# YELLOW LEAF DISEASE OF SUGARCANE BECOMING A THREATENING DISEASE OF SUGARCANE

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## ABSTRACT

Yellow leaf disease (YLD) caused by *Sugarcane yellow leaf virus*(SCYLV) is recorded in almost all the sugarcane growing countries in the world. The disease was observed during 1999 in India for the first time and later its wide spread occurrence throughout country was found. In popular varieties upto 100% disease intensity is noticed. The expression of symptoms under field conditions is influenced to a limited extent by different biotic and abiotic factors. Severe infection of YLD was found to significantly affect cane yield and juice quality in popular varieties. The affected plants recorded lesser photosynthetic activity and reduced mobilization of photosynthates from the leaves to sink, thereby reducing the photosynthetic efficiency. Virus-infected planting materials have been found as the main source of disease introduction in the field and secondary transmission of the disease occurs through aphid vectors to varying extent. RT-PCR assays have been standardized to detect the virus from the suspected sugarcane varieties and tissue culture derived seedlings. Efforts are being made to identify the disease-resistant sources to YLD in sugarcane germplasm and to breed YLD-resistant varieties. Elimination of the virus through meristem culture combined with molecular diagnosis has been demonstrated to eliminate the virus from the infected planting materials and establishment of disease-free nurseries in largescale is to be popularized to sustain sugarcane cultivation in different states.

## INTRODUCTION

Yellow leaf disease (YLD) in sugarcane earlier described as yellow leaf syndrome (YLS) is characterized by a yellowing of the midrib and lamina from in most of the sugarcane growing countries. The name was given to a disease that appeared in Hamakua (Hawaii) on variety H65-7052 in 1989 (Schenck, 1997). However, genesis of yellow leaf syndrome started most likely earlier because earlier reports of sugarcane leaf yellowing exist, such as yellow wilt in East Africa in the 1960s (Ricaud, 1968). In India, the disease symptoms occurred in most of the sugarcane growing regions of the country and the disease intensity was recorded up to 100 per cent in certain susceptible varieties (Rao *et al.*, 2001, Viswanathan *et al.*, 1999). However, the author reported the occurrence of the disease with detailed account of symptomatology in India (Viswanathan, 2001, 2002). It is now widely distributed in most sugarcane growing countries from all the continents. With the advent of more precise molecular diagnostic techniques, SCYLV was found to be widespread in most sugarcane producing countries. This chapter reviews the disease scenario, symptoms, associated pathogens, impact on sugarcane yield and physiological changes, diagnostic techniques and management strategies.

## SYMPTOMATOLOGY

The symptoms appear initially on matured leaves three through five usually in maturing plant or ratoon crop. The symptoms could be very clear after 5 to 6 months of crop growth. On the leaves, the symptom appears as yellowish midrib on the lower surface. The yellowing may be confined to midrib region or the yellow discolouration may spread laterally to adjoining laminar region parallel to midrib upto a distance of 2.0 cm. Reddish discolouration of midrib and laminar region is also noticed in certain varieties. In most susceptible varieties, typical yellowing of midribs and laminar region is noticed on upper surface of the leaves. Finally,

ymptoms of necrosis of discoloured laminar region from leaf tip to bottom along the mid rib and subsequent ying of entire leaf is noticed. The sugarcane varieties showing mild symptoms of midrib yellowing usually ord normal cane growth. In severely infected clumps with yellowing of mid rib along with laminar region, ne thickness and stalk height are significantly affected. Severe infection of the disease leads to shortening of ternodes in the top. This effect culminates in bunching of leaves at the top. Usually such infection results in ying of entire clumps. Usually the expression of the symptoms will be more severe in ratoon crops than plant op.

Before the clear-cut establishment of etiology and epidemiology, it was been reported that part of YLD mptom expression could be related to other biotic or abiotic factors such as water logging, drought and cool nters (Comstock *et al.*, 1994, Lockhart *et al.*, 2000, Schenck *et al.*, 1997, Schenck, 2001, Izaguirre-Mayoral *et al.*, 2002). Now it is established that no nutritional, environmental or field factor could be identified which early influence symptom expression. It is speculated that the symptom expression is elicited by assimilate ckup in the stalks and that the fluctuation of symptom expression is caused by the growth rhythm of mature garcane stalks (Lehrer and Komor, 2008).

Yellowing of leaf midrib, its expanding to adjoining lamina and necrosis of discoloured tissues from the portion are the common disease symptoms observed in tropical climatic conditions in the country. As symptoms this disease vary among the varieties, the progress of these symptoms was studied at Coimbatore from lisease free status in the plant, using a newly devised 0-5 YLD severity scale. Progress of recovery of symptoms n infected leaves was monitored with a 0-4 symptom severity scale for four seasons (Viswanathan et al., 2012). Detailed observations with 8 infected varieties and 2 genotypes have very clearly indicated a clear variation in ymptom expression among them. Apart from yellow discoloration of midrib, reddish discoloration, pinkish nidrib and laminar discoloration were recorded in cvs B38192, Co 86010 and Co 85019, respectively. Progress f laminar discoloration on both sides parallel to the midrib varied from ~0.5 to 3.0 cm in different varieties. Apart from severe laminar discoloration progressive leaf drying from the tip region along the midrib towards eaf base and bunching of leaves in the crown were identified as the severe form of disease in sugarcane varieties. For such leaf drying with characteristic pattern of 'V' or 'Y' shape and in a bunchy top, leaf yellowing was recorded up to -7th leaf and drying was recorded up to -1st leaf in severely affected varieties (Chinnaraja and Viswanathan, 2015a). Further progressive increase in disease symptom was noticed in most of the varieties till nine months after planting and later a fluctuation in disease expression was noticed among the varieties. Subsequently decrease of yellowing was observed leaving behind the dried laminar region at the tip. The correlation and regression analyses with meteorological parameters established that ~ 50 % of variability in disease severity grades is explained by minimum temperature and relative humidity in the afternoon. This study on the disease symptomology has clearly revealed variation in YLD symptom expressions among the varieties and impact of prevailing weather factors on the fluctuation of disease expressions.

