Mixed Infection of *Sugarcane Yellow Leaf Virus* and Grassy Shoot Phytoplasma in Yellow Leaf Affected Indian Sugarcane Cultivars

Kadirvel Nithya¹, Balasubramaniam Parameswari², and Rasappa Viswanathan¹*

¹ICAR-Sugarcane Breeding Institute, Coimbatore 641 007, Tamil Nadu, India
²ICAR-Sugarcane Breeding Institute, Regional Centre, Karnal 132 001, Haryana, India

(Received on June 3, 2020; Revised on June 17, 2020; Accepted on June 25, 2020)

Sugarcane is an important sugar crop contributes more than 80% of world sugar production. Mosaic, leaf fleck, and yellow leaf (YL) are the major viral diseases affecting sugarcane, amongst YL occurrence is widely reported in all the sugarcane growing countries. It is caused by *Sugarcane yellow leaf virus* (SCYLV) and detailed works were done on complete genome characterization, transmission, and management. However, in countries like Egypt, South Africa, Cuba, Mauritius and Hawaii, the disease was reported to the cause of sugarcane yellow phytoplasma (SCYP) and/or SCYLV as single/combined infections. Hence, we have investigated in detail to identify the exact *Candidatus* phytoplasma taxon associated in Indian cultivars affected with YL. The sequencing results and the restriction fragment length polymorphism pattern of the PCR products using the universal phytoplasma primers confirmed presence of sugarcane grassy shoot (SCGS) phytoplasma (16SrXI group) in the YL-affected plants. Mixed infection of SCYLV and SCGS phytoplasma was estimated as 32.8% in YL affected plants. Evolutionary genetic relationship between SCYP and SCGS phytoplasma representatively taken from different countries showed that SCYP from South Africa and Cuba were diverged from others and had a higher similarity with SCGS phytoplasma. Although we wanted to identify SCYP from YL affected Indian sugarcane cultivars, the study clearly indicated a clear absence of SCYP in YL affected plants and we found SCYLV as the primary cause for the disease.

**Keywords**: nested PCR, phylogeny, restriction fragment length polymorphism, sugarcane grassy shoot phytoplasma, *Sugarcane yellow leaf virus*

**Handling Editor**: Young-Su Seo

Sugarcane is one of the most important commercial crops grown mainly for sugar in many countries and also for bio energy production from its by-products such as, bagasse and molasses. It is being affected by several biotic factors like fungi, bacteria, viruses and phytoplasma causing reduction in production and productivity according to its severity. Among the viral diseases affecting sugarcane, yellow leaf (YL) (showing yellowing of midrib and lamina and in severe cases drying of entire leaf canopy) occurrence is reported in most of the sugarcane growing countries sometimes with up to cent percent incidence (Lehrer et al., 2008; Rassaby et al., 2004; Viswanathan, 2016). In 1960s, it was suspected to occur from East Africa and authentic reports of the disease had come from Hawaii in 1980s (Schenck 1990) followed by South Africa (Cronje et al., 1998) and Cuba (Arocha et al., 1999) and now distributed in all the sugarcane-growing countries causing losses up to 60% in susceptible varieties (Arocha et al., 2000; Comstock et al., 1998). In India, it was first reported during the year 1999 (Viswanathan, 2002; Viswanathan et al., 1999) since then its occurrence and severity continued in all the varieties (Viswanathan et al., 2006). The causative *Sugarcane yellow leaf virus* (SCYLV) belonging to *Polerovirus*; family *Luteoviridae* was reported in Hawaii, Brazil, Florida, and Australia whereas only phytoplasma association was reported from South Africa and Cuba and named as Sugar-
Mixed Infection of SCYLV and SCGS Phytoplasma in YL Affected Indian Sugarcane Cultivars

The SCGS disease causing phytoplasma is a small, oval to spherical shaped, obligate, endophytic, cell wall-less intracellular parasites resides in the phloem sieve tube and nearby companion cells, and vascular bundles of the host with 0.816-1.603 µm diameter in size (Nithya, unpublished) and have very small genome size of 505.17 kb (Kirdat et al., 2020). They are capable of invading and replicating in both plant and insect hosts. In plants, they inhabit in the nutrient-rich phloem tissues and spread systemically through sieve pores and induce various symptoms such as chlorotic leaves, stunting, and proliferation of axillary shoots, etc. due to abnormal endogenous phytohormone signaling (Bertaccini et al., 2014). In India, sugarcane is widely cultivated in ~5.2 M ha in varying climatic conditions and YL has been distributed throughout the country. Although SCYLV has been found in all the states, association of SCYP is not known except the report of Gaur et al. (2008) and Kumar et al. (2015) from subtropical India. Hence, an attempt was made to investigate the presence of SCYP in YL affected sugarcane cultivars from different geographical locations in India. Our studies clearly proved that SCYLV is the primary cause of YL disease in sugarcane cultivars and no SCYP association was found in any of the YL-affected plants, whereas some of the SCYLV infected plants had SCGS phytoplasma infection.

Materials and Methods

Sample collection. During the year 2016-2018, sugarcane leaves showing YL symptoms with severity grades of 3-4 with intense yellowing of leaf mid rib spread along the lamina region and severe drying of leaf from tip to downwards (Viswanathan et al., 2016) (Fig. 1A and B) were collected from Plant Pathology Farm, ICAR-Sugarcane...
Breeding Institute (ICAR-SBI) Coimbatore, and at ICAR-SBI regional Centre, Agali, Kerala and stored at –80°C deep freezer until further processing. Amongst, the cv. Co 10031 in advanced varietal trial was strongly suspected for phytoplasma association as it had YL symptoms with severity grade of 4 along with stunting compared to other YL plants. All the leaves, roots and different internode (3rd, 6th, and 9th) samples were taken for the diagnosis. The sample collection details were given in Table 1.

