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Studies on Distribution of Sugarcane Yellow Leaf Virus in Commercially Grown Sugarcane Varieties in Andhra Pradesh

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ABSTRACT

Background: Among the diseases of sugarcane, Sugarcane yellow leaf disease (YLD) is one of the most prevalent diseases of sugarcane posing serious threat to the sugarcane cultivation worldwide. The current study aims at studying the distribution of SCYLV in the major sugarcane-growing of Andhra Pradesh and determining sequence identities, sequence variations and phylogenetic relationships between the SCYLV isolates reported earlier from India and worldwide.

Methods: ORF1 and ORF2 regions of the viral genome coding for RNA dependent RNA Polymerase (RdRP) was amplified from twelve of the samples using the gene specific primers *viz*., SCYLV-F3 and SCYLV-R3. The ORF1 and ORF2 regions were studied for sequence similarity at nucleotide and aminoacid level and phylogentic relationships were established with the other SCYLV isolates.

Result: The multiple sequence analysis of the isolates under the study with 22 similar sequences of SYLCV isolates reported from India and worldwide revealed 90.6-100% identities with IND, REU, BRA, CHN, HAW, CUB, COL and PER genotypes reported worldwide. The Indian isolates showed close phylogenetic 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.

Key words: Molecular Characterization, Phylogenetic analysis, RdRp protein, Sugarcane yellow leaf virus.

INTRODUCTION

Sugarcane (*Saccharium officinarum*) is an important commercial crop of the world belonging to C4 family and is principal sources of sugar, ethanol and jaggery globally (Gulati *et al.* 2015; Misbah *et al.* 2017). India ranks second in sugarcane production after Brazil and first in sugar production. Sugarcane crop is attacked by more than 200 diseases caused by fungi, bacteria, viruses, phytoplasma and nematodes resulting in severe yield losses worldwide. Among the diseases of sugarcane, Sugarcane yellow leaf disease (YLD) is one of the most prevalent diseases of sugarcane worldwide. *Sugarcane yellow leaf virus* (SCYLV) is a distinct member of the *Polerovirus* genus of the *Luteoviridae* family. SCYLV is the major limitation to sugarcane production worldwide and presently posing a major threat to sugarcane cultivation. The disease was first reported in Hawaii in the late 1988 and was subsequently observed in almost all sugarcane growing countries (Comstock *et al*., 2002). In India, it was reported during 1999 (Viswanathan, 2002). Later, 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), India (Viswanathan 2002), Island of Reunion (Rassaby *et al*., 2004) and in Thailand (Lehrer *et al*., 2008).

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 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

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three untranslated regions (UTRs) consisting of ~5.8 kb nucleotides. In Andhra Pradesh, sugarcane is grown in an area of 1.26 lakh hectares with average production of 76.14t/ha {Source: Cooperative Sugar Vol 51(6) Feb, 2020)}. The disease was first recorded in Nizamabad area during 2004 (Bharathi and Kishan Reddy, 2007). The wide prevalence of the disease was observed during 2008-09 season in various sugarcane varieties used in All India Coordinated trails at Agricultural Research Station, Perumallapalle, Tirupati, (Andhra Pradesh). Later, the disease was noticed on other commercially grown varieties *viz*., 2003 V46, 86 V 96, Co 7219, 87 A 298, Co 86032 in Coastal and Southern regions of Andhra Pradesh. 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 (Hemalatha *et al.,* 2014). The per cent disease incidence up to 30.6% in plant crop and 51.0% in ratoon crops was recorded in most of the cultivated varieties in Andhra Pradesh (Suresh *et al*., 2020; Hemalatha *et al*., 2021). At present scenario, the disease has attained epidemic status in the country and the situation warranted management approaches to sustain sugarcane productivity. Hence establishing disease-free healthy nurseries is suggested to the sugar industries to manage the disease and the strategy has found success in different states. An assay for viruses plays an important role to prevent the disease during germplasm exchange and seed cane production. Many methods to overcome viral infection have become ineffective. Since last two decades, production of disease free seedlings through tissue culture techniques is playing a significant role in solving the problems of viral infection in plants. This technique is applied successfully to a wide range of agricultural crops and horticultural plants to eliminate the viral infection (Rupinder and Manish, 2017; Misbah *et al*., 2017). 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 (Elsayed *et al*., 2010; Gao *et al*., 2012, Chinnaraja *et al.,* 2013; Lin *et al.,* 2014). 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.

