

RESEARCH ARTICLE

A Comprehensive Study on the 16S rRNA Gene of Major Phytoplasma Diseases in Sri Lanka with their Close Relatives

Y.A.P.K. Dayasena

Department of Biosystems Technology, Faculty of Technology, Sabaragamuwa University of Sri Lanka, Belihuloya, 70140, Sri Lanka

Submitted: February 04, 2024; Revised: June 11, 2024; Accepted: October 21, 2024

*Correspondence: prasanna@tech.sab.ac.lk,  <https://orcid.org/0000-0002-7264-824X>

ABSTRACT

Phytoplasmas are plant pathogenic mollicutes that have a significant impact on the sugarcane and coconut plantations in Sri Lanka. Sugarcane and coconut have been reported as hosts for many phytoplasmas in Asia and Africa that show almost similar symptoms. A comprehensive study on the phytoplasma disease reported in Sri Lanka will be useful for prospective disease management practices. Therefore, this study aimed to analyze the 16S rRNA gene identity of phytoplasmas associated with major diseases in Sri Lanka and compare them with their close relatives worldwide to assess their genetic relationships. Seventeen phytoplasma isolates 16S rRNA gene sequences ranging from 773 bp to 1768 bp including eight SCWL and SCGS, three WCLW and four other different coconut phytoplasma, and two different grass phytoplasma sequences were retrieved from NCBI-GenBank. The 'Candidatus' phytoplasma species assignment and phytoplasma groups and subgroups were determined by the iPhyClassifier tool. The sequence identity was studied by the Clustal Omega tool and phylogenetic analysis was done with the Maximum Likelihood method. All the SCWL, SCGS, CKW, and CYD belonged to 'Ca. *P. cynodantis*' species. The WCLW isolates and SCWL Sri Lankan isolates showed a very close sequence identity, ranging from 98.84% to 99.89%, and clustered within the same monophyletic group in the phylogenetic tree. The two grass phytoplasma isolates found in Sri Lanka showed sequence identities ranging from 96.06% to 98.32% with WCLW phytoplasmas, which is lower than their identity with SCWL phytoplasmas. The WCLW phytoplasma also showed a close genetic and phylogenetic relationship with CKW and CYD diseases reported in neighboring countries. The two coconut phytoplasma diseases reported in African countries were more distantly related to all other phytoplasmas isolates and many INDELS were observed in the 16S rRNA gene.

Keywords: 16S rRNA gene Phylogenetic relationships, Phytoplasma, Sugarcane white leaf disease, Weligama coconut leaf wilt disease

INTRODUCTION

Fungi, bacteria, viruses, and phytoplasma are the predominant types of plant pathogens that cause many diseases in plants. Among these, Phytoplasmas that belonged under the 'Mollicutes' is often associated with rapidly spreading severe diseases in agriculturally important crops and forest plants also (Bertaccini *et al.*, 2014; Bertaccini and Lee, 2018; Rao *et al.*, 2018). Phytoplasmas show a wide range of host compatibility though they have a small genome. Therefore, many commercial palm species including coconut are being affected by serious

phytoplasma diseases around the world (Gurr *et al.*, 2016). Some of the symptoms resulting from phytoplasma diseases are similar to the viral diseases in plants. Therefore, some of the diseases thought to be plant-viral diseases are now confirmed as phytoplasma diseases (De Costa, 2009).

Sugarcane and coconut are the two important plantation crops that are being severely affected by two different phytoplasma diseases in Sri Lanka. There are no effective measures available to eliminate diseases from infected plants instead of controlling them. There are two major phytoplasma diseases in sugarcane namely sugarcane white leaf disease (SCWL) and sugarcane yellow leaf syndrome (SCYLS) caused by the phytoplasmas in Sri Lanka. Among these diseases, SCWL was first reported in 1972 and has been spread in almost all sugarcane plantations now (Jayathilaka, 1973; Seneviratne, 2008; Keerthipala, 2016). Dayasena *et al.* (2021) revealed that the phytoplasma causes for SCWL in all sugarcane-growing areas of Sri Lanka belonged to the 16SrXI-B subgroups and there is no significant genetic variation among them based on their 16S rRNA gene sequence.

The lethal yellowing-like diseases (LYDs) of coconut and other palm species have led to the losses of millions of coconuts and other palm species (Jones, 2002; Eziashi and Omamor, 2010). In Sri Lanka, the Weligama Coconut Leaf Wilt Disease (WCLWD) was first recorded in the Weligama area in the southern coastal region in 2006 and it was declared a serious disease in coconut plantations in the country after a few years after the discovery (Wijesekara *et al.*, 2008; Perera *et al.*, 2012). Several studies have been done to compare the similarity of the 16 rRNA gene of the WCLWD, SCWL, and other phytoplasmas diseases reported in the same host. The partial sequence of 16 rRNA gene sequence of WCLWD (EU635503) showed 99% sequence identity with SCWL isolates (GQ121046) and Sugarcane grassy shoot phytoplasma (SCGS) Maharashtra, India isolates (AM261831) (Jung *et al.*, 2003; Firrao *et al.*, 2004; Ariyaratne *et al.*, 2007). Furthermore, it belonged to the 16SrXI phytoplasma group that consists of the *Candidatus* phytoplasma ('Ca. P) oryzae and further suggested that WCLWD phytoplasma has divergent from the mutated version of SCWL phytoplasmas in Sri Lanka (Perera *et al.*, 2012). Nair *et al.* (2016) suggested the coconut Kerala wilt disease phytoplasma recorded in Southern India grouped into the 16SrXI phytoplasma group. The same group contains both SCWL and SCGS phytoplasmas (Viswanathan and Rao, 2011; Rao *et al.*, 2014). However, a sequence that is enough for the identification of the phytoplasmas subgroup of the WCLWD has not been submitted to the GenBank database so far. Dayasena *et al.* (2021) revealed the presence of two phytoplasma diseases associated with wild grass species near sugarcane growing fields in Sri Lanka. These phytoplasmas, belonging to the 16SrXIV-A subgroup, showed 97.51% to 98.15% identity in their 16S rRNA gene sequences with SCWL phytoplasma isolates reported in Sri Lanka. Therefore, the similarities of the 16 rRNA gene of the different phytoplasma diseases associated with the several host plants in Sri Lanka have been shown in a few previous studies but those are not specified for

