96.0	97.0	96.0	95.2	100	97.0	100	97.0	100	96.7	97.0	99.7	99.4	99.0	100		
GU570006 Haw	99.7	97.2	99.7	99.7	97.0	100	97.0	97.0	97.0	97.2	96.9	97.4	97.2	96.9	96.9	95.2	97.0	96.2	96.4	97.0	97.0	97.0	97.0	97.0	97.0	95.7	100	97.0	100	97.0	100	97.2	97.0	99.7	99.4	99.0	99.0	100	
AJ582772- BRA1	100	96.9	100	100	97.0	100	97.0	97.0	97.0	96.9	96.7	97.2	96.9	96.7	96.7	95.4	96.0	95.9	96.2	97.0	97.0	97.0	97.0	98.0	97.0	95.4	100	97.0	100	97.0	100	96.9	97.0	100	99.7	99.0	100	100	100
AM072756- REU-YL2	100	96.9	100	100	97	100	97.0	97.0	97.0	96.9	96.7	97.2	96.9	96.7	96.7	95.4	96.0	95.9	96.2	97.0	97.0	97.0	97.0	98.0	97.0	95.4	100	97.0	100	97.0	100	96.9	97.0	100	99.7	99.0	100	100	100	100

Table 4: Percent identities of SCYLV isolates for ORF 3 &4 regions under studies with other reported SCYLV isolates at aminoacid level