**RNA extraction and RT-PCR for SCYLV diagnosis.**

Total RNA was extracted from the collected leaf samples,

| No. | Cultivar   | Origin of the genotype/variety     | SCYLV | SCGS phytoplasma | Positive to both universal and custom designed phytoplasma primers |
|-----|------------|-----------------------------------|-------|------------------|---------------------------------------------------------------------|
| 1   | 57 NG 56   | Coimbatore                        | +     | +                | –                                                                   |
| 2   | B 38192    | Coimbatore                        | +     | –                | –                                                                   |
| 3   | Co 86010   | Coimbatore                        | +     | +                | +                                                                   |
| 4   | Co 86032   | Coimbatore                        | +     | –                | –                                                                   |
| 5   | Co 87269   | Coimbatore                        | +     | +                | +                                                                   |
| 6   | Co 99004   | Coimbatore                        | +     | –                | –                                                                   |
| 7   | Co 10031   | Coimbatore                        | +     | +                | +                                                                   |
| 8   | Co 11015   | Coimbatore                        | +     | –                | –                                                                   |
| 9   | CoC 671    | Cuddalore, Tamil Nadu             | +     | +                | +                                                                   |
| 10  | CoS 767    | Shahjahanpur, Uttar Pradesh       | +     | +                | +                                                                   |
| 11  | CoBl 96009 | Buralikson, Assam                 | +     | –                | –                                                                   |
| 12  | CoPant 9421 | Pantnagar, Uttarakhand           | +     | +                | –                                                                   |
| 13  | CoS 767    | Shahjahanpur, Uttar Pradesh       | +     | +                | +                                                                   |
| 14  | CoT 8201   | Tirupathi, Andhra Pradesh         | +     | +                | –                                                                   |
| 15  | PI 11131   | EID Parry, Pugalur                | +     | –                | –                                                                   |
| 16  | BO 78      | Pusa, Bihar                       | +     | –                | –                                                                   |
| 17  | Co 449     | Coimbatore                        | +     | +                | +                                                                   |
| 18  | Co 740     | Coimbatore                        | +     | –                | –                                                                   |
| 19  | Co 875     | Coimbatore                        | –     | –                | –                                                                   |
| 20  | Co 1169    | Coimbatore                        | –     | –                | –                                                                   |
| 21  | Co 6304    | Coimbatore                        | +     | +                | –                                                                   |
| 22  | Co 8213    | Coimbatore                        | –     | –                | –                                                                   |
| 23  | Co 8342    | Coimbatore                        | +     | –                | –                                                                   |
| 24  | Co 85004   | Coimbatore                        | –     | –                | –                                                                   |
| 25  | Co 89003   | Coimbatore                        | –     | –                | –                                                                   |
| 26  | Co 94008   | Coimbatore                        | +     | –                | –                                                                   |
| 27  | Co 95007   | Coimbatore                        | +     | –                | –                                                                   |
| 28  | Co 88015   | Coimbatore                        | –     | –                | –                                                                   |
| 29  | Co 06027   | Coimbatore                        | +     | –                | –                                                                   |
| 30  | Co 06030   | Coimbatore                        | +     | –                | –                                                                   |
| 31  | Co 09014   | Coimbatore                        | –     | –                | –                                                                   |
| 32  | Co 09021   | Coimbatore                        | +     | –                | –                                                                   |
| 33  | Co 11001   | Coimbatore                        | –     | –                | –                                                                   |
| 34  | Co 11015   | Coimbatore                        | +     | –                | –                                                                   |
| 35  | Co 13015   | Coimbatore                        | –     | –                | –                                                                   |
| 36  | Co 85004   | Coimbatore                        | +     | –                | –                                                                   |
| 37  | CoA 8401   | Anakapalle, Andhra Pradesh        | +     | –                | –                                                                   |
| 38  | CoA 89081  | Anakapalle, Andhra Pradesh        | +     | –                | –                                                                   |

Continued
ground in liquid nitrogen and resuspended with 1 µl TRI reagent (Sigma, St. Louis, MO, USA) by following the manufacturer’s protocol. The pellet was dissolved in a final volume of 30 µl RNase free water and stored at –80°C. It was treated with DNase I (1 U/µl) (Thermo Fischer Scientific, Waltham, MA, USA) along with 10× reaction buffer with MgCl₂ and RNase free water to make up the final volume 10 µl, kept at 37°C for 2 h incubation in water bath and were enzyme de activated by adding 1 µl of 50 mM EDTA and continued incubation at 60-65°C for 10 min. The integrity, concentration and purity of RNA were assessed on 1% EtBr-stained agarose gel visualized under UV light and on a Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) and stored at –80°C. The SCYLV primer was designed from the consensus coat protein regions through primer 3.0 and sigma oligo evaluator, named as SCYLV-FP (5’-CTGAGAATTCTAGGATACGGGCGCTAACCGCCT-3’) and SCYLV-RP (5’-CTGCAAGCTTGGTATTTGGGATTCTGGAATA-3’) which can amplify complete ORF 3 of coat protein gene were used in this study. One microgram of total RNA per reaction was reverse transcribed using RevertAid H Minus First Strand cDNA Synthesis Kit (MBI, Fermentas, Waltham, MA, USA), primed with 50 pmol of SCYLV-615R by following the manufacturer’s protocol in a thermal cycler (Eppendorf, Hamburg, Germany). The PCR reaction was performed in a total volume of 25 µl containing 2.5 µl of 10× Taq buffer with 15 mM MgCl₂, 0.5 µl of 10 mM dNTP mix, 10 µM of each forward and reverse primers, 1.25 units of Taq polymerase (Origin, Kerala, India), 2 µl of cDNA and sterile MilliQ water to make up the final volume. The PCR programme was performed with initial denaturation