all major phytoplasma diseases in Sri Lanka with their close relative phytoplasma diseases reported in other countries. A comprehensive analysis of the genetic similarities and phylogenetic relationship of the major phytoplasmas diseases in the country such as SCWL, WCLW, and grass phytoplasmas with their relative phytoplasma diseases in the same host will be useful to presume the prospective pathogenic potential, potential vectors and get an insight of the management strategies for present and future outbreak. Therefore, the objective of the present study was to investigate the identity of the 16 rRNA genes of different phytoplasma isolates reported in Sri Lanka with their close relative phytoplasmas reported in other countries to assess the relationship among them.

MATERIALS AND METHODS

Selection of the phytoplasmas 16 rRNA gene sequences for the study

The 16 rRNA gene sequences of the phytoplasma isolate in Sri Lanka including SCWL, WCLWD, *Brachiaria* Grass White Leaf (*Bra*WL), and *Bermuda* Grass White Leaf (BGWL) and the similar phytoplasma diseases reported in the same host plants in the different countries of the Asian and African continent were used in the present study. The FASTA format of the lengthiest and most reliable sequence of the 16 rRNA gene of each phytoplasma isolate deposited in the GenBank database of the National Center for Biotechnological Information (NCBI) was retrieved for the study (Table 1).

Studies on 16S rRNA gene sequence identity and phytoplasma groups and subgroups

The phytoplasma 16SrRNA gene sequences have been amplified by phytoplasma-specific P1/P7 followed by R16F2n/R16R2 nested primer pairs (Gundersen and Lee, 1996) were used for the study. It is a preliminary requirement to reveal the respective '*Candidates* phytoplasma' and 16S ribosomal group or subgroups of the phytoplasma based on restriction fragment length polymorphism (RFLP) analysis on a 1.25 kb PCR product derived from their 16S rRNA gene in accordance to IRCPM (2004). The percentage of identity of the 16S rRNA gene sequence in different phytoplasma strains was studied using the Clustal Omega online tool (Sievers *et al.*, 2011). The identity among the 16SrRNA gene sequence was determined by using that percentage of the identity matrix. The respective '*Candidates* phytoplasma' species was determined based on the similarity of the 16 rRNA gene. *In-silico* RFLP analysis was performed to identify the 16Sr groups and subgroups of each isolate based on the virtual Restriction Fragments Length Polymorphisms (RFLP) gel patterns of 17 restriction endonucleases similarity coefficients by using iPhyClassifier online tool (Zhao *et al.*, 2009).

Table 1: The phytoplasmas isolates used for the study

No	Phytoplasma isolate	GenBank Acc. No	Length (bp)	Reference
01	WCLW isolates RDS1, Sri Lanka	MZ822450	865	De Silva <i>et al.</i> (2022)
02	WCLW isolates Sri Lanka	KF993431	894	Wijisekara <i>et al.</i> (2014)
03	WCLW isolates Matara, Sri Lanka	EU635503	773	Perera <i>et al.</i> (2008)
04	CKW isolate Kayankulam, India	JX273772	1246	Manimekalai <i>et al.</i> (2014)
05	CYD isolate, Malaysia	EU636906	1247	Nejat <i>et al.</i> (2009)
06	CPW isolate, Ghana	JQ868442.1	1768	Makarova <i>et al.</i> (2012)
07	CAW isolate, Nigeria	Y14175.1	1530	Tymon <i>et al.</i> (2008)
08	SCWL isolates Uda Walawe, Sri Lanka	MT811809	1298	Dayasena <i>et al.</i> (2021)
09	SCWL isolates Kantale, Sri Lanka	MT860714	1246	Dayasena <i>et al.</i> (2021)
10	SCWL isolates Hingurana, Sri Lanka	MT811811	1251	Dayasena <i>et al.</i> (2021)
11	SCWL isolates SCKK-BP, Thailand	FM208257	1247	Klinkong (2008)
12	SCGS isolate SC-Phy385, India	HF586648	1242	Yadav <i>et al.</i> (2013)
13	SCWL clone isolate LCYC9, China	MT649297	1247	Zhang <i>et al.</i> (2020)
14	SCWL isolate DNAI-NTTT, Vietnam	KC295286	1263	Nguyen <i>et al.</i> (2013)
15	SCGS isolate Sharanpur, India	MT510165	1557	Sharma <i>et al.</i> (2020)
16	BraWL isolate, Sri Lanka	MT862162	1365	Dayasena <i>et al.</i> (2021)
17	BGWL isolate, Sri Lanka	MT872407	1268	Dayasena <i>et al.</i> (2021)

WCLW- Weligama Coconut Leaf Wilt phytoplasma, CKW- Coconut Kerala Wilt phytoplasma, CYD- Coconut Yellow Decline phytoplasma, CPW- Coconut Cape St. Paul Wilt phytoplasma, CAW- Coconut Awka Wilt phytoplasma, SCWL- Sugarcane White Leaf phytoplasma, SCGS- Sugarcane Grassy Shoot phytoplasma, BraWL- Brachiaria Grass White Leaf phytoplasma, BWL- Bermuda Grass white leaf phytoplasma.