MATERIALS AND METHODS

During 2017-18, 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 districts). The collected leaf samples were stored at -20°C until further processing. The details of the SCYLV isolates used in the study are given in Table 1.

RNA extraction and RT-PCR

RNA from the infected leaf samples were isolated using TRI reagent (Sigma, USA) as per the method described by Singh *et al.,* (2011). The quality of the RNA was checked by Nanospectrophotometer. The forward primer SCYLV- F3: 5′ -GCAGCAGAACGGAGGGAAGAAGTC-3′ and reverse primer SCYLVR3: 5′-TGAGTTTGGGCGTACAR GACACCG CC-3′ designed by Viswanathan *et al*. (2008) were used to amplify ~1110 bp of ORF1 and ORF2 regions of SCYLV genome. The cDNA was synthesized from total RNA of all the 25 samples separately using Revert Aid H Minus first stand cDNA synthesis kit (MBI Fermentas, USA) as per manufactures protocol.

Sequence analysis and phylogenetic relationship

cDNA from 12 positive samples were amplified with SCYLV-F3 and SCYLV-R3 by RT-PCR. The amplicon of 1110 bp from 12 positive samples were eluted using GenElute Gel Extraction Kit (Sigma, USA). The nuleotide sequence of each virus isolate was sequenced with virus specific primers for ORF1 and ORF2 regions. The nucleotide sequence data of 12 SCYLV isolates were analyzed using Bioedit and MEGA 7.0 (Kumar *et al.,* 2016) to study the sequence identities/similarities with the other 22 SCYLV isolates available in GenBank database (Table 2) including partial sequence data of RdRp of representative genotypes (BRA from Brazil, CUB from Cuba, PER from Peru and REU from Reunion Island). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches and phylogenetic tree was generated with Neighbour-Joining method.

RESULTS AND DISCUSSION

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 1). In some sugarcane cultivars, leaves show a pinkish discoloration of the midrib on the adaxial surface (Fig 2). The disease incidence was significantly high (60-70%) at later stage (6-7 months of age) of the crop.

Detection of SCYLV by RT-PCR

The expected size (1110 bp) fragment was successfully amplified with SCYLV-F3 and SCYLV-R3 primers by RT-PCR in 12 symptomatic samples collected from major sugarcane growing regions of Andhra Pradesh (Fig 3). Successful PCR detection of RdRp gene 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 (Holkar *et al*., 2020).

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

SCYLV isolate	Location	District	Variety	Accession number
SCYLV-SKHT1	Srikalahasti	Chittoor	2003V46	MN865128
SCYLV-YPD	Yerpedu	Chittoor	83V15	MN909725
SCYLV-PTR	Puttur	Chittoor	2005T16	MN900951
SCYLV-YPD1	Yerpedu	Chittoor	CoT 8201	MT060294
SCYLV-SKHT2	Srikalahasti	Chittoor	86V96	MN972615
SCYLV-ANK1	Anakapalle	Vishakapatnam	87A298	MT074088
SCYLV-ANK2	Anakapalle	Vishakapatnam	93A145	MN857470
SCYLV-PPL1	Perumallapalle	Chittoor	92A30	MN909726
SCYLV-PPL2	Perumallapalle	Chittoor	Co 86032	MN865129
SCYLV-PPL3	Perumallapalle	Chittoor	CoC671	MN865130
SCYLV-ANK3	Anakapalle	Vishakapatnam	2000A240	MT992722
SCYLV-VYV	Vuyyur	Krishna	2009V89	MW032686

Sequencing and phylogenetic analysis

The amplified fragments of 1110 bp with prominent intensity obtained from infected leaf samples were sequenced. The BLAST analysis of partial RdRp gene sequences obtained from 12 different cultivars showing >90% sequence identity with SCYLV genomes from NCBI database were deposited in GenBank. The sequence data of 12 isolates under study was deposited in GenBank database with accession numbers mentioned in Table 1.

