Natural occurrence and diagnosis of yellow leaf disease affecting sugarcane genotypes under tropical and sub-tropical conditions in India

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Abstract

Aim: To diagnose the cause of yellow leaf disease (YLD) and to record its incidence in the 189 sugarcane genotypes planted under tropical (n= 47) and sub-tropical (n= 142) conditions in India during 2016–17 and 2017–18 crop growing seasons.

Methodology: The symptomatology, disease incidence and diagnosis of YLD was carried out in all 189 genotypes comprising of 47 tropical and 142 sub-tropical genotypes during 2016–17 and 2017–18 crop growing seasons. Further, all the YLD symptomatic samples were subjected to nested PCR assay for detection of the associated phytoplasma, and to study its distribution in different YLD affected plant parts using universal primers.

Results: YLD affected genotypes were characterized by the mild to prominent yellowing of the midribs, extending the yellowing toward leaf lamina followed by leaf-tip drying from top towards the leaf base, bunching at the crown region and subsequently complete drying of the leaves and stunted growth. Of the total 189 genotypes, disease incidence in 142 sub-tropical genotypes during 2016 – 17 and 2017 – 18 crop growing seasons ranged from 1.17% to 65.38% and 7.95% to 67.44%, respectively. Similarly, of the total 47 tropical genotypes, disease incidence was found in 39 genotypes and ranged from 3.89% to 55.58% and 2.38% to 62.96% in 2016-17 and 2017 – 18 crop growing season. All the 95 symptomatic samples from sub-tropical and tropical genotypes were tested by nested PCR assay of which 94 samples showed ~1.2 Kb size amplicons.

Interpretation: From the present study, it was concluded, that among the total 189 genotypes, 94 genotypes were found affected due to YLD and presence of phytoplasma in these genotypes were diagnosed. Remaining 95 genotypes which were found apparently healthy would be a good source of resistance and can be further involved for the development of YLD resistant sugarcane progenies in India.

Key words: Disease incidence, Nested PCR, *Sugarcane yellows phytoplasma*, Yellow leaf disease

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Introduction

Sugarcane (*Saccharum* hybrid) is one of the most important cash crops of India. It belongs to the genus *Saccharum* and family *Poaceae*. Sugarcane is a long duration crop and needs around 12-18 months to mature, based upon the geographical conditions *i.e.*, tropical and sub-tropical. It requires hot and humid climate with average temperature of 21°C-27°C and 75 cm -150 cm rainfall. In tropical conditions, three planting methods are recommended because of conducive weather for sugarcane growth and development. Three planting methods *viz.*, seasonal (crop duration 14-15 months), pre-seasonal (12 months) and kharif/ adsali (18 months). Whereas, under sub-tropical conditions sugarcane faces the high temperature during summer and low temperature during winter season therefore, mainly two planting times are recommended in sub-tropics *i.e.*, autumn and spring plantings. Autumn planting is done during October and delay in autumn planting affects cane germination and yield whereas, spring planting is done in February – March to avoid heat stress during April-May.

Worldwide, sugarcane is known to infect by many fungal, bacterial, and viral diseases and are the major constraints in its production and productivity. Besides these fungal, bacterial and viral diseases, phytoplasma are one of the major constraints in sugarcane production including *Sugarcane white leaf phytoplasma* (SCWLP) (Sdoodee, 2001, Rao *et al.*, 2008, Soufi *et al.*, 2013), *Sugarcane grassy shoot phytoplasma* (SCGSP) (Singh *et al.*, 2002, Rao *et al.*, 2008) and *Sugarcane yellows phytoplasma* (SCYP) (Cronje *et al.*, 1998; Arocha *et al.*, 2000; Gaur *et al.*, 2008; Kumar *et al.*, 2015). Of which SCYP is one of the serious pathogens causing severe reduction in cane growth and yield contributing parameters (Cronje *et al.*, 1998). Association of phytoplasma with YLD was detected from different sugarcane growing countries including, Australia, Cuba and Mauritius (Cronje *et al.*, 1998; Arocha *et al.*, 1999; Aljanabi *et al.*, 2001). Moreover, latent infections of 16SrI and 16SrXI subgroups of phytoplasma have also been reported from asymptomatic sugarcane cultivars in Hawaii (Soufi and Komor, 2014). Subsequently, association of '*Candidatus phytoplasma asteris*' subgroup (16Sr I-B) was reported from Brazil, Egypt and India (Silva *et al.*, 2009; Elsayed and Boulila 2014; Kumar *et al.*, 2015) and white leaf phytoplasma in Thailand (Soufi *et al.*, 2013).

Worldwide, first evidence of YLD was reported in Hawaii in sugarcane (*S. officinarum*) cv. H 65-7052 (Schenck, 1990). Subsequently, identical visual symptoms were also reported in Florida (Comstock *et al.*, 1994). Since then, symptoms of YLD have been recorded and diagnosed in more than 20 countries including Australia, Brazil, Cuba, Mauritius, Reunion, South Africa and the USA (Bailey *et al.*, 1996; Cronje *et al.*, 1998). YLD, earlier known as yellow leaf syndrome (YLS) is caused by SCYP and *Sugarcane yellow leaf virus* (SCYLTV). In India, YLD is caused by SCYLTV (Viswanathan *et al.*, 1999, Rao *et al.*, 2000, 2001, Viswanathan *et al.*, 2002). Later, Gaur *et al.* (2008) for the first time reported the association of SCYP with YLD in India.