Phylogenetic analysis

Phylogenetic relationships among the 16S rRNA gene of the different phytoplasma isolates were studied to find their evolutionary relationship. Multiple sequence alignment was done by using the ClustalW matrix to identify the divergent sites of the 16S rRNA gene among the different isolates and the phylogenetic analysis was done with the Maximum Likelihood (ML) method and Tamura-Nei model (Tamura and Nei, 1993) with a 1000 bootstrap replicate value of the Molecular Evolutionary Genetic Analysis, version X (MEGA X) program (Kumar *et al.*, 2018). The *Acholeplasma laidlawii* (Acc No. AB680603) strain was used as an outgroup for the phylogenetic analysis.

RESULTS AND DISCUSSION

Candidates phytoplasma species and phytoplasma subgroups

The length of the retrieved phytoplasma 16S rDNA sequences varied between 773 bp to 1768 bp and there were no gaps or undefined nucleotides in the GenBank retrieved FASTA files. Three WCLW phytoplasmas sequences were short in length compared to the other phytoplasma sequences that ranged from 773 bp to 894 bp. All the sugarcane, grass, CKW, and CYD phytoplasmas isolates were assigned to *Candidates phytoplasma cynodontis*. The phytoplasma isolates belonged to different 16Sr subgroups of the 16SrXI group based on the iPhyClassifier 'Ca. phytoplasma species assessment. The RFLP similarity coefficient ranged from 0.7 to 1.0 which means some of the phytoplasmas isolates could have been grouped in new a 'Ca. new species if more 'Ca. phytoplasma species have been defined (Table 2).

Table 2: Summary of the *Candidatus* phytoplasma species assessment and 16Sr subgroups.

No	Phytoplasmas isolates	'Ca. phytoplasma Assessment	RFLP similarity coefficient	16Sr group/ Subgroup
01	WCLW RDS1, Sri Lanka	-	-	-
02	WCLW Sri Lanka	-	-	-
03	WCLW Matara, Sri Lanka	-	-	-
04	CKW Kayankulam, India	'Ca.P. cynodontis'	1.0	16SrXI-B
05	CYD Malaysia	'Ca.P. cynodontis'	0.97	16SrXIV-A
06	CPW Ghana	'Ca.P. palmicola'	1.0	16Sr XXII-B
07	CAW Nigeria	'Ca.P. palmicola'	1.0	16SrXXII-A
08	SCWL Uda Walawe, Sri Lanka	'Ca.P. cynodontis'	1.0	16SrXI-B
09	SCWL Kantale, Sri Lanka	'Ca.P. cynodontis'	1.0	16SrXI-B
10	SCWL Hingurana, Sri Lanka	'Ca.P. cynodontis'	1.0	16SrXI-B
11	SCWL SCKK-BP, Thailand	'Ca.P. cynodontis'	0.7	16SrXI-B
12	SCGS SC-Phy385, India	'Ca.P. cynodontis'	1.0	16SrXI-F
13	SCWL clone LCYC9, China	'Ca.P. cynodontis'	1.0	16SrXI-B
14	SCWL DNAI-NTTT, Vietnam	'Ca.P. cynodontis'	0.88	16SrXI-D
15	SCGS Sharanpur, India	'Ca.P. cynodontis'	-	-
16	BraWL Sri Lanka	'Ca.P. cynodontis'	1.0	16SrXIV-A
17	BGWL Sri Lanka	'Ca.P. cynodontis'	1.0	16SrXIV-A

A few previous studies suggested that the 'Ca. phytoplasma species of the SCGS and SCWL is 'Ca. phytoplasma *Oryza* that belonged to the 16Sr XI namely the Rice Yellow Dwarf group (Nasare *et al.*, 2007; Rao *et al.*, 2008; Viswanathan *et al.*, 2011). However, all phytoplasmas isolates in our study belonged to the 'Candidates phytoplasma *cynodontis* species (Table 2). A few previous studies have made controversy about the 'Ca. phytoplasma species assessment between the 16Sr XI and 16SrXIV groups which represent most of the phytoplasma disease reports in sugarcane and grasses. Nevertheless, Kirdat *et al.*, (2021) suggested that a close relationship between the phytoplasmas strains have been categorized under the 16Sr XI and 16SrXIV groups. Furthermore, they recently defined a novel *Ca. phytoplasma* species associated with SCGS disease. Therefore, a finer taxonomic resolution to differentiate these two phytoplasma species is needed. Moreover, the anomalies in their taxonomy can be resolved by defining new 'Candidates for phytoplasma' species for the phytoplasma presence in the 16Sr XI group after doing a comprehensive study on the variability in the 16SrRNA gene.