| No. | Cultivar   | Origin of the genotype/variety | SCYLV | SCGS phytoplasma | Positive to both universal and custom designed phytoplasma primers |
|-----|------------|-------------------------------|-------|-----------------|------------------------------------------------------------------|
| 39  | CoA 89082  | Anakapalle, Andhra Pradesh    | +     | +               | -                                                                |
| 40  | CoA 90081 (1) | Anakapalle, Andhra Pradesh    | +     | -               | -                                                                |
| 41  | CoA 90081 (2) | Anakapalle, Andhra Pradesh    | +     | -               | -                                                                |
| 42  | CoA 92082  | Anakapalle, Andhra Pradesh    | -     | -               | -                                                                |
| 43  | CoA 01082  | Anakapalle, Andhra Pradesh    | -     | -               | -                                                                |
| 44  | CoBln 9006 | Buralikson, Assam              | +     | -               | -                                                                |
| 45  | CoBln 9101 | Buralikson, Assam              | +     | -               | -                                                                |
| 46  | CoBln 9104 | Buralikson, Assam              | +     | -               | -                                                                |
| 47  | CoC 779    | Cuddalore, TamilNadu           | +     | -               | -                                                                |
| 48  | CoC 99061  | Cuddalore, TamilNadu           | +     | +               | +                                                                |
| 49  | CoC 01061  | Cuddalore, TamilNadu           | +     | +               | +                                                                |
| 50  | CoG 93076  | Gudiyattam, Tamil Nadu          | -     | -               | -                                                                |
| 51  | CoLk 8002  | Lucknow, Uttar Pradesh         | +     | +               | +                                                                |
| 52  | CoM 7712   | Padegaon, Maharashtra          | -     | -               | -                                                                |
| 53  | CoPant 84214 | Punnaguar, Uttarakhand         | -     | -               | -                                                                |
| 54  | CoS 8342   | Shahjanhanpur, Uttar Pradesh   | +     | -               | -                                                                |
| 55  | CoS 8436   | Shahjanhanpur, Uttar Pradesh   | +     | +               | +                                                                |
| 56  | CoSe 92423 | Seorahi, Uttar Pradesh         | +     | -               | -                                                                |
| 57  | CoSnk 03754 | Sankeswar, Kamataka            | +     | -               | -                                                                |
| 58  | CoSi 86071 | Sirugamani, Tamil Nadu          | +     | +               | +                                                                |
| 59  | CoV 89101  | Vuyyuru, Andhra Pradesh        | +     | +               | +                                                                |
| 60  | CoV 92101  | Vuyyuru, Andhra Pradesh        | +     | +               | +                                                                |
| 61  | CoV 92102  | Vuyyuru, Andhra Pradesh        | +     | +               | -                                                                |
| 62  | CoV 94101  | Vuyyuru, Andhra Pradesh        | +     | +               | +                                                                |
| 63  | 51 NG 156  | Coimbatore                     | -     | -               | -                                                                |
| 64  | 57 NG 77   | Coimbatore                     | -     | -               | -                                                                |

YL, yellow leaf; SCYLV, Sugarcane yellow leaf virus; SCGS, sugarcane grassy shoot; RT-PCR, reverse transcription PCR.
aCoimbatore: Germplasm material.
at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 4 min, annealing at 56°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. All the amplified products were run on 1.5% agarose gels stained with ethidium bromide (0.5 μg/ml) and gel images were documented in a gel documentation unit (G-BOX EF, Syngene, Cambridge, UK).

**DNA extraction and nested PCR for phytoplasma diagnosis.** Total genomic DNA was extracted from 0.5 g leaves, ground in liquid nitrogen by following the cetyl trimethyl ammonium bromide method (Doyle and Doyle, 1990), treated with RNase I (10 U/μl) (Thermo Fischer Scientific) and purified. The integrity, concentration and purity of DNA was assessed on 0.8% EtBr-stained agarose gel visualized under UV light and on a Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) and stored at –20°C. Based on the genomic DNA concentration it was diluted up to 50 ng/μl for the PCR analysis. PCR was performed using the universal phytoplasma specific primers P1/P7 (Deng and Hiruki, 1991) in the first round PCR and R16F2n 5′-GAAACGACTGCTAAGACTGG-3′ and R16R2 5′-TGACCGGCGGTGTGATCAAAACCCCG-3′ primer pairs were used in the nested PCR (Gunderson and Lee, 1996; Lee et al., 1993) for the amplification of both large (23S), small (16S), and 16S-23S ribosomal spacer regions. The PCR reaction was performed in a total volume of 25 μl containing, 2.5 μl of 10× Taq buffer with 15 mM MgCl₂, 2 μl of 2.5 mM dNTP mix, 10 μM of each forward and reverse primers, 0.33 units of Taq polymerase (Origin), 1 μl of genomic DNA and sterile MilliQ water to make up the final volume.

The PCR cyclic conditions for the P1/P7 primer was initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 1 min 30 s, extension at 72°C for 1 min 30 s, and a final extension at 72°C for 10 min. The three PCR amplified products were run on 1.2% agarose gels stained with ethidium bromide (0.5 μg/ml) and gel documented. All the PCR amplified products were run on 1.2% agarose gels stained with ethidium bromide (0.5 μg/ml) and gel documented. The positive samples were eluted from the low melting agarose gel using GenElute Gel Extraction Kit (Sigma), purified and was further checked in agarose gel and quantified in Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific). The positive samples were sent for double pass sequencing to commercial sequencing firm Bioserve Technologies, Hyderabad, India.

**Restriction fragment length polymorphism (RFLP) analyses.** Restriction fragment length polymorphism analysis was performed to the nested PCR product of the YL samples amplified by R16F2n/R16R2n primers. The restriction enzymes Bfa I (FspB I), Msp I (Hpa II), Mse I, Hpa I (KspA I), and Hinf I (10 U/μl) (Thermo Fischer Scientific) were selected based on the in silico virtual RFLP iphycalculator enzymes list to identify the phytoplasma. The reaction was performed with reaction volume of 20 μl containing 2 μl of 10× restriction digestion buffer with bovine serum albumin (Tango/R/B buffers according to the enzymes), 3 μl of PCR product (500-1,000 ng/μl), 2 μl of restriction enzymes and sterile MilliQ water to make up the final volume kept at 37°C for 2 h incubation in water bath and were run on 1.5% agarose gels and documented. SCGS phytoplasma cv. CoV 92101 was used as a positive control to confirm the restriction pattern of the phytoplasma in YL samples.

**Phylogenetic analysis.** Quality of all the samples sequences were confirmed based on the q-value ( > 30). All the sequences were subjected into BLASTn searches at NCBI website (Altschul et al., 1990) and pair wise multiple sequence alignment of the selected sequences were made through Bio edit (Hall, 1999) and maximum likelihood Tamura-Nei model based phylogenetic tree analysis was performed with nearest neighbour interchange tree options (MEGA X v.10.1.6) with 1,000 bootstrap replications to know the evolutionary genetic relatedness (Kumar et al., 2018) of all the samples collected from different varieties and fields.