Recently, from our earlier study, first report of '*Ca. phytoplasma asteris*' (subgroup: 16SrI-B) was detected in the two YLD affected sugarcane varieties *i.e.*, CoLk94184 and CoSe 92423 by Kumar *et al.* (2015) and in other four popular sugarcane varieties by Kumar *et al.* (2018). Further, disease incidence and distribution of phytoplasma in large number of genotypes and identification of resistant sources for YLD have not yet been well studied under sub-tropical conditions. Therefore, in the present study, systematic and regular studies were conducted to ascertain the prevalence of YLD in different sugarcane genotypes and detected the presence of pathogen in different plant parts during 2016 – 17 and 2017 – 18 crop growing seasons.

The primary natural transmission of phytoplasma is known to occur by vegetative propagation and secondary by insect-vectors from infected to healthy sugarcane crop. Phloem-feeding planthoppers (family: *Cicadellidae*) are the most effective insect-vectors for the transmission of phytoplasma. (Maixner and Reinert, 1999; Gatineau *et al.*, 2001). SCYP belongs to Aster yellows phytoplasma 16SrI-A subgroup and transmitted naturally by delphacid planthoppers (*Saccharosydne sachharivora*) (Arocha *et al.*, 2005). In India, association of 16SrXII group of phytoplasma was detected in the YLD affected sugarcane genotypes (Gaur *et al.*, 2008; Rao *et al.*, 2012). Insect transmission of phytoplasma causing grassy shoot disease (GSD) has been studied (Singh *et al.*, 2002; Srivastava *et al.*, 2006) but information on insect transmission of SCYP causing YLD is meagre. Since last two decades, YLD has become a serious concern for researchers and sugarcane growers, and this has generated keen interest to understand the disease epidemiology and development of the better management tactics. Further, screening of sugarcane genotypes against YLD would be future line of research. Therefore, the present study was undertaken to detect the associated pathogen in large number of sugarcane genotypes and its prevalence in these genotypes in the tropical and sub-tropical conditions of India.

Materials and Methods

Planting of sugarcane genotypes: A total of 189 sugarcane genotypes were planted for evaluating of YLD under field conditions, of which 142 genotypes were planted at the experimental farm, Division of Crop Improvement, Indian Institute of Sugarcane Research (IISR), Lucknow, during 2016–17 and 2017–18 crop growing seasons in randomized block design (Table 1). Whereas, 47 sugarcane genotypes were planted during 2016–17 and 2017–18 crop growing seasons at the experimental farm of the Padmashri Dr. Vitthalrao Vikhe Patil Sahkari Sakhar Karkhana (PDVVPSSK) Ltd, Pravaranagar, Maharashtra in alpha design (Table 1).

Symptoms and disease incidence: Based on the visual YLD symptoms, the disease incidence (%) under field condition was recorded during both the crop growing seasons 2016–17 and 2017–18 on the total 189 sugarcane genotypes planted at IISR, Lucknow, and PDVVPSSK, Ltd., Pravaranagar. Undersub-

tropical conditions, YLD incidence was recorded in September, November, January, and March during both the 2016–17 and 2017–18 crop growing seasons. Similarly, under tropical condition, the evidence of the occurrence of YLD was recorded during 2016–17 and 2017–18 in 47 sugarcane genotypes.

Sample collection and genomic DNA extraction: In order to detect the phytoplasma infection, a total of 56 YLD affected sugarcane genotype samples including BO 91, BO 111, BO 120, BO 128, BO 129, BO 130, BO 136, BO 137, BO 138, BO 153, CoP 9113, Co 0233, Co 419, Co 453, Co 1336, Co 1148, Co 05011, Co 06425, Co 89003, Co 98014, Co 0118, Co 0238, CoH 92, CoH 99, CoH 119, CoLk 8112, CoLk 94184, CoPant 96219, CoS 245, CoS 510, CoS 687, CoS 797, CoS 91230, CoS 90269, CoS 90265, CoS 95222, CoS 94270, CoS 93259, CoS 02258, CoS 02264, CoS 8207, CoS 119, CoS 98259, CoSe 00235, CoSe 01235, CoSe 98231, CoSe 01421, Up 05125, CoS 08234, CoPb 08211, CoPb 08217, CoH 08262, CoH 08263, CoLk 09202, CoLk 09204 and CoS 09232, and 39 tropical genotypes exhibiting YLD symptoms were collected and stored in deep freezer (-80°C ULT, Haier, India) for further analysis.

Further, these YLD infected leaf samples were subjected for isolation of genomic DNA by CTAB (Cethyl Trimethyl Ammonium Bromide) method. To avoid any cross-contamination in the extracted genomic DNA, all the collected leaf samples were surface cleaned with 70% ethanol. Genomic DNA extraction was carried out by mixing 500 mg leaf midrib powder in 750 µl extraction buffer containing 2% polyvinylpyrrolidone, incubating at 65°C for 60 min, followed by the purification step by addition of equal volume of chloroform-isoamyl alcohol (24:1) and centrifuged for 15 min at 10000 rpm. The supernatant was transferred to a new centrifuge tube. DNA was precipitated by adding equal volume of chilled isopropanol followed by centrifugation at 12000 rpm for 10 min and discarding the supernatant and by two successive washing of DNA pellets with 70% ethanol. The purified transparent pellet was eluted in 100 µl of TE buffer contained (10 mM Tris, 1 mM EDTA, pH 7.6 (Ahrens and Seemuller, 1992) and quality of DNA was checked on 0.8% agarose gel electrophoresis.