## ASSOCIATED PATHOGEN(S)

Detailed studies conducted in India and other countries characterized the virus to the complete genome level. The virus is a monopartite and consists of a positive-sense single stranded RNA of 5,895–5,898 nucleotides. The viral genome encodes at least six open reading frames (ORFs 0–5) and shows a genome organization typical of Poleroviruses. Nucleotide sequence similarities suggest that at least two independent recombinations have occurred during evolution of the SCYLV genome. SCYLV is therefore considered to be an emerging virus that has evolved by recombination between ancestors of the three genera (*Luteovirus*, *Polerovirus*, and *Enamovirus*) forming the family *Luteoviridae* (Moonan *et al.*, 2000; Smith *et al.*, 2000). Using reverse transcription-polymerase chain reaction (RT-PCR) with specific primer pairs the virus genome was amplified

and characterized in different countries. Abu Ahmad *et al.* (2006a,b) described four genotypes viz. BRA, CUB, PER and REU of SCYLV were distinguished. Later, Viswanathan *et al.* (2008) reported the fifth genotype of SCYLV viz. IND. The name given to each of these genotypes was based on the geographical location from where it was first detected: Brazil, Cuba, Peru, Reunion and India, respectively. Recently complete genome of four SCYLV isolates from Coimbatore were characterized at SBI after complete genome sequencing (~ 5875 nt) (Chinnaraja et al., 2013). These isolates (SCYLV-IND) exhibited amino acid (aa) sequence differences of 29.2-31.8, 28.1-34.4 and 30.7-33.4% with REU, HAW-PER and BRA in partial ORF0 sequences, respectively. Similarly IND isolates have 21.4-23.7, 22.5-25.0 and 21.4-23.9% aa sequence differences with REU, HAW-PER and BRA, respectively in partial ORF 1. The genotype reported from China, CHN1 shared a very close relationship with IND isolates with minimum differences of 4.3-5.3%, 4.8-5.8% and 2.5-3.0% in ORF0, 1 and 5 in aa sequences, respectively and 4.4-5.3% in complete nucleotide sequences.

There is a strong evidence that Sugarcane yellow leaf phytoplasmas (SCYP) are also associated with leaf yellowing in some countries viz.,South Africa, Mauritius and in Cuba. Two strains of phytoplasma were reported and provisionally named as sugarcane yellow leaf phytoplasma I and II (SCYP I and SCYP II) based on their RFLP profiles. After detailed studies on the etiology of YLD in Mauritius, Aljanabe *et al.* (2001) concluded that either SCYLV or SCYP or their combination is associated with YLD symptoms; however, SCYLV is more widely distributed than ScYP in Mauritius.Recently phytoplasma association with leaf yellows and leaf lamina with yellow patches symptomatic samples was confirmed by Kumar et al. (2015) in India through PCR and nested PCR assays using phytoplasma specific primers. Further studies characterized the phytoplasma strains into subgroup 16SrI-B and phylogeneticanalyses confirmed that both the phytoplasmaswere closely related to 16SrI group. This is the first reportof association of 16SrI-B subgroup phytoplasma withsugarcane leaf yellows disease in India.

**Transmission and virus –vector relationship**

Two aphid species viz., sugarcane aphid- *Melanaphis sacchari* (Zehntner) and corn leaf aphid-*Rhopalosiphum maidis* (Fitch) transmit the virus from infected to healthy plant (Scagliusi and Lochkart, 2000, Schenck and Lehrer, 2000). However, *M. sacchari* is the most important and efficient vector of SCYLV in Hawaii than *R. maidis*, which is common in sugarcane fields. Moreover, *R. maidis* infects sugarcane occasionally and sugarcane is not a preferred host of *R. maidis*. Conversely, Rassaby *et al.* (2004) reported that although both *M. sacchari* and *R. maidis* were found on sugarcane, only the former tested positive for SCYLV in RT-PCR. The artificial insect transmission studies carried out by Schenck and Lehrer (2000) revealed that *M. sacchari* transmits SCYLV to wheat seedling to 96 per cent, while *R. maidis* transmitted the virus by 20 per cent. Although the sugarcane yellow aphid, *Sipha flava* is a common aphid multiplies to high populations in sugarcane field did not transmit SCYLV. A high percentage of virus transmission to sugarcane was only observed with the sugarcane aphid *M. sacchari*.Some other phloem-feeding aphids common on sugarcane and other plants in Hawaii also transmitted SCYLV, but much less efficiently (Lehrer *et al.*, 2007). Recently, *Ceratovacuna lanigera*, a new aphid vector was reported to be transmitting SCYLV in South China (Zhou *et al.*, 2006). At SBI, detailed studies were conducted on the virus transmission by sugarcane aphid *M. sacchari* in sugarcane by inoculating virus-free meristem derived from micro-propagated plants of sugarcane cv Co 86032 with viruliferous aphids. Virus transmission was confirmed through RT-PCR assays and subsequently SCYLV population was established through RT-qPCR. A maximum of $22.3 \times 10^3$, $3.16 \times 10^6$ and $4.78 \times 10^6$ copies of SCYLV-RNA targets was recorded in the plants after 7, 180 and 300 days respectively. This study showed that the aphid acts as an effective vector to transmit SCYLV from one plant to another in sugarcane fields in India. The relative standard curve method in RT-qPCR efficiently detected the increment in SCYLV copy numbers in sugarcane after virus inoculation (Chinnaraja and Viswanathan, 2015b).