The multiple sequence analysis of the isolates under the study with 22 similar sequences of SYLCV isolates reported from India and abroad revealed 90.6-100% identities with IND, REU, BRA, CHN, HAW, CUB, COL and PER genotypes reported from other countries. Comparisions of the nucleotides (nt) and their deduced amino acid (aa) sequences of ORF 1 and 2 of the isolates under the study with similar sequences of SCYLV isolates available in GenBank database revealed 95.7-100% nucleotide identities among them and with other isolates SCYLV reported from India (Table 2). All the Indian isolates shared maximum identity with SCYLV-CUB isolate (MF622079) and SCYLV-COL isolate (MF622078) with 96.6-99.0 % while they shared 90.6-92.2% nucleotide identity with SCYLV isolates (REU, BRA and PER genotypes) from other parts of the world. 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). The sequence comparisons reported in this study contribute to a better understanding of the taxonomic status of SCYLV isolates throughout the world.

Comparision of deduced amino acid sequence of RdRp among the 12 SCYLV isolates under the study revealed maximum similarity ranging from 93.4%- 100% with IND isolates while 93.4-98.3% similarity with SCYLV-CUB genotype (MF622079) and 93.0- 98.3% with SCYLV-COL genotype (MF622078).The other genotypes *viz*., CHN, REU,



Fig 1: Severely infected sugarcane crop with yellow leaf disease.



Fig 2: Symptoms of sugarcane yellow leaf disease with pinkish discoloration of the midrib on the adaxial surface.

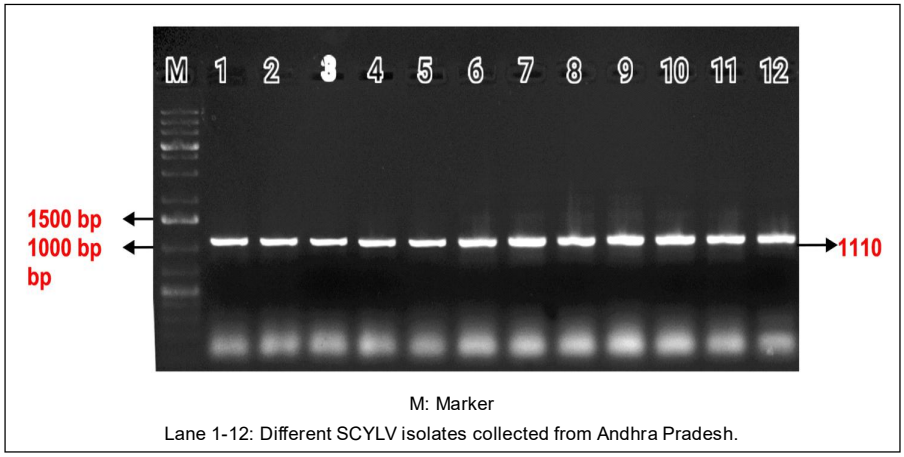
Table 2: Per cent identities of SCYLV isolates based on partial sequences of RdRP understudy with other SCYLV isolates at nucleotide level.