Nested polymerase chain reaction: The nested PCR assay was conducted for the amplification of ribosomal DNA (16S rDNA) using phytoplasma universal primers (P1: 5'-AAGAGTTTGATCCTGGCTCAGGATT-3' and P6: 5'-CGGTAGGGATCACTTGTTACGACTTA-3') (Deng and Hiruki, 1991) and R16F2n: 5'-GAAACGACTGCTAAGACTGG-3' and R16R2: 5'-TGACGGGCGGTGTGTACAAACCCCG-3') (Gunderson and Lee, 1996; Smart et al. (1996).

First round PCR amplification was performed in a 25 µl reaction mixture using ProFlex thermal cycler (ProFlex PCR, Thermo Fisher Scientific, USA). The PCR reaction mixture contained 2 µl of template DNA, 1.5 µl of MgCl₂, 0.5 µl of dNTP mixture, 0.5 µl of each primer, 0.5 U of TaqDNA polymerase (Dream Taq) and 2.5 µl of 10X buffer. The first round PCR

amplification protocol was carried out at different temperature conditions; 5 min hot start for initial denaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing for 1 min at 55°C and synthesis at 72°C for 1 min, and a cycle of final extension at 72°C for 10 min. Further, first round PCR product at a dilution of 1:10 was used as template for the second round PCR with same reaction as described for the first round PCR. The second round PCR amplification protocol was performed in thermal cycler at similar temperature followed for the first round PCR, except the annealing temperature at 56°C for 1 hr.

Detection of phytoplasma in different sugarcane plant parts:

Samples were collected from YLD affected different plant parts including roots, stem (basal, middle, and upper regions), and leaves (midribs and lamina) from Co 0238 variety. From each plant part, two replications were collected and subjected for isolation of genomic DNA using similar procedure as described above. Tissue culture raised healthy control of same variety Co 0238 was obtained from Division of Crop Improvement, IISR, Lucknow. Further, all the six genomic DNA samples were subjected to nested PCR assay using the similar protocol as described above.

Results and Discussion

Symptomatology: During 2016–17 and 2017–18 crop growing seasons, the YLD affected sugarcane genotypes exhibited various symptoms like mild yellowing of midribs and smaller leaves, bunching at crown region of plants along with shortened internodes and horizontal spread of yellowing across the leaf lamina, followed by necrosis from leaf tips which continued towards the leaf base. Complete necrosis led to wilting and stunted growth of plants and subsequent complete die-back of plants was observed during severe incidence of YLD under field conditions (Fig.1). Field view of genotypes severely affected due to wide spread occurrence of YLD at IISR, Lucknow and PDVVPSSK, Ltd., Pravar Nagar. Similar symptoms have been previously reported on different sugarcane genotypes viz., Co 86032 and CoV 92102 affected by YLD in India (Gaur et al., 2008; Viswanathan and Rao 2011; Rao et al., 2012; Holkar et al., 2016).

During 2013–14 crop growing season, symptoms of YLD were recorded at IISR, Lucknow experimental farm on six genotypes viz., CoS 767, CoJ 64, Co 419, Co 7717, CoLk 94184, Baragua, Khakai and CoSe 92423. Subsequently, YLD symptomatic samples from two sugarcane varieties i.e., CoLk 94184 and CoSe 92423 were collected and subjected to nested PCR assay followed by sequencing, which revealed the association of new group of phytoplasma (16Srl-B subgroup) with YLD on sugarcane (Kumar et al., 2015). Since, similar type of symptoms of YLD were recorded in more than 100 sugarcane genotypes at the IISR experimental farm (Anonymous, 2014–2015a, 2015–2016a). In the present study, during 2016–17 and 2017–18 crop growing seasons, symptoms were recorded at maturity stage of crop i.e., in September, November, January, and March and the symptoms observed were development from slightly yellowing of midribs to complete die-back of the crop as

Table 1: Yellow leaf disease incidence recorded on 189 sugarcane genotypes planted at experimental field of IISR, Lucknow and PDVPPSSK, Ltd., Pravaranagar, during 2016–17 and 2017–18 crop growing seasons