Since *M.sacchari* has been established as the vector of the disease further studies were conducted on population dynamics of the aphid during different growth stages in different varietal collections. Some genotypes recorded maximum aphid population of up to 621 per plant. However, it ranged from 742 to 920 per plant in severely infested varieties. There was a clear variation among the varieties for aphid colonization and also encountered season to season variation.

The sugarcane diseases caused by phytoplasmas include white leaf (SCWL) and grassy shoot (GSD) in Southeast Asia are transmitted by leafhopper, *Matsumuratettixhiroglyphicus*, Ramu stunt disease in Papua New Guinea, vectored by the delphacid, *Eumetopina flavipes* (Kuniata *et al.*, 1994) and green grassy shoot (SCGGS) in Thailand (Pliansinchai and Prammanee, 2000). A delphacid planthopper, *Saccharosydne saccharivora* was reported to transmit phytoplasma associated with YLD of sugarcane in Cuba (Arocha *et al.*, 2005) and this vector was also found in India. Further studies are required to identify the vector for SCYP from India.

## DISEASE EPIDEMIOLOGY

*Saccharum* species including traditional and modern sugarcane cultivars and wild relatives are the only known natural hosts of SCYLV (Lockhart and Cronje, 2000). Schenck and Lehrer (2000) found that in a field-grown collection of *Saccharum* and related species, 11 to 71% of the clones of four of the species were infected with SCYLV. None of the related genus *Erianthus* plants were infected, but four clones were infected experimentally by aphid inoculation. Apart from sugarcane, the other cereal crops viz., wheat, oats and barley were very susceptible to SCYLV infection than sorghum, which was moderately susceptible to SCYLV, and sweet corn and rice plants were also successfully inoculated with SCYLV. None of seven weeds common in sugarcane fields were infected with SCYLV (Schenck and Lehrer, 2000).

In Reunion, survey of Rassaby *et al.* (2004) for the disease in sugarcane plantations revealed that percentage of infected stalks range from 16 to 94% in cv. R570 and from 21 to 92% in cv. R579. They suggested that in Reunion: (i) infected sugarcane stools do not recover from the disease after harvesting; and (ii) the virus is mainly propagated by planting infected cuttings. When virus-free sugarcane plants of cv. R575 derived from meristem culture were transferred to the field, they found SCYLV in 14, 21 and 25% of the plants 2, 4 and 6 months after planting, respectively. These values remained unchanged 8, 10 and 12 months after planting. The infection rate reached 42% at 6 months of growth in the first ratoon crop. Spatial distribution of infected plants in the plant crop showed that SCYLV spread in the field decreased between 2 and 6 months after planting, and spread between 2 and 6 months mainly occurred around plants that had become infected between planting and 2 months of growth. This phenomenon may be explained by two complementary reasons. First, over flying aphids are most likely to land in crops at early stages of growth before crop cover eliminates the contrast between foliage and bare ground (Klingauf, 1987). Secondly, sugarcane plants may be more suitable for insect feeding and colonization at an early stage of growth, as is the case in broad beans (Bouchery, 1977). Additionally, when a plant was found infected with SCYLV, all the shoots or stalks collected from this plant also tested positive for the virus, with only a few exceptions. In first ratoon crop, newly infected plants were always adjacent to plants already invaded by SCYLV. Viswanathan *et al.* (2006) established that disease infected setts are the primary source for the disease in the field. Eighty percent YLDwasrecorded in 19 varieties, 50-80% in four and less than 50% in one among 28 varieties, when disease infected setts were planted. Whereas the plots planted with healthy seed cane were free either from the disease or with trace incidences.