SCYLV Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
MN097766-USA	100																																							
MF622078-COLM	96.1	100																																						
MF426270-MARITIUOS	100	96.1	100																																				
KY052166-REU	100	96.1	96.1	100																																			
KF680098-IND-LUNKW	96.1	98.4	96.1	96.1	100																																		
KR608791-KENYA	100	96.1	100	100	96.1	100																																	
JF925152-IND	96.1	98.4	96.1	96.1	98.4	96.1	100																																
EU647735-IND-TN	96.1	98.4	96.1	96.1	98.4	96.1	98.4	100																															
FJ430661-KER-IND	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	100																														
FJ430663 TN-IND-1	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	100	100																													
FJ430665 Hy-IND-1	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100																												
FJ430667-Pun-IND-1	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100																											
FJ430669-UP-IND-1	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	98.4	98.4	99.2	99.2	100																										
FJ430672 MP-IND	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	98.4	98.4	99.1	99.2	99.2	100																									
MN814252 IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100																								
MN850408 IND	93.1	95.4	93.1	93.1	96.9	93.1	95.4	95.4	96.1	96.1	96.9	96.9	96.1	96.1	96.9	100																							
MN962641 IND	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	98.4	98.4	99.2	99.2	98.4	98.4	99.2	96.1	100																						
MN922287 IND	92.3	94.6	92.3	92.3	96.1	92.3	94.6	94.6	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.8	95.4	100																					
MT929272 IND	93.1	95.4	93.1	93.1	96.9	93.1	95.4	95.4	96.1	96.1	96.9	96.9	96.1	96.1	96.9	94.6	96.1	99.2	100																				
MT124724 IND	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	98.4	98.4	99.2	99.2	98.4	98.4	99.2	96.1	98.4	95.4	96.1	100																			
MT060295 IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	99.2	99.2	96.1	96.9	99.2	100																		
MT913601 IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	96.9	99.2	96.1	96.9	99.2	100	100																	
MT882328 IND	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	98.4	98.4	99.2	99.2	98.4	98.4	99.2	96.1	98.4	95.4	96.1	98.4	99.2	99.2	100																
MT882329 IND	96.9	99.2	96.9	96.9	99.2	96.9	99.2	99.2	98.4	98.4	99.2	99.2	98.4	98.4	99.2	96.1	98.4	95.4	96.1	98.4	99.2	99.2	98.4	100															
MW118673IND	95.4	97.7	95.4	95.4	99.2	95.4	97.7	97.7	98.4	98.4	99.2	99.2	98.4	98.4	99.2	96.1	98.4	95.4	96.1	98.4	99.2	99.2	100	98.4	100														
MW309845IND	92.3	94.6	92.3	92.3	96.1	92.3	94.6	94.6	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.1	95.4	92.3	93.1	95.4	96.1	96.1	95.4	95.4	95.4	100													
EU647740-IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	96.9	99.2	96.1	96.9	99.2	100	100	99.2	99.2	99.2	96.1	100												
EU089689-IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	96.9	99.2	96.1	96.9	99.2	100	100	99.2	99.2	99.2	96.1	100	100											
KX260957 IND	93.1	95.4	93.1	93.1	96.9	93.1	95.4	95.4	96.1	96.1	96.9	96.9	96.1	96.1	96.9	93.8	96.1	93.1	93.8	96.9	96.9	96.9	96.1	96.1	96.1	93.1	96.9	96.9	100										
KF680098 IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	96.9	99.2	96.1	96.9	99.2	100	100	99.2	99.2	99.2	96.1	100	100	96.9	100									
JX287509 IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	96.9	99.2	96.1	96.9	99.2	100	100	99.2	99.2	99.2	96.1	100	100	96.9	100	100								
AJ582770-CUBA	96.1	98.4	96.1	96.1	98.4	96.1	98.4	98.4	97.7	97.7	98.4	98.4	97.7	97.7	98.4	95.4	97.7	94.6	95.4	97.7	98.4	98.4	97.7	99.2	97.7	94.6	98.4	98.4	95.4	98.4	98.4	100							
EU647740-IND	96.1	98.4	96.1	96.1	100	96.1	98.4	98.4	99.2	99.2	100	100	99.2	99.2	100	96.9	99.2	96.1	96.9	99.1	100	100	99.2	99.2	99.2	96.1	100	100	96.9	100	100	98.4	100						
GU190162-CHN	100	96.1	100	100	96.1	100	96.1	96.1	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.1	95.4	92.3	93.1	95.4	96.1	96.1	95.4	96.9	95.4	92.3	96.1	96.1	93.1	96.1	96.1	96.1	96.1	100					
AJ582767-PER1	100	96.1	100	100	96.1	100	96.1	96.1	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.1	95.4	92.3	93.1	95.4	96.1	96.1	95.4	96.9	95.4	92.3	96.1	96.1	93.1	96.1	96.1	96.1	96.1	100	100				
AM072751-CHN-YL1	97.7	93.8	97.7	97.7	93.8	97.7	93.8	93.8	93.1	93.1	93.8	93.8	93.1	93.1	93.8	93.8	90.8	93.1	9.0	90.8	93.1	93.8	93.8	93.1	94.6	93.1	9.0	93.8	90.8	93.8	93.8	93.8	93.8	97.7	97.7	100			
HQ245346-CHN	99.2	95.4	99.2	99.2	95.4	95.4	99.2	95.4	95.4	94.6	94.6	95.4	95.4	94.6	94.6	95.4	92.3	94.6	91.6	92.3	94.6	95.4	95.4	94.6	96.1	94.6	91.6	95.4	95.4	92.3	95.4	95.4	95.4	99.2	99.2	96.9	100		
GU570006 Haw	100	96.1	100	100	96.1	100	96.1	96.1	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.1	95.4	92.3	93.1	96.4	96.1	96.1	95.4	96.9	95.4	92.3	96.1	96.1	93.1	96.1	96.1	96.1	96.1	100	100	97.7	99.2	100	
AJ582772- BRA1	100	96.1	100	100	96.1	100	96.1	96.1	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.1	95.4	92.3	93.1	96.4	96.1	96.1	95.4	96.9	95.4	92.3	96.1	96.1	93.1	96.1	96.1	96.1	96.1	100	100	97.7	99.2	100	100
AM072756- REU-YL2	100	96.1	100	100	96.1	100	96.1	96.1	95.4	95.4	96.1	96.1	95.4	95.4	96.1	93.1	95.4	92.3	93.1	96.4	96.1	96.1	95.4	96.9	95.4	92.3	96.1	96.1	93.1	96.1	96.1	96.1	96.1	100	100	97.7	99.2	100	100	100