Genotype Planted at Lucknow (142) (2016 – 17 and 2017 - 18)	Genotype planted at Pravaranagar (47) (2016 – 17 and 2017-18)
BO 91, CoJ 89, CoS 96269, BO 99, CoLk 790, CoS 96275, BO 119, CoLk 8001, CoS 97258, BO 111, CoLk 8112, CoS 97261, BO 120, CoLk 94184, CoS 97264, BO 128, CoLk 9709, CoS 99259, BO 129, CoLk 07201, CoS 08272, BO 130, CoPant 03220, CoS 98259, BO 136, CoPant 05224, CoSe 00235, BO 137, CoPant 24211, CoSe 01235, BO 138, CoPant 84212, CoSe 98231, BO 139, CoPant 90223, CoSe 96436, BO 141, CoPant 96219, CoSe 95436, BO 145, CoPant 97222, CoSe 95422, BO 147, CoPant 99214, CoSe 92423, BO 153, CoPk 05191, CoSe 08457, CoP 9113, CoS 245, CoSe 05451, CoP 9206, CoS 443, CoSe 03234, CoP 9301, CoS 510, CoSe 01434, CoP 9302, CoS 514, CoSe 01424, CoP 9702, CoS 541, CoSe 01421, Co 0124, CoS 687, UP 9530, Co 0232, CoS 770, UP 05125, Co 0233, CoS 767, UP 0097, Co 0237, CoS 797, UP 05, Co 419, CoS 91269, UP 39, Co 453, CoS 91230, CoS 08279, Co 1336, CoS 90269, CoS 8233, Co 1148, CoS 90265, CoS 08234, Co 05011, CoS 88230, CoS 08235, Co 06425, CoS 8436, CoPb 08211, Co 6811, CoS 95270, CoPb 08212, Co 87263, CoS 95255, CoPb 08217, Co 872683, CoS 95222, CoH 08262, Co 89003, CoS 94270, CoH 08263, Co 89029, CoS 94257, CoH 08264, Co 98014, CoS 93259, CoLk 09202, Co 0118, CoS 01256, CoLk 09204, Co 0238, CoS 02258, CoPb 09181, Co 0239, CoS 02264, CoS 09232, CoB 94164, CoS 03279, Co 09022, CoH 56, CoS 07250, CoH 09264, CoH 92, CoS 7918, CoH 99, CoS 8118, CoH 111, CoS 8207, CoH 119, CoS 8315, CoJ 64, CoS 119, CoJ 83, CoS 8432, CoJ 85, CoS 96258, CoJ 88 and CoS 96268	CoSnK 05103, Co 14002, Co 14003, Co 14004, Co 14006, CoN 14071, CoN 14072, CoSnK 14101, CoSnK 14102, CoT 14366, CoT 14367, MS 14081, MS 14082, Co 13021, Co 13022, Co 14008, CoVc 14061, CoVc 14062, PI 14131, PI 14132, VSI 14121, VSI 14122, Co 86032, CoC 0671, Co 11005, Co 11007, Co 11012, Co 11019, Co 99004, CoM 11086, CoM 11085, Co 11001, Co 11004, CoM 11081, CoM 11082, CoM 11084, Co 85004, Co 94008, Co 12019, Co 12024, CoSnK 5103, CoM 12085, Co 12007, Co 12008, Co 12009, Co 12012, VSI 12121

the symptoms of YLD are visible at six to eight months after planting of sugarcane (Viswanathan and Rao, 2011; Holkar *et al.*, 2016). Recently, Kumar *et al.* (2018) have recorded the YLD symptoms on 48 commercial sugarcane varieties across eleven major sugarcane growing states of India viz., Uttarakhand, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Punjab, Bihar, Chhattisgarh, Haryana and Assam. This investigation has limited to only 48 genotypes as compared to 189 genotypes from the present study. In addition to this, similar symptoms of yellow leaf disease from different countries from where association of phytoplasma were described including Africa (Arocha *et al.*, 1999; Cronje and Bailey, 1999), Brazil (Silva *et al.*, 2009), Cuba (Aljanabi *et al.*, 2001), Egypt (Elsayed and Bouilila, 2014), the USA (Hawaii: Schenck, 1990; Florida: Comstock *et al.*, 1994), Mauritius (Aljanabi, 2001) and Thailand (Soufi *et al.*, 2013).

Disease incidence: Disease incidence was recorded in total 189 sugarcane genotypes planted under tropical and sub-tropical conditions as mentioned in (Table 1). Of the total 142 sub-tropical genotypes, 86 genotypes were found free of YLD whereas, 56 genotypes showed disease incidence ranging from 1.17% to 65.38% during 2016–17 crop growing season. Lowest disease incidence of about 1.17% was recorded on sugarcane cv. BO 91 whereas the highest disease incidence of about 65.38% was recorded on CoS 797 during 2016–17 crop growing season (Table 2). Similarly, during 2017–18 crop growing season disease incidence ranged from 7.95% to 67.44% among the same 56 genotypes (Table 2). During both crop growing seasons, 86 genotypes were found free of prominent YLD symptoms and were therefore treated as apparently healthy as compared to rest of 56 genotypes which showed prominent YLD symptoms under field conditions. Moreover, the disease incidence was also recorded in 47 tropical sugarcane genotypes during 2016–17 and 2017–18 crop growing seasons and of which 39 genotypes were found

affected due to YLD, and incidence ranged from 3.89% to 55.58% and 2.38% to 80.39%, respectively. No prominent visual symptoms were recorded on eight genotypes, including CoN 14072, MS 14082, Co11012, Co 12012, CoM11085, CoM 11082, CoSnK 5103, and CoM 12085 (Table 3).