When viruliferous *M. sacchari* were placed on the blade of the top-visible dewlap (TVD) leaf (leaf 1) the virus was first detected after 3 weeks in the root tips, in the stem tissue under the apical meristem and in a few bundles of a very young leaf ("4). Here confinement of the virus to the phloem was established by tissue-blot

and *in-situ* RT-PCR. It further appeared that the virus was detected exclusively in the companion cells; thus there was no detectable spread of SCYLV-RNA into cells outside of the phloem region. During the following weeks the newly emerging young leaves became infected. The entire green top of the sugarcane plant was eventually infected 9–11 weeks after inoculation (Lehrer *et al.*, 2007). The propagation of infection by aphids proceeds slowly and sporadically, in the range of a few metres per year in the test fields of Hawaii and Reunion Island (Lehrer *et al.* 2007; Rassaby *et al.*, 2004). However, the situation may be different in other sugarcane-growing countries, where infected aphids may be moved over greater distances by wind. SCYLV-infection proceeds at a rate of 20–80% in Florida within 18 months (Comstock and Miller, 2004). Edon-Jock *et al.* (2007) reported that mean virus incidence in plant crops was 6.4%, and it ranged from 0% to 21% according to cultivar and geographical location in Guadeloupe. Detailed surveys in different districts of Tamil Nadu revealed that occurrence of the YLD in moderate to severe form on Co 86032, the major variety under cultivation (Viswanathan et al., 2006). Further, it was found that the disease incidence was more severe in ratoons and in poorly maintained fields.

SCYLV often persists in the plants without being noticed by the growers, in fact, this non-symptomatic stage seems to be the most common epidemiological state for this viral pathogen. A screening in Hawaii revealed that all plants of susceptible cultivars were infected with SCYLV, but disease symptoms appeared only occasionally. The severity of symptom expression is varied to the seasonal variations as it is more pronounced during the cooler winter months. Symptoms also often appeared as plants aged or when they suffered from drought stress (Schenck and Lehrer, 2000).

It has been commonly found that individuals of the same variety do not develop disease symptoms in spite of being infected by the SCYLV. Observations that both symptomatic and asymptomatic plants are located next to each other without a discernible pattern of distribution and that plots were subjected to similar levels of irrigation, fertilization and control of pests, suggest that environmental conditions are not responsible for the onset of symptoms in symptomatic plants. Rather, the polyploid characteristic of sugarcanes could determine that certain SCYLV-infected individuals express a post-transcriptional gene silencing and homology-dependent virus resistance and becoming asymptomatic plants (Ingelbrecht *et al.*, 1999). Cells of asymptomatic plants might produce factors that confer resistance to virus products that would otherwise, lead to symptom expression. The suggestion that the genetic background of the plants determines the appearance or absence of symptoms is further supported by the fact that symptoms in symptomatic plants are not reverted by increasing fertilization and heavy irrigation. Another possibility to consider for the existence of asymptomatic plants is the resistance to diseases induced by the root colonization of selected sugarcane individuals by rhizobacteria (Izaguirre-Mayoral *et al.*, 2002). It was noted, however, that under extreme drought conditions all field-grown SCYLV-infected plants were symptomatic, meaning that the proposed genetic or bacteria-induced resistances were overcome by the imposed water stress.

Detailed studies of the author revealed that ratoon stunting disease (RSD) infection in sugarcane varieties favours severity of YLD and he suggested that infection of sugarcane by YLD and RSD causes varietal deterioration more rapidly which may lead to poor performance of the variety and consequently its replacement (Viswanathan, 2004). He found the clones with severe ratoon stunting disease had severe symptoms of YLD in varieties such as CoS 767, CoS 802, CoJ 64 etc under field conditions. Additionally he found that stunting and poor performance of certain sugarcane clones in different trials as well as in the fields in Karnataka is primarily due to their susceptibility to RSD and YLD. Adverse effect on sugarcane growth was noticed when SCYLV infection was combined with Lxx in many of the varieties. Poor or excess nutrition, drought, water logging and infected with other fungal and bacterial pathogens may aggravate the severity of the disease incidence. Earlier Borth *et al.* (1994) reported that the appearance of YLD in some cultivars may be ameliorated to some extent by high input fertilization and irrigation.

The influence of time and distances on the rate of disease spread conducted by Lehrer *et al.* (2007) found that the heavily aphid-infested and SCYLV-infected sites, such as a sugarcane breeding station and a field station, the infection pressure was very high and 80% of virus-free plants became infected within 4 months. However, virus-free plants at a distance of 1 km from the breeding station remained completely virus-free after 4 months. A large plot of virus-free cane 15 km away from infected sugarcane plants remained virus-free after 4 years. They found that sugarcane plants of a susceptible variety at a distance of 7 km from infected plantation fields are still virus-free some 20 years after the field had been abandoned. When a 100-m-wide area of resistant varieties was planted between a plot of infected cane and a plot of susceptible, virus-free cane, it proved sufficient to completely prevent infection of the virus-free plants for at least 15 months. Subsequently, Lehrer and Komor (2008) observed that SCYLV-infection together with plant and cultivation factors lead to YLD outbreak on Hawaiian fields. They found that occurrence of the disease under field conditions is not uniform in their spread and frequency of expression. They found that severely affected fields have the highest drought stress due to hot climate, sandy soil and strong winds, whereas the crop with the lowest drought stress and the richest (humous) soil have low symptom expression. One reason for the symptom fluctuations may lie in the yearly seasons although seasonal differences in Hawaii are small.