The 'Candidates phytoplasmas' species assessment and phytoplasma subgroup studies have not been done so far for the WCLW phytoplasmas since the unavailability of the 1.25 kb, 16S rRNA fragment for the R16F2n/R16R2 primer pair. However, Perera *et al.* (2012) revealed that the phytoplasma group of WCLW phytoplasma is 16SrXI. Indeed, a proper study is needed to identify the 'Ca. p. species and phytoplasmas subgroups for WCLW phytoplasma.

The two grass phytoplasmas reported in Sri Lanka belonged to the 16SrXIV-A subgroup with CYD Malaysia isolates which suggests the relationship between coconut and grass phytoplasma diseases. The CAW and CPW phytoplasma strains belonged to the 16SrXXII-A and 16SrXXII-B subgroups which is a distinct group to the 16Sr XI group, respectively. Therefore, it was not closely related to the SCWL and SCGS phytoplasma subgroups (Table 2).

The sequence similarity study was done to find the identity of the 16SrRNA gene among the different phytoplasma sequences used for the study. The three different WCLW isolates reported in Sri Lanka (MZ822450, KF993431, EU635503) showed a 98.84% to 99.89% sequence identity with SCWL Sri Lankan isolates (MT811809, MT860714, MT811811) that belonged to 16SrXI-B subgroups. That was a significant identity between two phytoplasmas isolates, which revealed a closeness of the conserved genomic region of two phytoplasma isolates. Moreover, the WCLW phytoplasma shared a 97.69% to 99.87% sequence identity with the SCWL and SCGS phytoplasma isolate reported in other counties that belonged to 16SrXI-B subgroup except one of the Indian SCGS isolate (HF586648) that belonged in the 16SrXI-F subgroup. Furthermore, the same isolates showed a 98.73% to 99.74% sequence identity with CKW-Kerala, India isolates (JX273772) which also belonged to the 16SrXI-B subgroup (Table 3).

In addition, the symptoms of affected palms were similar to the coconut root (wilt) disease reported in Kerala, India (Wijesekara *et al.*, 2008). These are the

strong evidence to show the closeness among the SCWL the WCLW and CKW phytoplasmas. The finding suggested that there is a fair likelihood to categorize WCLW phytoplasmas under the same phytoplasma subgroups if a proper study is to find the phytoplasma subgroup. Abeysinghe *et al.*, (2016) revealed >99% sequence identity between the SCWL/SCGS phytoplasma and WCLW phytoplasma reported from Sri Lanka. The WCLWD isolates shared a 96.06% to 98.32 % identity with the two-grass white leaf disease phytoplasma isolates reported in Sri Lanka (MT862162, MT872407) which belonged under the 16SrXIV-A subgroup. Moreover, the results suggested the two grass phytoplasmas species are distantly related to the WCLW phytoplasma since they share the minimal sequence identity and further indicated that they should be placed in two different *Candidates* phytoplasma species. One of the prime requirements to categorize two phytoplasmas isolates in the same *Candidates* phytoplasma species is, that both isolates should share >97.5% sequence identity, as per the IRPCM (2004) phytoplasmas taxonomy guideline.

The multiple-sequence alignment results showed a locus that is significantly divergent from the other loci of the 16SrRNA gene which is located between 825 bp and 873 bp. A few INDEL sites were shown for most phytoplasmas isolates studied here in that particular locus. Especially, the sequence was 'AATACT' between 830 and 836 bp for all SCWL and WCLW Sri Lankan isolates, but some of the other SCWL, SCGS reported in other countries were shown SNPs in the particular locus with a few INDELs. This is good evidence to show a high level of identity of the Sri Lankan SCWL and WCLW isolates even though the sites are polymorphic for other phytoplasma isolates. Furthermore, CKW isolates showed identical sequences to all Sri Lankan WCLW and SCWL isolates while showing a few SNPs for other coconut and grass phytoplasmas isolates studied here. The CPW and CAW have been more divergent sites from other phytoplasma isolates since a few insertions were identified between 825 and 830 bp and a unique transversion mutation also showed at 833 bp that "A" was replaced by "C". Furthermore, CYD isolates also showed significant divergence from other phytoplasmas strains since transversion occurred at 830 bp and substitutions occurred at 831-832 bp (Figure 1).

Table 3: Percentage similarity matrix of the 16S rRNA gene among the different phytoplasma isolates