In India, YLD incidence was recorded and found varied with genotype to genotype and location to location during 2012–13 to 2016–17 including, 10–80% at Anakapalle, 2% at Akola, 2–5% at Buralikson, 5–40% at Cuddalore, 2–11% in Karnataka, 2–25% at Kolhapur, 5–11% at Padegaon, Traces–15% at Powarkheda, 1–5% at Pune, 3–20% in Bihar (Pusa), 2–11% at Sankeshwar, 10–15% at Seorahi, 1–30% at Shahjahanpur, Traces–5% at Uchani, traces–4% at Hoshangabad, Betul and Bankhedi, 10–60% at Vijayanagaram, Vishakhapattanam, Karimnagar, Nizamabad, East Godavari and Chittoor, 2–11% at Haveri, Bagalkot, Dharwad and Belgaon, 15–30% at Pondicherry, Villupuram, Seithiathope, Mundiypakkam, and in traces at Thiruvalla, Shahabad, Rohtak, Sitarganj, Kiccha, Gadarpur, Kashipur, Bardoli, Iramallikk, Thiruvandoor and Vallamkulam (Anonymous, 2011-2012, 2012-2013, 2013-2014, 2014-2015b, 2015-2016b).

In India, incidence of YLD has been recorded up to 100% in different susceptible varieties (Viswanathan *et al.*, 1999; Rao *et al.*, 2001). Since its first record, incidence of YLD on sugarcane was observed in the major sugarcane growing states viz., Uttar Pradesh, Maharashtra, Tamil Nadu and Haryana (Viswanathan and Rao, 2011; Holkar *et al.*, 2016). Similarly, YLD incidence was also observed up to 60% in different susceptible varieties in Andhra Pradesh and Telangana states (Rajkumar *et al.*, 2014). Holkar *et al.* (2016) recorded up to 29.95% of YLD incidence in more than 40 sugarcane genotypes from sub-tropical India. In addition to this, YLD incidence was reported from the USA in five *Saccharum* species viz., *S. spontaneum* (7.0%), *S. officinarum*

Table 2: Yellow leaf disease incidence and nested PCR assay of 142 sub-tropical sugarcane genotypes planted at IISR, Lucknow, during 2016–17 and 2017–18 crop growing seasons

Genotype	Disease incidence (%)		Nested PCR assay (2016 - 17)		Genotype	Disease incidence (%)		Nested PCR assay (2017-18)	
	2016-17	2017-18	First round	Second round		2016-17	2017-18	First round	Second round
BO 91	1.17	7.95	-ve	+ve	CoS 687	20.08	30.29	-ve	+ve
BO 99	0	0	-ve	-ve	CoS 770	0	0	-ve	-ve
BO 119	0	0	-ve	-ve	CoS 767	0	0	-ve	-ve
BO 111	1.72	12.74	-ve	+ve	CoS 797	65.38	63.92	+ve	+ve
BO 120	7.74	19.83	-ve	+ve	CoS 91269	0	0	-ve	-ve
BO 128	9.67	25.17	-ve	+ve	CoS 91230	38.71	67.44	+ve	+ve
BO 129	9.06	33.03	-ve	+ve	CoS 90269	26.04	30.84	-ve	+ve
BO 130	8.7	20.2	-ve	+ve	CoS 90265	30.80	37.26	+ve	+ve
BO 136	9.66	23.67	-ve	+ve	CoS 88230	0	0	-ve	-ve
BO 137	8.27	26.38	-ve	+ve	CoS 8436	0	0	-ve	-ve
BO 138	7.39	25.04	-ve	+ve	CoS 95270	0	0	-ve	-ve
BO 139	0	0	-ve	-ve	CoS 95255	0	0	-ve	-ve
BO 141	0	0	-ve	-ve	CoS 95222	2.18	10.87	-ve	+ve
BO 145	0	0	-ve	-ve	CoS 94270	10.52	18.01	-ve	+ve
BO 147	0	0	-ve	-ve	CoS 94257	0	0	-ve	-ve
BO 153	2.91	16.46	-ve	+ve	CoS 93259	18.07	39.45	+ve	+ve
CoP 9113	2.72	11.84	-ve	+ve	CoS 01256	0	0	-ve	-ve
CoP 9206	0	0	-ve	-ve	CoS 02258	27.94	48.77	+ve	+ve
CoP 9301	0	0	-ve	-ve	CoS 02264	27.42	53.39	+ve	+ve
CoP 9302	0	0	-ve	-ve	CoS 03279	0	0	-ve	-ve
CoP 9702	0	0	-ve	-ve	CoS 07250	0	0	-ve	-ve
Co 0124	0	0	-ve	-ve	CoS 7918	0	0	-ve	-ve
Co 0232	0	0	-ve	-ve	CoS 8118	0	0	-ve	-ve
Co 0233	22.12	33.53	+ve	+ve	CoS 8207	13.88	21.65	-ve	+ve
Co 0237	0	0	-ve	-ve	CoS 8315	0	0	-ve	-ve
Co 419	23.15	43.55	-ve	+ve	CoS 119	15.03	22.43	-ve	+ve
Co 453	32.95	49.48	-ve	+ve	CoS 8432	0	0	-ve	-ve
Co 1336	20.24	31.91	-ve	+ve	CoS 96258	0	0	-ve	-ve
Co 1148	25.16	43.05	+ve	+ve	CoS 96268	0	0	-ve	-ve
Co 05011	24.68	38.04	-ve	-ve	CoS 96269	0	0	-ve	-ve
Co 06425	28.12	37.86	-ve	+ve	CoS 96275	0	0	-ve	-ve
Co 6811	0	0	-ve	-ve	CoS 97258	0	0	-ve	-ve
Co 87263	0	0	-ve	-ve	CoS 97261	0	0	-ve	-ve
Co 872683	0	0	-ve	-ve	CoS 97264	0	0	-ve	-ve
Co 89003	20.38	31.21	-ve	+ve	CoS 99259	0	0	-ve	-ve
Co 89029	0	0	-ve	-ve	CoS 98259	18.09	26.88	-ve	+ve
Co 98014	34.13	48.51	-ve	+ve	CoSe 00235	9.75	26.11	-ve	+ve
Co 0118	17.85	34.39	-ve	+ve	CoSe 01235	27.05	41.04	-ve	+ve
Co 0238	22.26	29.81	+ve	+ve	CoSe 98231	23.73	38.77	-ve	+ve
Co 0239	0	0	-ve	-ve	CoSe 96436	0	0	-ve	-ve
CoB 94164	0	0	-ve	-ve	CoSe 95436	0	0	-ve	-ve
CoH 56	0	0	-ve	-ve	CoSe 95422	0	0	-ve	-ve
CoH 92	33.85	50.44	+ve	+ve	CoSe 92423	0	0	-ve	-ve
CoH 99	39.28	40.39	+ve	+ve	CoSe 08457	0	0	-ve	-ve
CoH 111	0	0	-ve	-ve	CoSe 05451	0	0	-ve	-ve
CoH 119	28.46	49.96	-ve	+ve	CoSe 03234	0	0	-ve	-ve
CoJ 64	0	0	-ve	-ve	CoSe 01434	0	0	-ve	-ve
CoJ 83	0	0	-ve	-ve	CoSe 01424	0	0	-ve	-ve