Under the continental climatic conditions of Louisiana spread of YLD and dispersal of its aphid vector showed mainly a random distribution with some cases of aggregation (McAllister et al. 2008). However, Louisiana has a marked winter which results in a rupture of the aphid population dynamics, and the climatic conditions of Louisiana are therefore not representative of the usual tropical or subtropical sugarcane growing areas. A spatio-temporal analysis to characterize SCYLV spread in a disease- free plot in the humid tropical environment of Guadeloupe showed a two-phase disease development in this geographical location. The first one is random due to alate aphid arrivals from outside of the field, in the early stage of plant growth, before the soil is covered by the leaf canopy. The second phase is aggregative and due to neighbourhood colonization over short distances by apterous aphids (Edon-Jock et al. 2009). Daugrois et al. (2011) established a correlation between the aphid dynamics in the field and yellow leaf progress. Additionally, they found anegative correlation between rainfallduring the first weeks after transferring sugarcaneplants to the field and aphid dispersal within the field.This later result revealed an impact of rainfall onaphid invasion and subsequent plant infection bySCYLV. If aphids are the key factor for diseasespread, plant response varied also according tocultivar resistance with high variation depending onrain conditions.

Recent studies of Akbar et al. (2014) suggest non-preference by the aphid towards resistant sugarcane varieties. They found that absence of important free amino acids in the phloem sap of resistant variety and the inability of*M.sacchari*and its endosymbionts (e.g.,*Buchnera*) to derive specific free essential and non-essential amino acids from other ingested molecules,.possibly along with other unidentified factors, underlie the pest's decreased phloem sap ingestion and consequently reduced growth potential on the resistant variety.

## IMPACT OF YLD IN SUGARCANE

The incidence of SCYLV in commercial fields can reach upto 100 per cent in susceptible cultivars (Comstock *et al.*, 1999; 2001; Viswanathan, 2002; Rassaby *et al.*, 2004). Grisham *et al.* (2001) found yield losses of 6%, 11% and 14% in the plant crop, first and second ratoon, in Louisiana with cultivar LCP82-89, which is susceptible to YLD. Cane quality parameters did not differ between SCYLV-infected and non-infected plants of cultivar LCP82-89, but stalk number and tonnage were reduced in virus-infected plants. In contrast, SCYLV had a positive impact on several juice parameters like Brix, sucrose % and purity % were higher in juice from virus-infected green leaf tissue compared to healthy leaf tissue (Grisham *et al.*, 2001). Similar observations were made with the SCYP in Cuba: % Brix is always equal or more to 8 in SCYP-infected leaves

(Peralta *et al.*, 2000). Although the number of internodes was about the same in the infected plant as in the healthy plant they were shorter and lighter in weight. Fresh weights of comparable internodes of the infected plants were only 20–65% the weight of healthy plants, whereas the sugar concentration (nearly exclusively sucrose) per gram fresh weight of the internodes was higher than in the healthy plant (Lehrer *et al.*, 2007).

Rassaby *et al.* (2003) conducted detailed studies on the impact of SCYLV infection on sugarcane growth and yield in Reunion. Comparison of healthy and virus-infected canes from three varieties showed difference for 7 out of the 10 measured parameters in the cv R577. A greater impact of SCYLV on yield of cultivar R577 was found in the first ratoon crop compared to the plant crop: 46% reduction of stalk weight (vs. 28% in the plant crop), 13% reduction of stalk diameter (vs. 7% in the plant crop), and significant reduction in tonnage (37%). However, they found that the number of stalks per stool was not affected in either crop. The stalk height reduction was lower in the first ratoon crop when expressed as a percentage (18% vs 28% in the plant crop). Although no impact of SCYLV was detected in other two varieties in the plant crop, several yield components in them in the first ratoon crop were significantly lower in virus-infected than in virus-free plants. They concluded that the impact of SCYLV and tolerance of sugarcane to the virus vary according to sugarcane cultivar. In healthy sugarcane plants, root dry weight was positively correlated with fresh weight, stalk number, Brix and sucrose content. In contrast, in SCYLV infected plants, root dry weight was negatively correlated with fresh weight and stalk number. These results suggest that in healthy plants, a well-formed root system is crucial for plant development as it provides aboveground plant parts with sufficient water and nutrients for proper growth (Vasconcelos *et al.*, 2007).

Studies conducted at SBI indicated that healthy crops recorded more millable canes than the respective diseased crops except in seven varieties. Drastic reduction in NMC was recorded in Co 6511, Co 86032, CoS 687 and CoV 92101 due to disease infection (Viswanathan *et al.*, 2006). Earlier studies conducted by the author (Viswanathan, 2002) showed that the disease infection results in reduction in cane diameter and photosynthetic rate in a set of sugarcane varieties. The data indicated that cane diameter was reduced in all the affected varieties. However, the reduction was significant in CoS 510, CoS 767, CoS 8407 and Co 775 as compared to other varieties 84A125 and CoJ 84191. In many situations the author has observed severe growth retardation of virus infected sugarcane in the country in the field and in search stations. This can be seen more clearly in various varietal trials being conducted across the states where entries from different locations are planted and compared for their performance. He has observed that the virus strain introduced through the infected seed materials express the disease in severe manner in the new location.