	WCL W SL1	WCL W SL 2	WCL W SL 3	CKW Ind	CYD Mala	CPW Gha	CAW Nig	SCWL Uw SL	SCWL Kt SL	SCWL Hn SL	SCWL Thai	SCGS Ind	SCWLC hina	SCWL Viet	SCGS Sha Ind	<i>Bra</i> WL SL	BG WL SL
WCLW SL 1	100																
WCLW SL 2	99.60	100															
WCLW SL 3	99.87	99.73	100														
CKW Ind	98.73	99.78	99.74	100													
CYD Mala	97.46	98.21	98.32	97.43	100												
CPW Gha	92.72	93.51	93.27	93.50	92.62	100											
CAW Nig	92.49	93.51	93.01	93.50	92.62	99.54	100										
SCWL Uw SL	98.84	99.89	99.87	99.76	97.67	93.61	93.61	100									
SCWL Kt SL	98.84	99.89	99.87	99.76	97.67	93.66	93.66	100	100								
SCWL Hn SL	98.84	99.89	99.87	99.68	97.67	93.61	93.61	99.92	99.92	100							
SCWL Thai	98.84	99.78	99.87	99.12	97.51	93.18	93.18	99.36	99.36	99.36	100						
SCGS Ind	97.69	98.77	98.71	97.50	96.70	92.43	92.43	97.75	97.75	97.67	97.10	100					
SCWL China	98.84	99.89	99.87	99.44	98.00	93.34	93.34	99.68	99.68	99.68	99.28	97.42	100				
SCWL Viet	95.25	96.3	95.85	97.01	95.40	92.14	92.14	97.06	97.26	97.18	96.94	95.39	96.94	100			
SCGS Sha Ind	96.06	97.2	96.76	97.59	95.50	89.76	90.98	97.92	97.83	97.76	97.19	95.56	97.51	94.90	100		
<i>Bra</i> WL SL	97.34	98.21	98.32	97.91	99.20	92.67	92.75	98.19	98.15	98.08	97.75	97.02	97.83	95.89	96.26	100	
BGWLSL	97.34	98.21	98.32	97.91	99.20	92.8	93.06	98.19	98.15	98.08	97.75	97.02	97.83	95.89	96.05	100	100

*The GenBank accession No of the sequences used for the study (Table 1) is shown in the brackets

WCLW SL 1 (MZ822450), WCLW SL 2 (KF993431), WCLW SL 3 (EU635503), CKW Ind (JX273772), CYD Mala (EU636906), CPW Gha (JQ868442), CAW Nig (Y14175), SCWL Uw SL (MT811809), SCWL Kt SL (MT860714), SCWL Hn SL (MT811811), SCWL Thai (FM208257), SCGS Ind (HF586648) SCWLChina (MT649297), SCWL Viet (KC295286), SCGS Sha Ind (MT510165), BraWL SL (MT862162), BGWLSL (MT87

Phylogenetic study

All the phytoplasmas isolates used for the study were clustered into three monophyletic groups (Figure 2). All the Sri Lankan SCWL, WCLW, and some other SCWL and SCGS isolates reported in other countries were clustered in a single monophyletic group in the tree with an 85% bootstrap confidence value. The results revealed a close phylogenetic relationship between the SCWL and WCLW phytoplasmas isolates present in Sri Lanka with other sugarcane and coconut phytoplasma isolates present in neighbouring countries. The CKW phytoplasma reported in the coconut plantation in Kerala also reported in the same clade and showed a close phylogenetic relationship among the WCLW and SCWL phytoplasmas. However, two Sri Lankan WCLW phytoplasma isolates were clustered in a sub-clade with two sugarcane phytoplasma strains with low bootstrap values. This could be due to the length of that WCLW sequence being insufficient or the presence of evolutionary processes that complicate the interpretation of relationships. Almost similar phylogenetic relationships have been reported by previous studies which showed a close evolutionary relationship between the two major phytoplasma diseases reported in Sri Lanka such as SCWL and WCLW (Perera *et al.*, 2012; Abeyasinghe *et al.*, 2016). The two grass phytoplasma isolates reported in Sri Lanka were separately clustered in the 2nd monophyletic group and only two coconut phytoplasma namely CAW and CPW which were reported in African countries clustered in the 3rd monophyletic group. They showed a phylogenetic distance with the phytoplasmas grouped under monophyletic group 01.

Based on all facts gathered in the present study, very close relationships of the 16SrRNA genes of the SCWL and WCLW phytoplasmas were reported in Sri Lanka. In addition, other related phytoplasmas strains reported from sugarcane and coconut in Sri Lanka, India showed a significant similarity in many features. The outcome of the present study will be useful in identifying the common vector/s that can be transmitted to both SCWL and WCLW diseases. There is no record available so far on a common vector transmission of both diseases. Furthermore, due to the genetic closeness of the WCLW phytoplasma diseases with other coconut phytoplasma diseases in neighbouring countries, better to give attention to the prospective potential outbreak due to transmission of those diseases by the vectors of WCLW. Earlier, *Proutista moesta* has been identified as a major vector of transmitting the CKW of India (Koshy *et al.*, 2001). A few recent studies have identified, *Proutista moesta* as one of the major putative vectors of WCLWD in Sri Lanka (Kumara *et al.*, 2015) and SCGS/SCWL in India (Tiwari *et al.*, 2016). Hence, the facts of the present study may be supportive for confirming a new hypothesis that *Proutista moesta* as a super vector to transmit closely related phytoplasma diseases. Therefore, prospective studies are needed to reveal whether one or more common vectors are transmitting both WCLWD and SCWL since they show a close relationship.