Sequence	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
AM072627-PER	ID																																	
AM072632-USA	97.4	ID																																
JX287508-IND-AP	91.8	91.9	ID																															
JF925153-IND-TN	91.1	90.9	97.8	ID																														
AM072642-REU29	95.5	95.5	93.0	92.5	ID																													
EU624496-IND-TN	91.0	91.4	98.9	97.2	92.7	ID																												
EU624499-IND	91.3	91.4	99.1	97.5	92.7	98.6	ID																											
MF622078-COL	91.5	91.8	98.5	96.8	93.4	98.3	98.2	ID																										
AF369925-BRA	97.1	99.0	91.8	90.7	95.3	91.3	91.3	91.7	ID																									
KF680098-IND-LCKNW	91.1	91.3	99.0	97.9	92.6	98.5	98.7	98.1	91.1	ID																								
MN097766-USA	97.0	99.1	91.9	90.9	95.5	91.4	91.4	91.8	99.3	91.3	ID																							
GU190162CHN-GX2	97.2	99.1	92.2	91.1	95.7	91.7	91.7	92.1	99.5	91.5	99.4	ID																						
MN857470-93A145-IND	91.7	92.1	99.3	97.6	93.2	99.0	99.0	98.5	91.9	98.9	91.8	92.3	ID																					
MN865128-2003V46-IND	91.1	91.3	99.0	97.9	92.6	98.5	98.7	98.1	91.1	100.0	91.3	91.5	98.9	ID																				
CO86032-MN865129-IND	91.3	91.4	99.1	98.6	92.7	98.6	98.9	98.2	91.3	98.7	91.4	91.7	99.0	98.7	ID																			
MN865130-COC671-IND	91.0	91.1	98.9	97.2	92.5	98.3	99.7	97.9	91.0	98.5	91.1	91.4	98.7	98.5	98.6	ID																		
MN900951-2005T16-IND	91.8	91.9	99.0	97.4	93.6	98.7	98.7	99.4	91.8	98.6	91.9	92.2	98.9	98.6	98.7	98.5	ID																	
MN909725-83V15-IND	90.9	90.9	97.5	98.0	92.5	97.0	97.2	96.6	90.7	97.9	90.9	91.1	97.4	97.9	97.2	97.0	97.1	ID																
MN909726-92A30-IND	90.8	90.7	98.0	96.4	92.5	97.8	97.8	97.4	90.8	97.6	91.0	91.3	97.9	97.6	97.8	97.5	97.9	96.6	ID															
MN972615-86V96-IND	91.0	91.4	98.9	97.2	92.7	98.6	98.9	98.0	91.3	98.5	91.4	91.7	99.0	98.5	98.6	98.6	98.5	97.0	97.5	ID														
MT074088-87A298-IND	91.0	91.4	98.9	97.2	92.7	100.0	98.6	98.3	91.3	98.5	91.4	91.7	99.0	98.5	98.6	98.3	98.7	97.0	97.8	98.6	ID													
MT060294-COT8201-IND	91.3	91.4	98.7	97.1	92.7	98.2	98.5	97.8	91.3	98.3	91.4	91.7	98.6	98.3	98.5	98.2	98.3	96.8	97.4	98.2	98.2	ID												
MT992722-2000A240-IND	91.5	91.7	98.9	97.4	93.2	98.3	98.6	98.2	91.5	98.5	91.7	91.9	98.7	98.5	98.6	98.3	98.7	97.4	97.8	98.3	98.3	98.2	ID											
MW032686-2009V89-IND	91.3	91.4	98.7	97.1	92.7	98.2	98.5	97.8	91.3	98.3	91.4	91.7	98.6	98.3	98.5	98.2	98.3	96.8	97.4	98.2	98.2	100.0	98.2	ID										
MF426270-MARITIUOS	95.5	95.6	92.5	92.0	98.3	92.2	92.2	92.9	95.7	92.4	95.9	95.9	92.6	92.4	92.2	91.9	93.0	92.2	91.9	92.2	92.2	92.2	92.6	92.2	ID									
KY052166-REU	95.5	95.2	92.9	92.5	98.5	92.6	92.6	93.3	95.1	92.8	95.2	95.5	93.0	92.8	92.6	92.3	93.4	92.8	92.3	92.6	92.6	92.6	93.0	92.6	98.9	ID								
EU624497-IND	91.3	91.4	99.1	98.6	92.7	98.6	98.9	98.2	91.3	98.7	91.4	91.7	99.0	98.7	100.0	98.6	98.7	97.2	97.8	98.6	98.6	98.5	98.6	98.5	92.2	92.6	ID							
JF925152-IND	90.7	90.7	97.1	97.4	92.6	96.6	97.1	96.7	90.6	97.8	90.7	91.0	97.0	97.8	96.8	96.8	97.2	97.6	95.7	96.8	96.6	96.7	97.0	96.7	92.4	92.9	96.8	ID						
GU570006-Haw	97.9	97.6	91.7	90.9	95.2	90.8	91.1	91.4	97.5	91.0	97.6	97.6	91.3	91.0	91.1	90.8	91.7	90.9	91.1	90.8	90.8	91.1	91.4	91.1	95.2	94.9	91.1	90.5	ID					
AM072751-CHN-YL1	96.8	99.0	91.9	90.9	95.1	91.4	91.4	91.8	99.1	91.3	99.3	99.3	92.1	91.3	91.4	91.1	91.9	91.1	91.0	91.4	91.4	91.4	91.7	91.4	95.5	94.8	91.4	91.0	97.2	ID				
MF622079-CUB-YL1a	91.4	91.7	98.6	97.0	93.2	98.5	98.3	99.0	91.5	98.2	91.7	91.9	98.6	98.2	98.3	98.0	99.3	96.8	97.8	98.2	98.5	97.9	98.5	97.9	92.6	93.0	98.3	96.8	91.3	91.7	ID			
JF925155-IND	90.9	90.9	97.5	98.0	92.5	97.0	97.2	96.6	90.7	97.9	90.9	91.1	97.4	97.9	97.2	97.0	97.1	100.0	96.6	97.0	97.0	96.8	97.4	96.8	92.2	92.8	97.2	97.6	90.9	91.1	96.8	I		
EU624502-IND	91.4	91.5	98.9	97.2	92.6	98.3	98.6	98.0	91.4	98.5	91.5	91.8	98.7	98.5	98.6	98.3	98.5	97.0	97.5	98.3	98.3	98.2	98.3	98.2	92.1	92.7	98.6	96.6	91.3	91.5	98.0	97.0	ID	
HQ342888-CHN-HN1	97.4	99.3	92.1	91.0	95.6	91.5	91.5	91.9	99.4	91.4	99.3	99.5	92.2	91.4	91.5	91.3	92.1	91.0	91.1	91.5	91.5	91.5	91.8	91.5	95.7	95.3	91.5	90.9	97.8	99.1	91.8	91.0	91.7	ID