Detailed studies were conducted on the physiology of YLD infected sugarcane in different countries. The translocation of sugars in sugarcane occurs through the leaf blade and leaf sheath to the stalk, via the phloem, then to the centre of the stalk and downward to roots. During the day, sugars are temporarily accumulated in leaf sheaths before their translocation to the stalk, with about 80% of the carbon fixed during early morning hours being exported after midday. Phloem functionality is therefore a requisite for appropriated sugar movement within the plant, and the phloem vessels are the main target of the SCYLV infection. Studies clearly proved that due to severe cytophatic alterations in the companion cells of the phloem a higher level of tannins accumulate and blockage of the sieve plates of the conducting vessels occur (Izaguirre-Mayoral *et al.*, 2002). Thus, TRS accumulation in leaf blades and sheaths of symptomatic plants seems to be the result of the negative effect of SCYLV infection on the phloem-transporting functions. The accumulation of a-amino-N in the leaf blade supports the suggestion of impaired translocation in symptomatic plants. In conditions where TRS are accumulated, an excess of photosynthates are diverted, via the enzyme pyruvate kinase, toward the synthesis of a-amino-N. In addition, it is expected that photosynthetic rates in symptomatic plants are decreased because of the massive accumulation of glucan grains in the bundle-sheath chloroplasts and the low chlorophyll, P and N content in leaves (Meinzer and Zhu, 1998).

There are indications that SCYLV infection slows the export of assimilates from the source leaves to the sink resulting in assimilates backing up in the source leaves, eventually inducing degradation of chlorophyll and chloroplasts resulting in leaf yellowing (Lehrer *et al.*, 2001). Brix readings for carbohydrate content of juice of YLD symptomatic plants are two to three times higher in leaf midribs compared to healthy plants. The physiology of the infected plants become altered and that the infected leaves exhibit a higher carbohydrate level, a lowered chlorophyll a/b ratio and a smaller photosynthetic capacity (Comstock *et al.*, 1998; Lehrer *et al.*, 2001). HR brix recorded at 10th month of plant crop at different internodes showed markedly reduced brix levels in CoJ 84191, CoS 510, CoS 767 and CoS 8407 as compared to Co 775 and 84A125 due to YLD. These results indicated that YLD infection significantly affects cane growth and sucrose accumulation in some of the susceptible sugarcane varieties. When photosynthetic rate was recorded in six varieties showing characteristic YLD symptoms at 10th month, it was found that there was a reduction in the photosynthetic rate in leaves of all the disease infected varieties as compared to the respective disease free leaves (Viswanathan, 2002).

Lehrer *et al.* (2007) compared carbohydrate concentration of old and young sugarcane plants, virus-free or infected in Hawaii where, sugarcane crop is harvested after 15–26 months of growth followed by several weeks for maturation after the application of a chemical ripener. In spite of the higher sucrose concentration in the SCYLV-infected plant, the sugar content per internode was less because of the reduced internode weight. The changes of total carbohydrate content by SCYLV infection and/or symptoms were due to increases in sucrose and starch, hexoses played only a minor role.

The leaves of the symptomatic plant and, more so of the non-symptomatic plant, had much higher levels of carbohydrates than the virus-free plants. In addition, the infected plants with the higher overall level of carbohydrates (sucrose) across all internodes did not show carbohydrate peak in the middle-aged leaves as in virus-free plants. Starch increased in the infected plants, especially in the non-symptomatic plants. The hexose levels were highest in the symptomatic plants. The carbohydrate change from day to night in leaves of different age from virus-free or -infected plants did not show a clear pattern, whereas the variously aged leaves of the virus-free plants showed relatively small diurnal changes, those from the infected plants exhibited a wide diurnal fluctuation with no obvious pattern, some leaves from the infected plants even appeared to have lost carbohydrates during daytime (Lehrer *et al.* 2007). Healthy virus-free leaf tops managed to export a large fraction of the daily photo-assimilates during the daylight hours whereas leaves of non-symptomatic plants, where the carbon assimilation capacity may be still relatively intact, showed higher sugar accumulation in the leaves due to reduced translocation capacity. They concluded that SCYLV-infection, even when not yet expressing YLD symptoms, is not a silent infection, but has a small measurable effect on leaf and phloem physiology possibly exerting negative effects on plant performance resulting in yield loss.

Recently detailed studies on the impact of SCYLV were conducted at SBI by comparing physiological parameters in symptomatic and asymptomatic plants of 10 different cultivars and a genotype. In addition, similar comparisons were made between virus infected and virus free plants derived through meristem culture.Our studies established that among several physiological parameters, photosynthetic rate (A), stomatal conductance (gs) and SPAD meter values were significantly reduced in cultivars severely infected with SCYLV. Virus-infected cultivars exhibited significant reduction in growth/yield parameters, viz. stalk height, stalk thickness and number of internodes. Plant growth reductions were found to be 42.9, 42.3 and 38.9% in susceptible cultivars CoPant 84211, Co 86032 and CoC 671, respectively. In addition to reduction in stalk weight, height and girth, YLD also reduced juice yield in the affected canes up to 34.15% (Viswanathan et al., 2014). Similarly, comparison of diseased (virus-infected) and virus-free plants derived through meristem culture also revealed a significant reduction in cane growth/physiological parameters and juice yield due to virus infection. The results of these studies conclude that SCYLV seriously affects cane and juice yield in major sugarcane varieties in India.