	825				830				835				840				845				850				855				860				865				870			873											
Species/Abbrev	*	*	*						*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*									
1. WCLW isolates RDS1 Sri Lanka (MZ822450.2)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
2. WCLW isolates Sri Lanka (KF993431.1)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
3. WCLW isolates Matara Sri Lanka (EU635503.1)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
4. CKW isolate Kayankulam India (JX273772.1)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
5. CYD isolate Malaysia (EU636906.1)	G	G	G	-	-	G	A	A	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
6. CPW isolate Ghana (JQ868442.1)	G	G	G	G	G	T	T	T	C	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
7. CAW isolate Nigeria (Y14175.1)	G	G	G	G	G	T	T	T	C	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
8. SCWL isolates Uda Walawe Sri Lanka (MT811809)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
9. SCWL isolates Kantale Sri Lanka (MT860714)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
10. SCWL isolates Hingurana Sri Lanka (MT811811)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
11. SCWL isolates SCKK-BP Thailand (FM208257)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
12. SCGS isolate SC-Phy385 India (HF586648)	G	G	G	-	-	G	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
13. SCWL clone isolate LCYC9 China (MT649297)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
14. SCWL isolate DNAI-NTTT Vietnam (KC295286)	G	G	G	G	G	-	A	T	T	A	C	T	G	G	T	T	A	A	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	G	T	A	C	T	C	C	G	G	C	G	-	T	G
15. SCGS isolate Sharanpur India (MT510165)	G	G	G	-	-	A	T	T	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
16. BraWL isolate Sri Lanka (MT862162)	G	G	G	-	-	G	A	A	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		
17. BGWL isolate Sri Lanka (MT872407)	G	G	G	-	-	G	A	A	A	C	T	C	G	G	T	A	-	C	T	G	A	A	G	T	T	A	A	C	A	C	A	T	T	A	A	G	-	T	A	C	T	C	C	G	C	C	-	T	G		

Figure 1: The most polymorphic locus of the 16S rRNA gene among the phytoplasma isolates used in the study.

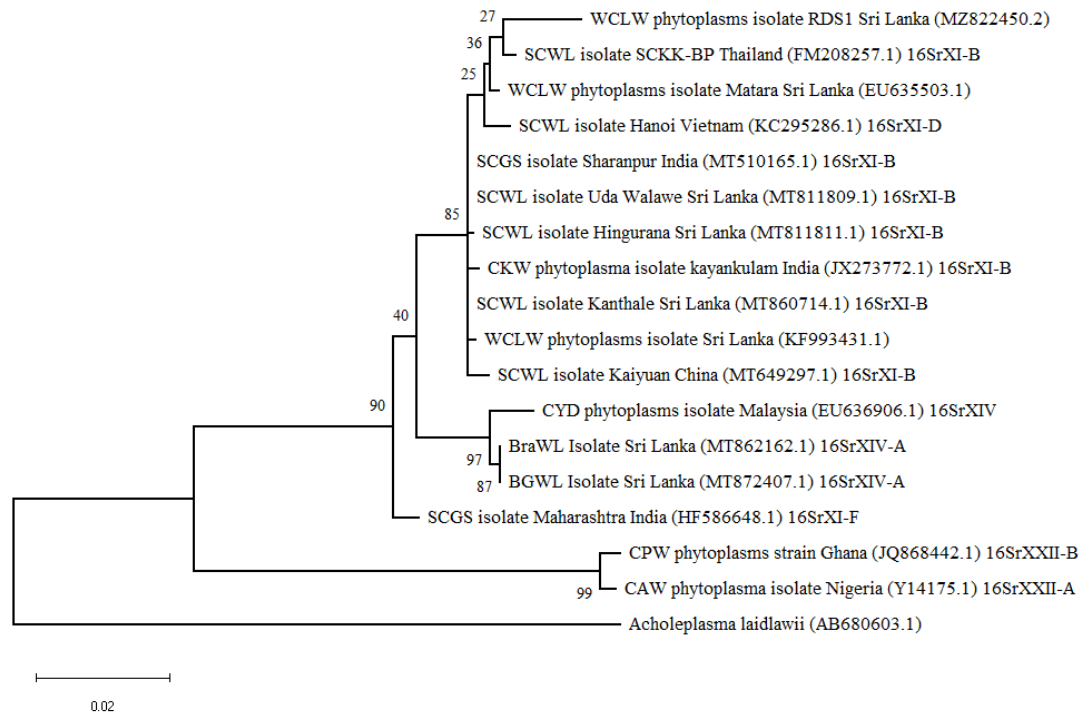


Figure 2: The phylogenetic relationship of the 16S rRNA gene sequence of phytoplasma isolates (*Acholeplasma laidlawii* is the outgroup and bootstrap replicates value is 1000).

Moreover, multilocus gene-based studies other than the 16SrRNA gene will be useful to get a clearer insight into the genetic closeness of the major phytoplasma diseases reported in Sri Lanka. Furthermore, the suggested study will be crucial to direct both SCWL and WCLW phytoplasma disease management strategies into a new and efficient pathway.

CONCLUSIONS

The sugarcane white leaf and Weligama coconut leaf wilt phytoplasmas are genetically and phylogenetically very close to each other based on their 16S rRNA gene. Therefore, a substantial potential has been identified in this study to categorize both phytoplasma in the same *Candidatus* phytoplasma species and subgroup in future studies. The other phytoplasma isolates including SCGS, CKW, and CYD reported in neighboring countries share significant similarities of the 16S rRNA gene with SCWL and WCLW phytoplasmas. Detailed studies on the phytoplasma taxonomy and subgroup/s of the WCLWD would also be crucial to further refine the genetic similarities of WCLW and SCWL phytoplasmas, which would be taking a new direction in those phytoplasma disease management studies.