Continued Table

Table 2: Yellow leaf disease incidence and nested PCR assay of 142 sub-tropical sugarcane genotypes planted at IISR, Lucknow, during 2016–17 and 2017–18 crop growing seasons

Genotype	Disease incidence (%)		Nested PCR assay (2016 - 17)		Genotype	Disease incidence (%)		Nested PCR assay (2017 - 18)	
	2016-17	2017-18	First round	Second round		2016 - 17	2017 - 18	First round	Second round
CoJ 85	0	0	-ve	-ve	CoSe 01421	44.92	54.45	+ve	+ve
CoJ 88	0	0	-ve	-ve	UP 9530	0	0	-ve	-ve
CoJ 89	0	0	-ve	-ve	UP 05125	2.29	8.34	-ve	+ve
CoLk 790	0	0	-ve	-ve	UP 0097	0	0	-ve	-ve
CoLk 8001	0	0	-ve	-ve	UP 05	0	0	-ve	-ve
CoLk 8112	12.23	24.9	-ve	+ve	UP 39	0	0	-ve	-ve
CoLk 94184	16.25	32.3	+ve	+ve	CoS 08279	0	0	-ve	-ve
CoLk 9709	0	0	-ve	-ve	CoS 8233	0	0	-ve	-ve
CoLk 07201	0	0	-ve	-ve	CoS 08234	19.21	31.23	-ve	+ve
CoPant 03220	0	0	-ve	-ve	CoS 08235	0	0	-ve	-ve
CoPant 05224	0	0	-ve	-ve	CoPb 08211	21.8	43.39	+ve	+ve
CoPant 24211	0	0	-ve	-ve	CoPb 08212	0	0	-ve	-ve
CoPant 84212	0	0	-ve	-ve	CoPb 08217	12.09	37.75	-ve	+ve
CoPant 90223	0	0	-ve	-ve	CoH 08262	23.48	45.04	+ve	+ve
CoPant 96219	19.41	32.87	+ve	+ve	CoH 08263	30.84	45.10	+ve	+ve
CoPant 97222	0	0	-ve	-ve	CoH 08264	0	0	-ve	-ve
CoPant 99214	0	0	-ve	-ve	CoLk 09202	1.64	9.65	-ve	+ve
CoPk 05191	0	0	-ve	-ve	CoLk 09204	3.43	12.65	-ve	+ve
CoS 245	30.24	45.13	+ve	+ve	CoPb 09181	0	0	-ve	-ve
CoS 443	0	0	-ve	-ve	CoS 09232	22.16	46.47	-ve	+ve
CoS 510	56.96	63.13	+ve	+ve	Co 09022	0	0	-ve	-ve
CoS 514	0	0	-ve	-ve	CoH 09264	0	0	-ve	-ve
CoS 541	0	0	-ve	-ve	CoH 09264	0	0	-ve	-ve

Table 3: Yellow leaf disease incidence recorded in 47 sugarcane genotypes planted at PDVSSK, Ltd., Pravar Nagar during 2017 – 18 crop growing seasons