Table 3: Per cent identities of SCYLV isolates based on partial sequences of RdRP understudy with other SCYLV isolates at aminoacid level.

Sequence	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
AM072627-PER	ID																																	
AM072632-USA	97.5	ID																																
JX287508-IND-AP	87.3	87.7	ID																															
JF925153-IND-TN	86.5	86.9	96.3	ID																														
AM072642-REU29	94.6	94.2	89.7	88.5	ID																													
EU624496-IND-TN	86.9	87.3	98.3	95.4	89.3	ID																												
EU624499-IND	86.5	86.9	98.3	95.4	88.9	97.5	ID																											
MF622078-COL	86.9	87.3	97.1	94.2	89.3	97.1	96.3	ID																										
AF369925-BRA	97.5	98.7	87.7	86.9	94.2	87.3	86.9	87.3	ID																									
KF680098-IND-LCKNW	86.5	86.9	98.3	97.1	88.9	97.5	97.5	96.3	86.9	ID																								
MN097766-USA	96.7	98.7	87.7	86.9	94.2	87.3	86.9	87.3	99.1	86.9	ID																							
GU190162CHN-GX2	97.5	98.7	87.7	86.9	94.2	87.3	86.9	87.3	100.0	86.9	99.1	ID																						
MN857470-93A145-IND	88.1	88.5	99.1	96.3	89.7	98.3	98.3	97.1	88.5	98.3	87.7	88.5	ID																					
MN865128-2003V46-IND	86.5	86.9	98.3	97.1	88.9	97.5	97.5	96.3	86.9	100.0	86.9	86.9	98.3	ID																				
CO86032-MN865129-IND	87.3	87.7	98.7	97.5	89.3	97.9	97.9	96.7	87.7	97.9	87.7	87.7	98.7	97.9	ID																			
MN865130-COC671-IND	86.1	86.5	97.9	95.0	88.5	97.1	99.5	95.9	86.5	97.1	86.5	86.5	97.9	97.1	97.5	ID																		
MN900951-2005T16-IND	87.7	88.1	98.7	95.9	90.2	98.7	97.9	98.3	88.1	97.9	88.1	88.1	98.7	97.9	98.3	97.5	ID																	
MN909725-83V15-IND	85.7	86.1	95.0	97.1	88.1	94.2	94.2	93.0	86.1	95.9	86.1	86.1	95.0	95.9	94.6	93.8	94.6	ID																
MN909726-92A30-IND	86.9	86.5	96.7	93.8	89.3	96.7	95.9	95.4	87.3	95.9	87.3	87.3	96.7	95.9	96.3	95.4	97.1	93.4	ID															
MN972615-86V96-IND	87.3	87.7	99.1	96.3	89.7	98.3	98.3	97.5	87.7	98.3	87.7	87.7	99.1	98.3	98.7	97.9	98.7	95.0	96.7	ID														
MT074088-87A298-IND	86.9	87.3	98.3	95.4	89.3	100.0	97.5	97.1	87.3	97.5	87.3	87.3	98.3	97.5	97.9	97.1	98.7	94.2	96.7	98.3	ID													