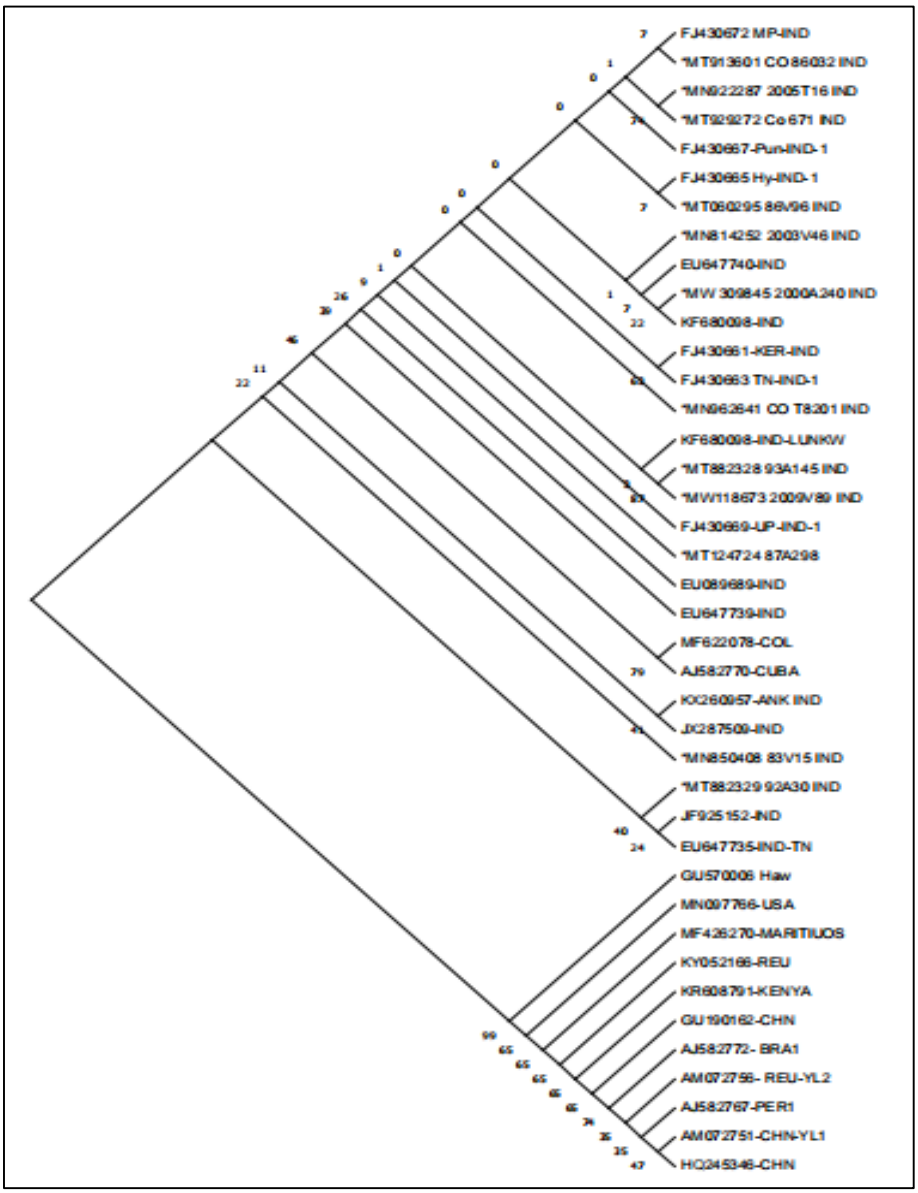

Fig 3: Phylogenetic relationships of SCYLV isolates under study with other SCYLV isolates reported from India and abroad at nucleotide sequence level as analyzed in Mega 7.0 using the neighbor joining method with the maximum composite likelihood model. The bootstrap values at the nodes indicate scores calculated in 500 replicates.

5. Conclusion

It is evident from the study that Sugarcane yellow leaf disease is threatening the sugarcane cultivation affecting almost all the varieties grown in India and abroad. The phylogenetic analysis revealed that the SCYLV isolates reported from India shared maximum nucleotide and amino acid similarity with the SCYLV-Cuban and Colombia isolates. The diversity among the SCYLV isolates used in the study showed a very less variation between them while the variability was greater with the BRA, CHN, REU, PER, HAW isolates.

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