## DIAGNOSIS

Presence of SCYLV was observed consistently by electron microscopy (EM) and immune specific electron microscopy (ISEM) in partially purified extracts of YLD-infected sugarcane but not of sugarcane that had no history of YLS (Vega et al., 1997; Scagliusi and Lockhart (2000). Antiserum specific to SCYLV was prepared by Lockhart from purified virus (Scagliusi and Lockhart, 2000). Schenk et al. (1997) developed tissue blot immunoassay (TBIA) technique using polyclonal antisera to detect SCYLV. Moutia and Saumtally (1999) reported suitability of double antibody sandwich-enzyme linked immunoassay (DAS-ELISA), immune specific electron microscopy (ISEM) and TBIA for the detection of the virus from the suspected sugarcane clones. They also found the presence of the virus in many of the asymptomatic plants through these techniques. DAS-ELISA has also been successfully used to detect the pathogen in infected plant material (Scagliusi and Lockhart, 2000; Viswanathan, 2002, 2004; Viswanathan and Balamuralikrishnan, 2004). Moutia and Saumtally (2001) standardized diagnosis of SCYLV by ELISA in infected juice collected from sugarcane stalk tissues. They established that SCYLV is present in all the stalks of infected stools. Korimbocus *et al.* (2002) expressed the coat protein and read through domain of SCYLV in a bacterial expression system and using the purified protein they have developed monoclonal antibodies. They have developed tissue blot assay to detect SCYLV using the serum. TBIA is probably the most widely used technique to detect the virus in different countries. Reliable results were also obtained through reverse transcription-polymerase chain reaction (RT-PCR) for detecting the virus in sugarcane. An RT-PCR technique was used to detect SCYLV in the sugarcane quarantine facility of CIRAD in Montpellier, France (Delage et al., 1999).

Diagnosis of YLD is being done for the past 15 years in India. Viswanathan et al. (1999) reported the disease for the first time in the country and the associated virus. Subsequently Viswanathan (2002) reported the disease occurrence in detail with detailed symptomatology and diagnosis using DAS-ELISA. Viswanathan and Balamuralikrishnan (2004) established that DAS-ELISA could be used to diagnose the virus infection in sugarcane using juice of sugarcane stalks. The reported SCYLV primers have shown non-specificity in our RT-PCR studies. Hence we have standardized RT-PCR technique with new set of specific primers to detect the virus in the suspected samples and meristem derived seedlings (Viswanathan *et al.*, 2008). The efficiency of the diagnostic primers *viz.*, SCYLV-615F and SCYLV-615R was established at SBI with a set of sugarcane samples collected before and after yellow leaf symptom expression. In pre-symptom expression, 34 of 44 samples gave a positive amplicon of ~615 bp in size in RT-PCR. The RT-PCR assay performed with the samples collected after symptom expression in the same set of varieties revealed that almost all the samples except one were found infected with SCYLV. Of the 43 positive samples, 10 were found apparently free from yellow leaf symptoms(Viswanathan et al., 2009). The RT-PCR assay established that 97.73 % of the samples were found to be infected with SCYLV and the diagnostic primers efficiently detecting all the SCYLV population even in asymptomatic plants. This study very clearly indicated that virus titre increases in most of the varieties from 6 to 11 month stage.

Now, RT-PCR assays are being routinely used to diagnose the presence of a virus in sugarcane with the primers specific to the virus. More recently, real-time RT-PCR assays are also developed at SBI. The assay was used to compare SCYLV in meristem-derived tissue culture raised *in vitro* plantlets and asymptomatic sugarcane plants by relative standard curve method. In this assay, copy number of virus population in *in vitro* plantlets and asymptomatic plants was estimated from 20,314.58 to 4,330.87 and from 8.96 to 0.27 million copies of viruses, respectively. Relative expression level of the virus between *in vitro* plantlets and asymptomatic plants was in the ratio 73.7 : 243,393.1 (Chinnaraja et al., 2014). The results clearly established that meristem-derived tissue culture significantly reduced SCYLV population and it is concluded that the relative standard curve method efficiently detects the copy numbers of the target virus in different sugarcane samples. Due to these diagnostic techniques, it is possible to demonstrate that most sugarcane varieties infected by SCYLV do not exhibit disease

symptoms. The virus was therefore spread undetected around the world in symptomless but infected material for many years, until efficient diagnostic tools were available, especially in quarantine.

**Disease management through healthy planting materials**

Since many of the viruses and phytoplasmas infecting sugarcane are systemically infecting sugarcane, their elimination through meristem-tip culture is being followed in many countries. *In vitro* culture techniques employed for virus elimination involve indirect morphogenesis. However, clonal fidelity is not assured when plants regenerate via a callus stage. Some viruses can be effectively eliminated from infected plants owing to their mode of replication and their mechanism of movement within the plant. Meristem tip culture is the most widely used method to eliminate the virus/phytoplasma. This technique takes advantage of the fact that many viruses are unable to replicate in this region. Transfer of the meristem dome, together with one or two leaf primordial, to a culture medium and development into a plantlet may lead to the elimination of a virus. Successful elimination of sugarcane mosaic virus and Fiji disease virus in sugarcane through apex or bud culture has been reported earlier.