**Results**

Out of 64 samples tested, SCYLV was detected from 48 samples with the expected amplification of 591 bp in RT-PCR assays (Fig. 2). In case of phytoplasma, none of the samples had shown any amplification in the first round of direct PCR using P1/P7 primers except the positive SCGS
phytoplasma cv. CoV 92101. Whereas, in nested PCR analysis 14 samples were found positive to both the phytoplasma primers R16F2n/R16R2 and YL FP1/RP1 with the expected amplification of 1,200 bp and 900 bp, respectively (Figs. 3 and 4). The YL affected cv Co 10031 leaf, root and internode (3rd, 6th, and 9th) samples were found positive to YL FP1/RP1 phytoplasma primers with the expected amplification of 900 bp (Fig. 5). The primers YL FP1/RP1 designed from ribosomal RNA regions from SCYP consensus sequences were found better than R16F2n/R16R2 in terms of intense amplification and wide sample coverage. Out of 64 samples tested, YL FP1/RP1 shown positive to 21 samples and R16F2n/R16R2 shown positive to 15 samples and both the primers shown intense amplification to 13 samples viz. 57 NG 56, Co 449, Co 6304, Co 86010, Co 86032, Co 87269, Co 10031, CoC 671, CoC 99061, CoPant 94211, CoS 8436, CoV 92102, and CoV 94101. Mixed infection of SCYLV and SCGS phytoplasma was estimated as 32.8% in YL affected plants. All the genomic sequences analyzed through Blastn had shown 98.92% to 99.82% similarity with 98.25-100% query coverage to other SCGS phytoplasma isolates, amongst maximum similarity was found with sugarcane SCGS phytoplasma sequences submitted from India, Thailand and with
white leaf phytoplasma from Sri Lanka (MN174860.2). All the sequences of both the primers have been submitted at GenBank with the accession numbers MN904464.1 to MN904486.1 (Table 2). Since, the SCGS phytoplasma was confirmed in the YL plants, its similarity with the available 22 SCYP sequences as well as from other SCGS and sugarcane white leaf (SCWL) phytoplasma sequences submitted from different countries were analyzed for evolutionary genetic relatedness. But, the SCYP sequences submitted from Cuba during the years 2001 (2 numbers), 2003 and 2005 and one SCYP isolate from Africa (Cronje at al., 1999) were < 250 nucleotides in length compared to others hence, were not considered for the analysis (Table 3).

The results of the remaining 15 SCYP sequences and other SCGS and SCWL phytoplasma sequences representationally taken from different countries such as India, Thailand, Hawaii, Sri Lanka, China, and Vietnam along with phytoplasma sequences obtained from the YL plants in this study were subjected to phylogenetic analyses. The results of 31 sequences segregated into three major clusters, the first one contained all the SCGS, SCWL phytoplasmas belonging to 16SrXI (Rice yellow dwarf) group and the second one contained all the SCYP belonging to 16SrI (aster yellows; Ca. Phytoplasma asteris) group and the third one contained the SCYP from South Africa and Cuba, both shared the maximum similarity with 16SrXI group. All the SCGS phytoplasma used in this study and SCWL phytoplasma reported from Thailand, Vietnam, and China showed highest similarity of >80% with SCYP India, 2014 followed by SCYP Cuba, 2010 (HM804282.1) and SCYP Brazil, 2009. Acholeplasma sp. was used as out group in the tree (Fig. 6, Supplementary Table 1).

Of the total 22 SCYP sequences, 20 only were analyzed in pairwise multiple sequence alignment using Clustal W (leaving the two sequences submitted from India during the year 2016 from see A gene and LeucyltRNA gene partial sequences) in that, the South African sequence submitted by Cronje in the year 2000 (AF056094.1) has the maximum length i.e. 1,929 nucleotides. Hence, all the SCYP sequences were compared by taking that one as a reference in SCGS, sugarcane grassy shoot; YL, yellow leaf.

---

**Table 2. SCGS phytoplasma sequences from YL plants submitted at NCBI, GenBank**

| No. | Isolate name | GenBank accession no. (R16F2n/R16R2) | No. | Isolate name | GenBank accession no. (YL FP1/RP1) |
|-----|--------------|-------------------------------------|-----|--------------|-------------------------------------|
| 1   | CB-57NG56    | MN904473.1                          | 11  | CB-Co 86010  | MN904486.1                          |
| 2   | CB-CoC 0106  | MN904472.1                          | 12  | CB-Co 6304   | MN904476.1                          |
| 3   | CB-CoC 671   | MN904471.1                          | 13  | CB-CoC 0106  | MN904482.1                          |
| 4   | CB-CoV 92101 | MN904470.1                          | 14  | CB-Co 10031  | MN904475.1                          |
| 5   | CB-CoC 99061 | MN904469.1                          | 15  | CB-CoC 99061 | MN904479.1                          |
| 6   | CB-CoS 8436  | MN904468.1                          | 16  | CB-CoS 8436  | MN904478.1                          |
| 7   | CB-Co 86010  | MN904467.1                          | 17  | CB-CoV 92101 | MN904480.1                          |
| 8   | CB-Co Pant 84211 | MN904466.1                | 18  | CB-CoC 671   | MN904481.1                          |
| 9   | CB-Co 6304   | MN904465.1                          | 19  | CB-CoPant 84211 | MN904485.1                 |
| 10  | CB-Co10031   | MN904464.1                          | 20  | CB-57NG56    | MN904483.1                          |
|     |              |                                     | 21  | CB-CoV 92102 | MN904484.1                          |

SCGS, sugarcane grassy shoot; YL, yellow leaf.

Discussion

SCYLV was confirmed as the causative agent of the YL disease in Indian cultivars and its characterization was done up to complete genome level and existence of different genotypes of the virus was reported including the new genotype SCYLV-IND (Chinnaraja et al., 2013, Viswanathan et al., 2008). Since mixed infections of SCYLV and SCYP were reported in some countries, we suspected the possible association of SCYP in YL of sugarcane hence, investigated on their probable mixed infections in Indian sugarcane cultivars, and importantly to develop effective diagnostics and to characterize exact Ca. phytoplasma taxon associated with Indian YL disease. SCYP was first reported in South Africa and Cuba (Arocha et al., 1999; Cronje et al., 1998), and in Mauritius mixed infections of SCYLV and SCYP...
Table 3. Sugarcane yellow leaf phytoplasma sequence submissions at GenBank, NCBI (as on January 2020)