Genotype	Disease incidence (%)	Genotype	Disease incidence (%)	Genotype	Disease incidence (%)
CoSnK 05103	12.00	CoVc 14061	2.50	Co 11004	15.56
Co 14002	11.11	CoVc 14062	7.32	CoM 11081	7.5
Co 14003	4.55	PI 14131	28.13	CoM 11082	0.00
Co 14004	8.00	PI 14132	13.89	CoM 11084	6.9
Co 14006	6.45	VSI 14121	5.13	Co 85004	32.08
CoN 14071	7.50	VSI 14122	62.96	Co 94008	36.67
CoN 14072	0.00	Co 86032	30.00	Co 12019	80.39
CoSnK 14101	20.69	CoC 0671	11.54	Co 12024	45.45
CoSnK 14102	4.76	Co 11005	32.56	CoSnK 5103	0.00
CoT 14366	8.57	Co 11007	28.57	CoM 12085	0.00
CoT 14367	2.63	Co 11012	0.00	Co 12007	36.84
MS 14081	11.43	Co 11019	11.76	Co 12008	13.89
MS 14082	0.00	Co 99004	25.71	Co 12009	5.13
Co 13021	25.00	CoM 11086	2.38	VSI 12121	39.47
Co 13022	37.93	CoM 11085	0.00	Co 12012	0.00
Co 14008	28.13	Co 11001	28.95		

(75.8%), *S. robustum* (62.5%), *S. sinense* (46.2%), and *S. barberi* (13.6%) (Comstock *et al.*, 2001) but they have detected the presence of SCYLV instead of phytoplasma as YLD is caused by single or mixed infection of SCYLV and phytoplasma. However,

scanty information is available on incidence of YLD in large number of genotypes from sub-tropical conditions in India. The disease incidence was recorded during 2016–2017 and 2017–2018 seasons, in which the variety BO 91 showed

Fig. 1: Symptoms of healthy and yellow leaf diseased sugarcane plants. (A) plant with green leaves and midribs; (B) mild yellowing of the midribs and smaller leaves; (C) bunching at the crown region of the plant along with shortened internodes; (D) yellowing was spread laterally across the leaf lamina; (E) necrosis starts from leaf tips which continued towards the leaf base and (F) complete necrosis led to the wilting and stunted growth and subsequently complete die-back of plants.

Fig. 2: 1.5% agarose gel electrophoresis of the first round nested PCR amplification of phytoplasma using P1/P6 universal primers showed amplification of ~1.5 Kb size amplicons in all the 19 samples. Lanes; 1: Co 1148, 2: Co 0238, 3: CoPant 96219, 4: CoS 02258, 5: CoS 02264, 6: Co 0233, 7: CoLk 94184, 8: CoH 92, 9: CoH 99, 10: CoS 245, 11: CoS 510, 12: CoS 797, 13: CoS 91230, 14: CoS 90265, 15: CoS 93259, 16: CoSe 01421, 17: CoPb 08211, 18: CoH 08262 and 19: CoH 08263; M= 100 bp plus DNA ladder in A and 1 Kb DNA ladder in B and C; H= healthy control.

minimum disease incidence of 1.17% and 7.95% whereas, CoS 797 showed maximum disease incidence of 65.38% and 67.44% in 2016–17 and 2017–18 seasons, respectively. It was observed that the disease incidence increased slightly in all genotypes cultivated during 2017–18 crop growing season as compared to 2016–17. This could be due to the transmission of phytoplasma via infected seed cane used for planting and/or by insect vector. Likewise, sub-tropical conditions, incidence was also recorded in 39 tropical genotypes exhibiting the similar symptoms.

Nested PCR assay: Total 56 genomic DNA samples isolated from YLD affected sub-tropical sugarcane plants were subjected to first round of nested PCR assay which resulted in the amplification of ~1.5 Kb size amplicons in the 19 samples indicated in (Fig. 2; Table 2). Whereas, second round PCR of these 56 samples using the first round PCR product as a template resulted in the amplification of ~1.2 Kb size amplicons in 55 samples except one sample as indicated (Fig. 3; Table 2). The YLD affected genotype *i.e.*, Co 05011 showed no amplification in either of the rounds of nested PCR assay. Thus, from the present

study it was evident that among the total YLD samples subjected to nested PCR, 98% samples ($n=55$) revealed the association of phytoplasma using nested PCR assay. Similarly, of the total 47 tropical genotypes, 39 genotypes showed the amplification of ~1.2 Kb size amplicons in second round nested PCR assay (*Data not shown*). Using similar procedure which used in the present study was earlier followed by different workers and obtained similar amplicon sizes of about 1.2 Kb in nested PCR.

The association of SCYP belonging to *Aster yellows phytoplasma* (16Srl-A subgroup) with YLD was first reported by Arocha *et al.* (1999). Subsequently, Cronje and Bailey (1999) detected different strains of phytoplasma associated with YLD belonging to *Western-X phytoplasma* of 16SrlII group. From Brazil, Silva *et al.* (2009) identified the association of SCYP belonging to *Aster yellows phytoplasma* (16Srl-B subgroup) with yellow leaf disease of sugarcane. Later, Soufi and Komor (2014) reported that the yellow leaf symptom of sugarcane was caused by SCYP of 16Srl group (*Aster yellows phytoplasma*) in Thailand. Whereas in Egypt, similar results have been submitted by