MT060294-COT8201-IND	86.5	86.9	98.3	95.4	88.9	97.5	97.5	96.3	86.9	97.5	86.9	86.9	98.3	97.5	97.9	97.1	97.9	94.2	95.9	98.3	97.5	ID												
MT992722-2000A240-IND	86.9	87.3	97.9	95.4	89.3	97.1	97.1	95.9	87.3	97.1	87.3	87.3	97.9	97.1	97.5	96.7	97.5	95.0	96.3	97.9	97.1	97.1	ID											
MW032686-2009V89-IND	86.5	86.9	98.3	95.4	88.9	97.5	97.5	96.3	86.9	97.5	86.9	86.9	98.3	97.5	97.9	97.1	97.9	94.2	95.9	98.3	97.5	100.0	97.1	ID										
MF426270-MARITIUOS	95.0	94.6	90.2	88.9	98.7	89.7	89.3	89.7	94.6	89.3	94.6	94.6	90.2	89.3	89.7	88.9	90.6	88.5	89.7	90.2	89.7	89.3	89.7	89.3	ID									
KY052166-REU	95.0	94.6	90.2	89.3	97.9	89.7	89.3	89.7	94.6	89.3	94.6	94.6	90.2	89.3	90.2	88.9	90.6	88.5	89.7	90.2	89.7	89.3	89.7	89.3	99.1	ID								
EU624497-IND	87.3	87.7	98.7	97.5	89.3	97.9	97.9	96.7	87.7	97.9	87.7	87.7	98.7	97.9	100.0	97.5	98.3	94..6	96.3	98.7	97.9	97.9	97.5	97.9	89.7	90.2	ID							
JF925152-IND	86.1	86.5	96.3	97.5	88.5	95.4	95.4	94.2	86.5	97.9	86.5	86.5	96.3	97.9	95.9	95.0	95.9	96.3	93.8	96.3	95.4	95.4	95.4	95.4	88.9	88.9	95.9	ID						
GU570006Haw	96.3	96.7	86.5	85.7	93.8	86.1	85.7	86.1	97.9	85.7	97.9	97.9	86.5	85.7	86.5	85.3	86.9	84.8	86.9	86.5	86.1	85.7	86.1	85.7	94.2	94.2	86.5	85.3	ID					
AM072751-CHN-YL1	96.7	98.7	87.7	86.9	93.4	87.3	86.9	87.3	99.1	86.9	99.1	99.1	88.5	86.9	87.7	86.5	88.1	86.1	87.3	87.7	87.3	86.9	87.3	86.9	93.8	93.8	87.7	86.5	97.1	ID				
MF622079-CUB-YL1a	86.5	86.9	97.1	94.2	89.3	97.1	96.3	97.5	86.9	96.3	86.9	86.9	97.1	96.3	96.7	95.9	98.3	93.4	95.9	97.1	97.1	96.3	96.3	96.3	89.3	89.3	96.7	94.2	85.7	86.9	ID			
JF925155-IND	85.7	86.1	95.0	97.1	88.1	94.2	94.2	93.0	86.1	95.9	86.1	86.1	95.0	95.9	94.6	93.8	94.6	100.0	93.4	95.0	94.2	94.2	95.0	94.2	88.5	88.5	94.6	96.3	84.8	86.1	93.4	ID		
EU624502-IND	86.9	87.3	97.5	95.0	88.5	96.7	96.7	95.9	87.3	96.7	87.3	87.3	97.5	96.7	97.5	96.3	97.1	93.4	95.0	97.5	96.7	96.7	96.3	96.7	88.9	89.7	97.5	94.6	86.1	87.3	95.4	93.4	ID	
HQ342888-CHN-HN1	97.5	98.7	87.7	86.9	94.2	87.3	86.9	87.3	100.0	86.9	99.1	100.0	88.5	86.9	87.7	86.5	88.1	86.1	87.3	87.7	87.3	86.9	87.3	86.9	94.6	94.6	87.7	86.5	97.9	99.1	86.9	86.1	87.3	ID