| No. | Sugarcane yellows phytoplasma | NCBI GenBank accession no. | Size (bp) | Country | Author            | Title                                                                 | Year |
|-----|--------------------------------|----------------------------|-----------|---------|-------------------|----------------------------------------------------------------------|------|
| 1   | Sugarcane yellows phytoplasma type I “ScYP I-Afr” | AF090169.1 | 275 | Africa | Cronje et al. | Association of a phytoplasma with a yellow leaf syndrome of sugarcane in Africa | 1999 |
| 2   | Sugarcane yellows phytoplasma type I strain ScYP 1-Barb | AF056094.1 | 1,929 | South Africa | Cronje CPR | Direct submission | 2000 |
| 3   | Cuban sugarcane yellow leaf phytoplasma isolate II | AF421545.1 | 234 | Cuba | Arocha et al. | Genetic characterization of phytoplasmas associated with yellow leaf syndrome (YLS) in Cuba | 2001 |
| 4   | Cuban sugarcane yellow leaf phytoplasma isolate I | AF421544.1 | 235 | Cuba | Arocha et al. | Genetic characterization of phytoplasmas associated with yellow leaf syndrome (YLS) in Cuba | 2001 |
| 5   | Cuban sugarcane yellow leaf phytoplasma | AY257548.1 | 226 | Cuba | Arocha et al. | Distribution and genetic characterization of phytoplasmas associated with YLS in Cuba | 2003 |
| 6   | Saccharosydne sugarcane yellow leaf phytoplasma | AY725237.1 | 226 | Cuba | Arocha et al. | Transmission of a sugarcane yellow leaf phytoplasma by the delphacid planthopper Saccharosydne saccharivora, a new vector of sugarcane yellow leaf syndrome | 2005 |
| 7   | Sugarcane yellows phytoplasma type I | EU170474.1 | 1,420 | India (CoLk 8102) | Gaur et al. | Sugarcane yellow leaf phytoplasma associated for the first time with sugarcane yellow leaf syndrome in India | 2008 |
| 8   | Sugarcane yellows phytoplasma type I clone ScYL-Br 2 | EU423901.1 | 1,030 | Brazil | da Silva et al. | Molecular identification of phytoplasma associated with sugarcane yellow leaf syndrome | 2008 |
| 9   | Sugarcane yellows phytoplasma type I clone ScYL-Br 1 | EU423900.1 | 1,250 | Brazil | Silva et al. | Molecular identification and phylogenetic analysis of a group 16SrI-B phytoplasma associated with sugarcane yellow leaf syndrome in Brazil | 2009 |
| 10  | Cuban sugarcane C1051-73 yellow leaf phytoplasma Sugarcane cultivar C1051-73 | HQ116553.1 | 1,082 | Cuba | Soufi and Komor | Identification of sugarcane yellow leaf phytoplasma in Hawaiian sugarcane cultivars and its elimination by hot water treatment | 2010 |
| 11  | Cuban sugarcane Ja60-5 yellow leaf phytoplasma | HM804282.1 | 1,089 | Cuba | Soufi and Komor | Identification of Sugarcane yellow leaf phytoplasma in Hawaiian sugarcane cultivars and its elimination by hot water treatment | 2010 |
| 12  | Sugarcane yellows phytoplasma type I strain ScYP 1-Afr | AF056095.1 | 1,891 | South Africa | Cronje CPR. | - | | |
| 13  | Egyptian sugarcane yellow leaf phytoplasma isolate GT54-9 | JX157630.1 | 1,246 | Egypt | ElSayed et al. | Molecular detection and identification of phytoplasma associated with sugarcane yellow leaf syndrome in Egypt | |
Table 3. Continued

| No. | Sugarcane yellows phytoplasma | NCBI GenBank accession no. | Size (bp) | Country | Author | Title | Year |
|-----|--------------------------------|---------------------------|----------|---------|--------|-------|------|
| 14  | Egyptian sugarcane yellow leaf phytoplasma isolate Cane1 | JX157631.1 | 1,247 | Egypt | ElSayed et al. | Molecular detection and identification of phytoplasma associated with sugarcane yellow leaf syndrome in Egypt | 2012 |
| 15  | Sugarcane yellows phytoplasma type I isolate CoLk 94184 Lucknow | KJ491100.1 | 1,184 | India Lucknow | Kumar et al | Characterization of 16SrI-B group phytoplasma associated with sugarcane leaf yellows disease in India | 2014 |
| 16  | Sugarcane yellows phytoplasma type I isolate CoSe 92423 Lucknow | KJ491099.1 | 1,196 | India Lucknow | Kumar et al | Characterization of 16SrI-B group phytoplasma associated with sugarcane leaf yellows disease in India | 2014 |
| 17  | Sugarcane yellows phytoplasma type I leucyl tRNA synthetase (leu) gene, partial cds | KT335269.1 | 867 | India (Kamal, Haryana) | Singh and Rao | Partial sequences of secA, leu and GyrB genes from Indian phytoplasmas | 2016 |
| 18  | Sugarcane yellows phytoplasma type I SecA (secA) gene, partial cds | KT335270.1 | 540 | India Karnal, Haryana | Singh and Rao | Partial sequences of secA, leu and GyrB genes from Indian phytoplasmas | 2016 |
| 19  | Sugarcane yellows phytoplasma type I strain MxScYL-2 partial sequence | MH891145 | 1,539 | Mexico | Perez-Lopez et al. | First report of sugarcane yellow leaf disease in Mexico and detection of a ‘Candidatus Phytoplasma asteris’-related strain in affected plants | 2018 |
| 20  | Sugarcane yellows phytoplasma type I strain MxScYL-2 partial sequence | MH891144.1 | 1,539 | Mexico | Perez-Lopez et al. | First report of sugarcane yellow leaf disease in Mexico and detection of a ‘Candidatus Phytoplasma asteris’-related strain in affected plants | 2018 |
| 21  | Sugarcane yellows phytoplasma type I strain MxScYL-3 | MH891146.1 | 1,539 | Mexico | Perez-Lopez et al. | First report of sugarcane yellow leaf disease in Mexico and detection of a ‘Candidatus Phytoplasma asteris’-related strain in affected plants | 2018 |
| 22  | Vietnamese sugarcane yellow leaf phytoplasma isolate SCYLNAVN | MF784512.1 | 1,210 | Vietnam | Hoat et al. | Molecular detection and identification of phytoplasma associated with sugarcane yellow leaf syndrome in Nghe An province, Vietnam | 2018 |
Mixed Infection of SCYLV and SCGS Phytoplasma in YL Affected Indian Sugarcane Cultivars

were reported (Aljanabi et al., 2001; Parmessur et al., 2002). In Africa, two different phytoplasma viz. western X-disease and SCWL phytoplasma were reported (Aljanabi et al., 2001; Cronje and Bailey, 1999) with 98.8% sequence similarity. In Australia, the single sugarcane plant was reported with multiple infections of two or more distinctly different phytoplasma groups, most of them were infected by the tomato big bud (TBB) and less frequent were sun hemp witches'-broom (SUNHP) of the Faba Bean Phyllox group, stylosanthes little leaf (StLL) of the loofah witches-broom (LfWB) group and Maryland aster yellows (AY1) of the AY group (Tran-Nguyen et al., 2000). Also, the less frequently detected phytoplasmas were mainly from asymptomatic plants while the TBB phytoplasma was detected with equal frequency in both symptomatic and asymptomatic plants (Schneider et al., 1999). Recently, ‘Ca. P. asteris’ subgroup (16Sr I-B) in Brazil and Egypt (Silva et al., 2009; ElSayed and Boulila, 2014); white leaf phytoplasma in Thailand (Soufi et al., 2013); latent infections of 16SrI (aster yellows) and 16SrXI (Rice yellow dwarf) groups in asymptomatic Hawaiian sugarcane cultivars (Soufi and Komor, 2014) and Egyptian sugarcane cultivars based on sequencing and RFLP analyses (ElSayed et al., 2016) were reported from YL affected plants. In India, 16SrXII (Stolbur group) (Gaur et al., 2008) and 16SrI-B (Aster Yellow group) (Kumar et al., 2015) were reported.