REFERENCES

- Abeyasinghe, S., Kanatiwala de Silva, C., Abeyasinghe, P.D., Udagama, P., Warawichane, K., Aljafar, N., Kawicha, P. and Dickinson, M. (2016). Refinement of the taxonomic structure of 16SrXI and 16SrXIV phytoplasmas of gramineous plants using multilocus sequence typing. *Plant Disease*. 100(10), 2001–2010.
- Ariyaratne H.A.C.K., Everard J.M.D.T. and Karunanayake E.H. (2007). Diseased sugarcane in Sri Lanka is infected with sugarcane grassy shoot and/or sugarcane white-leaf phytoplasma. *Australian Plant Disease Notes*. 2, 123–125.
- Bertaccini, A., Duduk, B., Paltrinieri, S. and Contaldo, N. (2014). Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. *American Journal of Plant Sciences*. 5(12), 1763–1776.
- Bertaccini, A and Lee, I.M. (2018). Phytoplasmas: an update pp 1-29. In: Rao, G.P., Bertaccini, A., Fiore, N., Liefting, L.W. (Ed.), *Phytoplasmas: Plant Pathogenic Bacteria* - I. Springer, Singapore.
- Dayasena, Y.A.P.K., Panda, P., Thushari, A.N.W.S. and Rao, G.P. (2021). Geographical Distribution and Identification of Phytoplasma Strain Associated with Sugarcane White Leaf Disease in Sri Lanka. *Sugar Tech*. 23, 1351–1358.
- De Costa, D.M.D. (2009). *Clinical plant pathology; principles and laboratory techniques*. ISBN 978-955-51849-0-8.
- De Silva, R., Attanayaka, R. and Perera, C. (2021). Weligama coconut wilt phytoplasma isolate RDS1 16s ribosomal RNA gene, National Center for Biotechnology Information. U.S. National Library of Medicine. Unpublished. (Accessed on 21.12.2022) Available at <https://www.ncbi.nlm.nih.gov/nuccore/MZ822450>

- Eziashi, E. and Omamor, I. (2010). Lethal yellowing disease of the coconut palms (*Cocos nucifera* L.): an overview of the crises. *African Journal of Biotechnology*. 9(54), 9122–9127.
- Firrao G., Marcone C. and Bertaccini A. (2004). Phytoplasma classification. *Journal of Plant Pathology*. 86(4), 299.
- Gundersen, D. and Lee, I.M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea*. 35(3), 144–151.
- Gurr, G.M., Johnson, A.C., Ash, G.J., Wilson, B.A., Ero, M.M., Pilotti, C.A., Dewhurst, C.F. and You, M.S. (2006). Coconut Lethal Yellowing Diseases: A Phytoplasma: Threat to Palms of Global Economic and Social Significance. *Frontire Plant Science*. 26(7), 1521–1541.
- Jayathilaka, P.K. (1973). Virus disease in seed cane. A report submitted to the General Manager of Sri Lanka Sugarcane Industry.
- Jones, P. (2002). Coconut lethal diseases associated with phytoplasmas: a global review. Proceedings of the Expert Consultation on Sustainable Coconut Production through Control of Lethal Yellowing Disease (CFC Technical Paper No. 18).
- Jung H.Y., Sawayanagi T., Wongkaew P., Kakizawa S., Nishigawa H., Wei W., Oshima K., Miyata S., Ugaki M., Hibi T. and Namba, S. (2003). ‘*Candidatus Phytoplasma oryzae*’, a novel phytoplasma taxon associated with rice yellow dwarf disease. *International Journal of Systematic and Evolutionary Microbiology*. 53(6), 1925–1929.
- Keerthipala, A.P. (2016). Development of the sugar industry in Sri Lanka. *Sugar Tech*, 18, 612–626.
- Kirdat, K., Tiwarekar, B., Thorat, V., Sathe, S., Shouche, Y. and Yadav. A. (2021). ‘*Candidatus Phytoplasma sacchari*’, a novel taxon associated with Sugarcane Grassy Shoot (SCGS) disease. *International Journal of Systematic and Evolutionary Microbiology*. 71(1), 004591.
- Klinkong, S. (2008). Sugarcane white leaf phytoplasma partial 16S rRNA gene, isolate SCKK-BP, NCBI, National Center for Biotechnology Information. U.S. National Library of Medicine Unpublished. (Accessed on 21.12.2022) Available at <https://www.ncbi.nlm.nih.gov/nuccore/FM208257>
- Koshy, P.K., Solomon, J.J. and Nair, C.P.R. (2001). Diseases. In: Raju, C.R., Mohan, C., Maheshwarappa, H.P., Anithakumari, P. and Gopal, M. (Ed.), *Central Plantation Crops Research Institute Regional Station, Kayangulam 690 533, Kerala, India*.
- Kumara, A., Perera, L., Meegahakumbura, M., Aratchige, N. and Fernando, L. (2015). Identification of Putative Vectors of Weligama Coconut Leaf Wilt Disease in Sri Lanka pp 137-146. In: Chakravarthy, A. (Ed.), *New Horizons in Insect Science: Towards Sustainable Pest Management*. Springer, New Delhi.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*. 35(6), 1547–1549.
- Makarova, O., Contaldo, N., Paltrinieri, S., Kawube, G., Bertaccini, A. and Nicolaisen, M. (2012). DNA Barcoding for Identification of ‘*Candidatus Phytoplasmas*’ Using a Fragment of the Elongation Factor Tu Gene. *PLOS One*. 7(12), 52092.