Fig. 3: 1.5% agarose gel electrophoresis of the second round nested PCR amplification of phytoplasma using R16F2n/R16R2 universal primers showed amplification of ~1.2 Kb size amplicons in all the 55 samples. Lanes; 1: BO 91, 2: BO 111, 3: BO 120, 4: BO 128, 5: BO 129, 6: BO 130, 7: BO 136, 8: BO 137, 9: BO 138, 10: BO 153, 11: CoP 9113, 12: Co 419, 13: Co 453, 14: Co 1336, 15: Co 06425, 16: Co 89003, 17: Co 98014, 18: Co 0118, 19: CoH 119, 20: CoLk 8112, 21: CoPant 96219, 22: Co 0233, 23: Co 1148, 24: Co 0238, 25: CoH 92, 26: CoH 99, 27: CoLk 94184, 28: CoS 510, 29: CoS 245, 30: CoS 687, 31: *Co 05011, 32: CoS 797, 33: CoS 91230, 34: CoS 90269, 35: CoS 90265, 36: CoS 95222, 37: CoS 94270, 38: CoS 93259, 39: CoS 02258, 40: CoS 02264, 41: CoS 8207, 42: CoS 119, 43: CoS 98259, 44: CoSe 00235, 45: CoSe 01235, 46: CoSe 98231, 47: CoSe 01421, 48: Up 05125, 49: CoS 08234, 50: CoPb 08211, 51: CoPb 08217, 52: CoH 08262, 53: CoH 08263, 54: CoLk 09202, 55: CoLk 09204 and 56: CoS 09232; M= 100 bp plus DNA ladder in A, B, D, E, F and G and 1 Kb DNA ladder in C and H; H= healthy sample, B= Blank and *= Not amplified.

Fig. 4: 1.5% agarose gel electrophoresis of the nested PCR amplification of phytoplasma from the YLD infected sugarcane samples originating from different plant parts of Co 0238. Lanes; 1: roots (R), 2: basal stem regions (S1), 3: middle stem regions (S2), 4: upper stem regions (S3), 5: leaf midribs (MR) and 6: leaf lamina (LL) of YLD affected samples and Lanes; 7: roots (R), 8: basal stem regions (S1), 9: middle stem regions (S2), 10: upper stem regions (S3), 11: leaf midribs (MR) and 12: leaf lamina (LL) of healthy control; M= 1 Kb DNA ladder.

Elsayed and Boulila (2014) on the association of *Aster yellows phytoplasma*. To detect the presence of phytoplasma in different sugarcane plant parts, samples were collected from the leaf midrib and leaf lamina of YLD affected Co 0238 variety which showed amplification of ~1.2 Kb size amplicons. Whereas, samples from roots, basal, middle, and upper stem regions and healthy control from same variety showed no amplification. This confirmed that the phytoplasma showed its presence in the midrib

and leaf lamina region of affected plants (Fig. 4; Lanes: 5 and 6). In India, Gaur *et al.* (2008) for the first time reported the association of 16SrXII group of phytoplasma with YLD in sugarcane. Later, the association of '*Ca. P. asteris*' of 16SrI-B subgroup was detected in two YLD infected sugarcane genotypes viz., CoLk 94184 and CoSe 92423 in India, during 2014–15 (Accession No.: KJ599656 and KJ599657) (Kumar *et al.*, 2015). Recently in India, Kumar *et al.* (2018) have further

confirmed the association of 16Srl-B subgroup of phytoplasma in four YLD affected sugarcane varieties. Therefore, in the present study 189 genotypes were selected for recording YLD incidence following Kumar *et al.* (2015) as baseline information. Further, sequence information of more phytoplasma isolates is required to have clear understanding about the existence of genetic diversity and association of single or mixed infections of SCYP, SCWLP and SCGSP in the YLD affected sugarcane genotypes in India.

As SCYLV is one of the causal pathogens of YLD, recently, Boukari *et al.* (2020) investigated the transmission efficiency of SCYLV by aphid (*Melanaphis sacchari*) on YLD susceptible sugarcane cultivar CP96-1252 and the inoculated plants were found negative for the presence of SCYLV by tissue blot immunoassay (TBIA) and reverse transcription polymerase chain reaction (RT-PCR). The results revealed that the *M. sacchari* is one of the prominent vector of SCYLV in different parts of the world but it is not a vector of SCYLV in Florida. Similarly, the phytoplasma-vector interaction studies and possibility of new vectors need to identify for devising suitable management strategies to stop the secondary spread of phytoplasma in India.

Moreover, all these 142 sub-tropical genotypes were also subjected to DAS-ELISA for detecting the presence of SCYLV but none of the samples reacted positively against specific antiserum (AC diagnostic, USA). Keeping in mind the heavy losses incurred in cane yield and yield contributing parameters due to YLD in sugarcane, it is important to assess its incidence and wide spread prevalence in different sugarcane genotypes. In the present study, of the 142 sub-tropical genotypes 38% (55 genotypes) of YLD affected genotypes were found positive for phytoplasma through nested PCR, while remaining 62% genotypes (87 genotypes) were found without any prominent YLD symptoms and needs further confirmation on possibility of latent infection in these genotypes. Similarly, of the 47 tropical genotypes 83% (39 genotypes) were found affected due to YLD, while eight genotypes were found healthy and needs further confirmation of latent infections of SCYLV and phytoplasma. Thus, findings from the present study showed that the association of phytoplasma with YLD of sugarcane in 55 and 39 genotypes from sub-tropical and tropical conditions of India. Apparently healthy genotypes need to be confirmed and further used in developing YLD resistant progenies and/or genotypes in India.

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