Fig. 6. Phylogenetic tree was constructed by maximum likelihood neighbour joining method using MEGA-X v10.1.6 showing the relationship between the sugarcane grassy shoot phytoplasma and sugarcane yellow leaf phytoplasma. Bootstrap values were expressed as percentage of 1,000 replications and branch lengths are proportional to the number of substitutions. Acholeplasma sp. was used to out group the tree.

from asymptomatic plants while the TBB phytoplasma was detected with equal frequency in both symptomatic and asymptomatic plants (Schneider et al., 1999). Recently, ‘Ca. P. asteris’ subgroup (16Sr I-B) in Brazil and Egypt (Silva et al., 2009; ElSayed and Boulila, 2014); white leaf phytoplasma in Thailand (Soufi et al., 2013); latent infections of 16SrI (aster yellows) and 16SrXI (Rice yellow dwarf) groups in asymptomatic Hawaiian sugarcane cultivars (Soufi and Komor, 2014) and Egyptian sugarcane cultivars based on sequencing and RFLP analyses (ElSayed et al., 2016) were reported from YL affected plants. In India, 16SrXII (Stolbur group) (Gaur et al., 2008) and 16SrI-B (Aster Yellow group) (Kumar et al., 2015) were reported.

Fig. 6. Phylogenetic tree was constructed by maximum likelihood neighbour joining method using MEGA-X v10.1.6 showing the relationship between the sugarcane grassy shoot phytoplasma and sugarcane yellow leaf phytoplasma. Bootstrap values were expressed as percentage of 1,000 replications and branch lengths are proportional to the number of substitutions. Acholeplasma sp. was used to out group the tree.
from sub-tropical cvs CoLk 8102, CoLk 94184, and CoSe 92423.

As on date, SCYP has been reported in seven countries viz. South Africa, Cuba, Brazil, Egypt, Mexico, India and recently in Vietnam. Although 22 SCYP partial sequences were submitted in GenBank database, no reports are available on any phytoplasma specific symptom changes in the YL plants other than the characteristic SCYLV symptoms. Also the first reports from South Africa and Cuba have not ruled out absence of SCYLV from the YL affected plants (Arocha et al., 1999; Cronje et al., 1998) hence, all these SCYP reports speculate that there could have been SCYLV infection with additional infection of phytoplasma as reported by different researchers.

Despite the report of different groups of phytoplasma as mixed infections, the white leaf phytoplasma was reported along with that in most of the countries. In sugarcane, grassy shoot disease (GSD) and white leaf disease (WLD) both were named according to its symptom expression on the infected plants. In case of GSD, the infected plant

![Fig. 7. Restriction fragment length polymorphism patterns of phytoplasma from yellow leaf affected sugarcane samples. M1, M2, 1 kb and 100 bp ladder (GeNei StepUp, India); lane 1-3, restriction digestion of nested PCR product of yellow leaf samples DNA amplified by R16F2n/R16R2 primers; lane 4, positive sugarcane grassy shoot (SCGS) sample cv CoV 92101; except 7b) Bfa I where, lane 2 is SCGS sample cv CoV 92101.](image)

![Fig. 8. Different sugarcane varieties showing both yellow leaf and grassy shoot symptoms in the same clump. Top leaf canopy showing the typical yellowing of leaf mid rib with yellow leaf symptoms and the same bottom were with chlorotic leaves and thin grassy tillers of grassy shoot disease symptoms.](image)
Mixed Infection of SCYLV and SCGS Phytoplasma in YL Affected Indian Sugarcane Cultivars

Mixed Infection of SCYLV and SCGS Phytoplasma in YL Affected Indian Sugarcane Cultivars

does not produce any millable canes, instead produces only grassy chlorotic tillers hence, named as grassy shoot disease (Viswanathan, 2000) and in case of WLD, the infected clump produces the same symptom with complete white/creamy tillers. In India, two kinds of phenotypes are very common and WLD is a misnomer for Indian scenario (Nasare et al., 2007; Viswanathan et al., 2011). However, both are belonging to the same rice yellow dwarf/16SrXI group. WLD occurrence is widely reported in South East Asian countries viz. Indonesia, Thailand, Sri Lanka, and also in China and GSD occurrence is reported more from India only.

The results obtained in this work clearly evidenced that YL in India is caused only by SCYLV and the phytoplasma associated in the YL plant is SCGS phytoplasma which has been confirmed based on the sequencing results, phylogenetic analysis as well as by RFLP using specific enzymes. In support of our findings, presence of both YL and GSD symptoms in the same clumps were recorded in many popular varieties such as, Co 86032, Co 88028, CoV 92101, CoV 94101, CoV 89101, CoV 09356, etc. in both plant and ratoon crops in the institute fields as well as in farmers’ holdings in that only few millable cane formation used to be there and the remaining tillers show grassy shoot symptoms (Fig. 8) whereas, in some cases, the sett material taken from the severely affected YL plant or the ratoon of the YL affected clump shows the GSD expression even in the tillering phase of the crop due to build up of high phytoplasma titre in the previous crop. Although our results were contradictory with SCYP (16Sr-I aster yellow group) association in YL plants of sub-tropical cultivars, only SCGS phytoplasma association was confirmed from both tropical and sub-tropical cultivars in India. Such type of both group of phytoplasma associations (16SrI and 16SrXI) were earlier reported from YL plants in Hawaii (Soufi and Komor, 2014) and, in Egypt, SCYLV as well as 16SrI and 16SrXI groups of phytoplasma were reported on cvs H73-6110, G03-47, G84-47 (ElSayed et al., 2016). All the sugarcane viruses Sugarcane Mosaic Virus (SCMV), Sugarcane Streak Mosaic Virus (SCSMV), and SCYLV were diagnosed even in asymptomatic plants in RT-PCR assays (Viswanathan et al., 2008, 2009; Viswanathan, 2016) due to latent stage or low titre of viruses, the same can be applicable to SCGS phytoplasma. Both, SCYLV and SCGS phytoplasma are phloem limited pathogens and the SCYLV titre might be more than the SCGS phytoplasma in all the collected YL plants in this study. Because of mixed infections of both the pathogens and further inoculum build up in subsequent ratoons, the growth and yield of many high yield and high sugar varieties were reduced.