- Manimekalai, R., Nair, S. and Soumya, V.P. (2014). Evidence of 16SrXI group phytoplasma DNA in embryos of root wilt diseased coconut palms. *Australasian Plant Pathology*. 43(1), 93–96.
- Nair, S., Manimekalai, R., Soumya, V.P. and Likhitha, K.C. (2016). Dual-labeled probe-based real-time PCR method for detection of 16SrXI-B sub-group phytoplasma associated with coconut root wilt disease in India. *Australasian Plant Pathology*. 45, 187-189.
- Nasare, K., Yadav, A., Singh, A.K., Shivasharanappa, K.B., Nerkar, Y.S. and Reddy, V.S. (2007). Molecular and symptom analysis reveal the presence of new phytoplasmas associated with sugarcane grassy shoot disease in India. *Plant Disease*. 91(11), 1413–1418.
- Nejat, N., Sijam, K., Abdullah, S.N.A., Vadamalai, G. and Dickinson, M. (2009). Phytoplasmas associated with disease of coconut in Malaysia: phylogenetic groups and host plant species. *Plant Pathology*. 58(6), 1152–1160.
- Nguyen, T.D., Mai, Q.V., Vu, H.D., Ngo, B.G., Ha, C.V. and Trinh, H.X. (2013). Detection and identification of phytoplasmas associated with longan witches' broom in Vietnam. *Phytopathogenic Mollicutes*. 2(1), 23-27.
- Perera, L., Meegahakumbura, M.K., Wijesekara, H.R.T., Fernando, W.B.S. and Dickinson, M.J. (2012). A phytoplasma is associated with the Weligama coconut leaf wilt disease in Sri Lanka. *Journal of Plant Pathology*. 94(1), 205-209.
- Rao, G. P., Srivastava, S., Gupta, P.S., Singh, A., Singh, M. and Marcone, C. (2008). Detection of sugarcane grassy shoot phytoplasma infecting sugarcane in India and its phylogenetic relationships to closely related phytoplasmas. *Sugar Tech*. 10, 74–80.
- Rao, G.P., Madhupriya, V., Tiwari, A.K. and Baranwal, V. (2014). Identification of sugarcane grassy shoot-associated phytoplasma and one of its putative vectors in India. *Phytoparasitica*. 42, 349–354.
- Rao, G.P., Alvarez, E. and Yadav, A. (2018). Phytoplasma diseases of industrial crops pp 91–122. In: Rao, G.P., Bertaccini, A., Fiore, N., Liefting, L.W. (Ed.), *Phytoplasmas: Plant Pathogenic Bacteria—I*, Springer, Singapore.
- Seneviratne, J.A.U.T. (2008). An investigation of the secondary transmission of sugarcane white leaf disease in Sri Lanka, PhD Thesis, University of Peradeniya, Sri Lanka.
- Sharma, P., Singh, J., Kumar, P., Kumar, R. and Baranwal, V.K. (2020). Emerging Incidence and Diversity of Sugarcane Grassy Shoot Phytoplasma in Western Uttar Pradesh, India. National Center for Biotechnology Information. U.S. National Library of Medicine. Unpublished. (Accessed on 21.12.2022) <https://www.ncbi.nlm.nih.gov/nuccore/MT510165>
- Sievers, F., Wilm, A., Dineen, D.G., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D. and Higgins, D. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*. 7(1), 539–544
- Tamura, K. and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*. 10(3), 512–526.

- Tymon, A.M., Jones, P. and Harrison, N.A. (2008), Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. *Annals of Applied Biology*. 132(3), 437–452.
- Wijesekara, H.T.R., Perera, L., Wickramananda, I.R., Herath, I., Meegahakumbura, M.K., Fernando W.B.S. and De Silva P.H.P.R. (2008). Preliminary Investigation on Weligama Coconut Leaf Wilt Disease: A New Disease in Southern Sri Lanka. *Proceedings of the Second Symposium on Plantation Crop Research- coconut Research Institute, Lunuwila, Sri Lanka*. 336-341.
- Viswanathan, R. and Rao, G.P. (2011). Disease scenario and management of major sugarcane diseases in India. *Sugar Tech*. 13, 336–353.
- Wijesekara, R.H.T., Sadapani, S. and Manimekalai, R. (2013). Weligama coconut wilt phytoplasma isolate RDS1 16s ribosomal RNA gene, National Center for Biotechnology Information. U.S. National Library of Medicine. Unpublished. (Accessed on 21.12.2022) Available at: <https://www.ncbi.nlm.nih.gov/nuccore/KF993431>
- Yadav, A., Thorat, V., Deokule, S., Shouche, Y. and Prasad, D.T. (2017). New subgroup 16SrXI-F phytoplasma strain associated with sugarcane grassy shoot (SCGS) disease in India. *International Journal of Systematic and Evolutionary Microbiology*. 67(2), 374–378.
- Zhang, R.Y., Huang, Y.K. and Wang, X.Y. (2020). Sugarcane white leaf phytoplasma clone LCYC9 16S ribosomal RNA gene, partial sequence - nucleotide - NCBI, National Center for Biotechnology Information. U.S. National Library of Medicine. Unpublished. (Accessed on 21.12.2022) Available at: <https://www.ncbi.nlm.nih.gov/nuccore/MT649297>
- IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma taxonomy group (2004). ‘Candidatus Phytoplasma’, a taxon for the wallless, non-helical prokaryotes that colonize plant phloem and insects. *International Journal of Systematics Evolutionary Microbiology*. 54(4), 1243–1255.