BRA, PER, HAW shared 86.1% - 90.6% amino acid similarity with the IND isolates under the study (Table 3). 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.,* 2006b). On the basis of SCYLV genotypes identified



M: Marker

Lane 1-12: Different SCYLV isolates collected from Andhra Pradesh.

Fig 3: Ampliûcation of ORF1 and 2 regions of 12 isolates of SCYLV using speciûc primers SCYLV-F3 and SCYLV-R3.

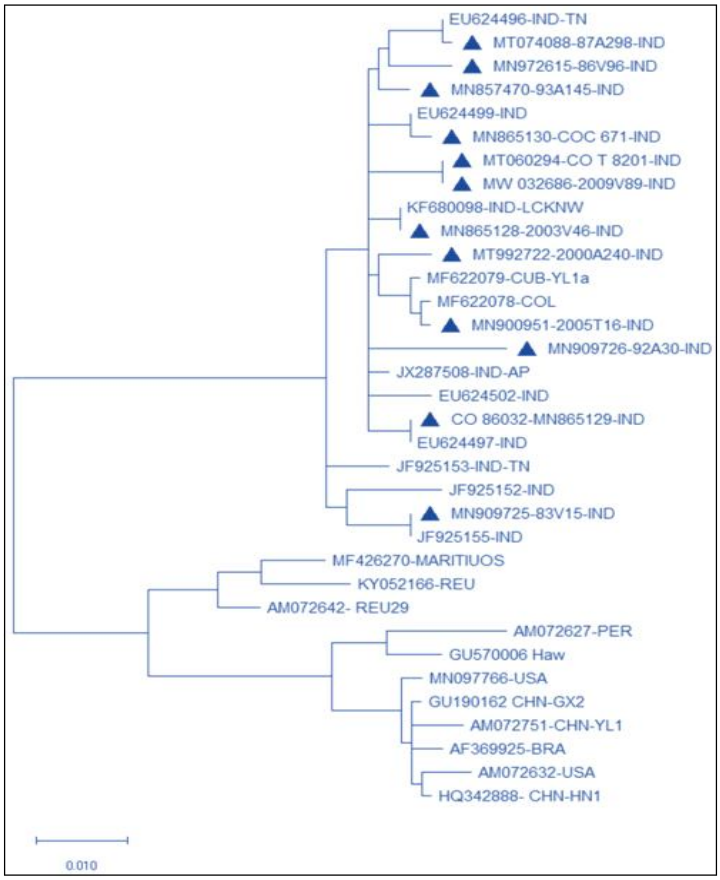


Fig 4: Phylogenetic tree (1000 boot strap replications) of partial RdRp sequences of SCYLV isolates under the study at nucleotide level.

in Brazil, Colombia, Cuba and Peru, Abu Ahmad *et al.* (2006 b) suggested that they may have been introduced through infected planting material imported from elsewhere. Phylogenetic analysis revealed that the SCYLV isolates under the study clustered into two major groups. SCYLV isolates from India, 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 (Fig 4). The 12 SCYLV isolates under study showed close relationship with Cuba isolate (MF622079) and Colombia (MF622078) isolate along with other 9 isolates from India. The 13 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.

Viswanathan *et al*. (2008) studied the phylogentic analysis of SCYLV isolates based on partial ORF 1 and 2 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 up to 99.2%. Similarly, The phylogenetic analyses of the entire genome of SCYLV described by Abu Ahmad *et al.* (2006a) 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).

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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Conflict of interest

The authors declare that they have no conflict of interest.

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