Non-fungal pathogens, especially virus and bacteria are associated with such varietal degeneration in India; pathogen elimination through tissue culture combined with molecular diagnosis has addressed such constraints under field conditions (Rao et al., 2012; Viswanathan, 2016; Viswanathan and Rao, 2011; Viswanathan et al., 2018). In this regard, our study clearly indicates SCYLV only causes the YL disease in sugarcane and SCYP association is not found with the disease.

Overall, the study has revealed lack of evidences for the occurrences of SCYP in YL affected sugarcane in India, the major sugarcane producer in the world after Brazil. A clarity on the YL associated pathogen has been brought out and incidentally we could detect mixed infections of SCYLV and SCGS-phytoplasma in YL affected sugarcane plants. Further studies are required on the combined infections of these phloem limiting pathogens in sugarcane on YL symptom expression, its severity and their interactions.

Acknowledgments

The authors thank the Director, ICAR- Sugarcane Breeding Institute, Coimbatore for providing all the facilities.

Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

References

Aljanabi, S. M., Parmessur, Y., Moutia, Y., Saumtally, S. and Dookun, A. 2001. Further evidence of the association of a phytoplasma and a virus with yellow leaf syndrome in sugarcane. Plant Pathol. 50:628-636.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Arocha, Y., Gonzalez, L., Peralta, E. L. and Jones, P. 1999. First report of virus and phytoplasma pathogens associated with yellow leaf syndrome of sugarcane in Cuba. Plant Dis. 83:1177.

Arocha, Y., Jones, P., Sumac, I. and Peralta, E. L. 2000. Detection of phytoplasmas associated to yellow leaf syndrome in Cuba. Rev. Prot. Veg. 15:81-86.

Bertaccini, A., Duduk, B., Paltrinieri, S. and Contaldo, N. 2014. Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. Am. J. Plant Sci. 5:1763-1788.

Chinnaraja, C. and Viswanathan, R. 2015a. Quantification of Sugarcane yellow leaf virus in sugarcane following transmission through aphid vector, Melanaphis sacchari. Virusdisease 26:237-242.
Chinnaraja, C. and Viswanathan, R. 2015b. Variability in yellow leaf symptom expression caused by the Sugarcane yellow leaf virus and its seasonal influence in sugarcane. Phytoparasitica 43:339-353.

Chinnaraja, C., Viswanathan, R., Karuppaiah, R., Bagyalakshmi, K., Malathi, P. and Parameswari, B. 2013. Complete genome characterization of Sugarcane yellow leaf virus from India: evidence for RNA recombination. Eur. J. Plant. Pathol. 135:335-349.

Chinnaraja, C., Viswanathan, R., Sathyabhama, M., Parameswari, B., Bagyalakshmi, K., Malathi, P. and Neelamathi, D. 2014. Quantification of Sugarcane yellow leaf virus in in vitro plantlets and asymptomatic plants of sugarcane by RT-qPCR. Curr. Sci. 106:729-734.

Comstock, J. C., Irey, M. S., Lockhart, B. E. L. and Wang, Z. K. 1998. Incidence of yellow leaf syndrome in CP cultivars based on polymerase chain reaction and serological techniques. Sugar Cane 4:21-24.

Cronje, C. P. R. and Bailey, R. A. 1999. Association of phytoplasmas with yellow leaf syndrome of sugarcane. Proc. Int. Soc. Sugar Cane Tech. 23:373-381.

Cronje, C. P. R., Tymon, A. M., Jones, P. and Bailey, R. A. 1998. Association of a phytoplasma with a yellow leaf syndrome of sugarcane in Africa. Ann. Appl. Biol. 133:177-186.

Deng, S. and Hiruki, C. 1991. Amplification of 16S rRNA genes from culturable and non culturable mollicutes. J. Microbiol. Methods 14:53-61.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

ElSayed, A. I. and Boulila, M. 2014. Molecular identification and phylogenetic analysis of sugarcane yellow leaf phytoplasma (SCYLP) in Egypt. J. Phytopathol. 162:89-97.

ElSayed, A. I., Soufi, Z., Wahdan, K. M. and Komor, E. 2016. Detection and characterization of phytoplasma and Sugarcane yellow leaf virus associated with leaf yellowing of sugarcane. J. Phytopathol. 164:217-225.

Gaur, R., Raizada, R. and Rao, G. P. 2008. Sugarcane yellow leaf phytoplasma associated for the first time with sugarcane yellow leaf syndrome in India. New Disease Rep. 16:43.

Gaur, R. K., Singh, A. P., Singh, M., Singh, A. K., Upadhyaya, P. P. and Rao, G. P. 2003. Reliability of serological identification of sugarcane mosaic potyvirus (SCMV) and sugarcane yellow leaf luteovirus (SCYLV) from cane stalk juice. Sugar Cane Int. 5:18-21.

Gundersen, D. E. and Lee, I. M. 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathology Mediterr. 35:144-151.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41:95-98.

Kirdat, K., Tiwarekar, B., Thorat, V., Narawade, N., Dhotre, D., Sathe, S., Shouche, Y. and Yadav, A. 2020. Draft genome sequences of two phytoplasma strains associated with sugarcane grassy shoot (SCGS) and bermuda grass white leaf (BGWL) diseases. Mol. Plant-Microbe Interact. 33:715-717.

Kumar, S., Tiwari, A. K., Hollar, S. K., Duttamajumder, S. K. and Rao, G. P. 2015. Characterization of a 16SrI-B subgroup phytoplasma associated with Sugarcane leaf yellows disease in India. Sugar Tech 17:156-161.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol Evol. 35:1547-1549.

Lee, I. M., Hammond, R. W., Davis, R. E. and Gundersen, D. E. 1993. Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopathology 83:834-842.

Lehrer, A. T., Kusalwong, A. and Komor, E. 2008. High incidence of Sugarcane yellow leaf virus (SCYLV) in sugar plantations and germplasm collections in Thailand. Australas. Plant Dis. Notes 3:89-92.

Nasare, K., Yadav, A., Singli, 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 Dis. 91:1413-1418.

Parmessur, Y., Aljanabi, S., Saumtally, S. and Dookun-Saumtally, A. 2002. Sugarcane yellow leaf virus and sugarcane yellows phytoplasma: elimination by tissue culture. Plant Pathol. 51:561-566.

Rao, G. P., Gaur, R. K., Singh, M., Srivastava, A. K., Virk, K. S., Singh, N., Viswanathan, R., Patil, A. S. and Jain, R. K. 2000. Occurrence of sugarcane yellow leaf virus in India. Sugar Tech 2:37-38.

Rao, G. P., Madhupriya, Thorat, V., Manimekalai, R., Tiwari, A. K. and Yadav, A. 2017. A century progress of research on phytoplasma diseases in India. Phytopathogenic Mollicutes 7:1-38.

Rao, G. P., Madhupriya, Tiwari, A. K., Kumar, S. and Baramwal, V. K. 2014. Identification of sugarcane grassy shoot-associated phytoplasma and one of its putative vectors in India. Phytoparasitica 42:349-354.

Rao, G. P., Mall, S. and Marcone, C. 2012. Recent biotechnological approaches in diagnosis and management of sugarcane phytoplasma diseases. In: Functional plant science and biotechnology. Recent trends in biotechnology and microbiology, Vol. 6, special issue 2, eds. by R. Viswanathan and A. R. Sundar, pp. 19-29. Global Science Books, New York, USA.

Rassaby, L., Girard, J.-C., Lemaire, O., Costet, L., Irey, M. S., Kodjia, H., Lockhart, B. E. L. and Rott, P. 2004. Spread of Sugarcane yellow leaf virus in sugarcane plants and fields on the island of Réunion. Plant Pathol. 53:117-125.

Schenck, S. 1990. Yellow Leaf Syndrome: A New Disease of Sugarcane. Report of HSPA Experiment Station. Hawaii Sugar Planters Association, Honolulu, HI, USA. 98 pp.

Schneider, B., Padovan, A., de La Rue, S., Eichner, R., Davis, R., Bernetz, A. and Gibb, K. S. 1999. Detection and differentiation of phytoplasmas in Australia: an update. Aust. J. Agric. Res. 50:333-342.

Silva, E. G., Bedendo, I. P., Casagrande, M. V. and Moraes, V. A. 2011b. Phytoplasma diseases. Mol. Plant-Microbe Interact. 33:715-717.
2009. Molecular identification and phylogenetic analysis of a group 16SrI-B phytoplasma associated with sugarcane yellow leaf syndrome in Brazil. *J. Phytopathol.* 157:771-774.
Soufi, Z. and Komor, E. 2014. Latent infection of asymptomatic Hawaiian sugarcane cultivars with 16SrI and 16SrXI phytoplasmas. *J. Gen. Plant Pathol.* 80:255-263.
Soufi, Z., Sakuanrungsirikul, S., Wongwarat, T., Hamarn, T., Srisink, S. and Komor, E. 2013. Sugarcane yellow leaf symptomatic plants in Thailand are infected by white leaf phytoplasma, not by leaf yellows phytoplasma. *Australas. Plant Pathol.* 42:723-729.
Tran-Nguyen, L., Blanche, K. R., Egan, B. and Gibb, K. S. 2000. Diversity of phytoplasmas in northern Australian sugarcane and other grasses. *Plant Pathol.* 49:666-669.
Viswanathan, R. 2000. Grassy shoot. In: *A guide to sugarcane diseases*, eds. by P. Rott, R. A. Bailey, J. C. Comstock, B. J. Croft and A. S. Saumsteel, pp. 215-220. CIRAD, Montpellier, France.
Viswanathan, R. 2002. Sugarcane yellow leaf syndrome in India: incidence and effect on yield parameters. *Sugar Cane Int.* 20:17-23.
Viswanathan, R. 2016. Varietal degeneration in sugarcane and its management in India. *Sugar Tech* 18:1-7.
Viswanathan, R. and Balamuralikrishnan, M. 2004. Detection of *Sugarcane yellow leaf virus*, the causal agent of yellow leaf syndrome in sugarcane by DAS-ELISA. *Arch. Phytopathol. Plant Prot.* 37:169-176.
Viswanathan, R., Balamuralikrishnan, M. and Karuppaiah, R. 2006. Yellow leaf disease of sugarcane: occurrence and impact of infected setts on disease severity and yield. *Proc. Sugar Technol. Assoc. India* 67:74-89.
Viswanathan, R., Balamuralikrishnan, M. and Karuppaiah, R. 2008. Identification of three genotypes of *sugarcane yellow leaf virus* causing yellow leaf disease from India and their molecular characterization. *Virus Genes* 37:368-379.
Viswanathan, R., Chinnaraja, C., Karuppaiah, R., Kumar, V. G., Rooba, J. I. and Malathi, P. 2011. Genetic diversity of sugarcane grassy shoot (SCGS)-phytoplasmas causing grassy shoot disease in India. *Sugar Tech* 13:220-228.
Viswanathan, R., Chinnaraja, C., Malathi, P., Gomathi, R., Rakkikappan, P., Neelamathi, D. and Ravichandran, V. 2014. Impact of *Sugarcane yellow leaf virus* (ScYLV) infection on physiological efficiency and growth parameters of sugarcane under tropical climatic conditions in India. *Acta Physiol. Plant.* 36:1805-1822.
Viswanathan, R., Karuppaiah, R., Malathi, P., Kumar, V. G. and Chinnaraja, C. 2009. Diagnosis of *Sugarcane yellow leaf virus* in asymptomatic sugarcane by RT-PCR. *Sugar Tech* 11:368-372.
Viswanathan, R., Malathi, P. and Neelamathi, D. 2018. Enhancing sugarcane yield per hectare through improved virus-free seed nursery programme. *ICAR NEWS* 24:4-5.
Viswanathan, R., Padmanaban, P., Mohanraj, D., Ramesh Sundar, A. and Premachandran, M. N. 1999. Suspected yellow leaf syndrome in sugarcane. *Sugarcane Breed. Inst. Newsl.* 18:2-3.
Viswanathan, R., Chinnaraja, C., Parameswari, B. and Chhabra, M. L. 2016. Status of yellow leaf resistance in sugarcane germplasm and parental clones at Sugarcane Breeding Institute, India. *Int. Sugar J.* 115:60-71.
Viswanathan, R. and Rao, G. P. 2011. Disease scenario and management of major sugarcane diseases in India. *Sugar Tech* 13:336-353.