Even since YLD became a serious disease studies were conducted to eliminate the associated pathogens SCYLV and SCYP by tissue culture from infected sugarcane plants worldwide. In Mauritius, the tissue culture derived regenerated plants were remained free from the respective pathogens over a period of one year in the glasshouse, confirming that the pathogens had been eliminated by tissue culture. Attempts made from CIRAD, France achieved virus elimination of 92% however, only 64 % disease free plantlets were achieved. Hence stringent seed indexing methods have to follow while screening of the regenerated plantlets. The potential for eradicating pathogens via rapid regeneration of plants directly from leaf roll discs was explored in South Africa. The technique, NovaCane®, has been used successfully to remove SCYLV from sugarcane. Plantlets were transferred to seedling trays after ten weeks and acclimatized in the glasshouse. Two months later, tests for the presence of the disease causal agents in selected plants were performed by RT-PCR for SCYLV and it was found that the process eliminated SCYLV. In addition, this process enabled elimination of bacterial pathogens from diseased sugarcane plants while simultaneously enabling large-scale micro-propagation. As disease eradication was not 100% effective, they have suggested that donor plants require conventional screening for the presence of known causal agents prior to micro-propagation.

Detailed studies were conducted at SBI to eliminate SCYLV from infected sugarcane. Meristem culture combined with viricide Ribavirin has effectively eliminated the virus and reverse transcription polymerase chain reaction (RT-PCR) is being used routinely to index the tissue culture materials (mother plants or seedlings) for the virus. Production of SCYLV-free seedlings has ensured supply of YLD-free planting materials to the growers fields and such fields showed renewed vigour in the crop. Overall, virus elimination through meristem culture combined with molecular diagnosis has been demonstrated as a viable strategy to manage YLD, which occurred in epidemic form in sugarcane in the recent years.

All the tissue culture seedlings supplied form the institute are indexed for the viruses. In addition, SBI offers virus-indexing service to tissue culture production units in the country and many laboratories are utilizing the service. Multiplication of virus-free planting materials in the nurseries ensures crop vigour in the field. Detailed studies conducted in the factory areas in Tamil Nadu revealed that adopting tissue culture derived YLD-free nurseries resulted in better crop stand with good vigour. Such fields recorded higher yields compared to the YLD infected crops in the same region (Viswanathan, 2012, 2013, 2015). There is also apprehension from the factories that the virus-free crop would acquire virus through aphid vectors subsequently. Yes, it would happen in the field. However build-up of adequate virus titre in the disease-free plants would take about 5-6 years. By the time it is due for new seed through the seed cycle and the fields are to be replaced with fresh seed.

SBI offers indexing of seedlings for viruses causing mosaic (SCMV and SCSMV) and YLD (SCYLV) and phytoplasma causing grassy shoot. During the last five years many tissue culture production units in the country utilized the services from SBI to produce disease-free planting materials (Table 1). Hence this approach is sustainable to manage sugarcane from non-fungal diseases and to prevent varietal degeneration in popular varieties.

**Disease resistance**

Sugarcane response to infection by SCYLV and the disease varies according to the variety, and numerous varieties can be infected by the pathogen without exhibiting disease symptoms. Researchers have found that all clones in the varietal development programme become infected by the end of the 10-year programme. In the world collection of sugarcane and related grasses in Florida, incidence of SCYLV ranged from 7% in *S. spontaneum*, the most resistant group, to 76% in *S. officinarum*, the most susceptible group (Comstock *et al.*, 2001). Differences in virus infection rates between different species of *Saccharum* were also reported in Hawaii. Resistance to sugarcane infection by SCYLV and to YLD therefore appears the most promising method to control the disease. In Colombia, virus infection varied between 0% and 100% and a cross between a susceptible female parent and a resistant male parent resulted in mostly resistant progenies (Victoria *et al.*, 2005). Similarly studies are in progress in Hawaii, Louisiana, Brazil and other countries to develop YLD resistance in sugarcane, where artificial inoculation techniques through insect vectors was standardized. Development of a new disease rating system is in progress to identify disease resistant and tolerant varieties in sugarcane to the virus.

Earlier studies of Viswanathan (2002) revealed that more than 30% of the varietal collections at Coimbatore are infected with YLD. Further studies carried out to identify disease resistance in sugarcane led to identifying varieties which are most susceptible and resistance to the disease. However there is a need to develop disease rating scale to quantify the disease resistance in sugarcane germplasm and progenies. A disease rating scale to YLD was developed (Viswanathan et al., 2012) and this score is being used at SBI to assess and screen sugarcane germplasm for YLD resistance. The same is also used under AICRP on sugarcane in different AICRP centres to identify YLD resistance in new sugarcane varieties.

**Transgenic approaches**

Transgenic sugarcane lines were produced by biolistic bombardment with an untranslatable virus coat protein construct in Hawaii (Zhu *et al.*, 2007). The presence of the transgene was confirmed with PCR and Southern blot analyses. Viral resistance of the transgenic plants was evaluated by inoculation with viruliferous aphid vectors followed by tissue blot immunoassay (TBIA), and inoculation results indicated the resistance levels in transgenic sugarcane were significantly higher than non-transformed controls. The resistance level of the seven transgenic lines varied from complete resistance, where no SCYLV was detected in any of the three inoculation experiments, to slight resistance, where only 20 to 50% of the tested plants showed SCYLV infection. Detailed studies were initiated at SBI to develop transgenic sugarcane for resistance to SCYLV through RNAi approach.

## CONCLUSION

Overall, the investigations conducted during the last 16 years across the countries show that SCYLVinfection, even when not yet expressing YLD symptoms, is not a silent infection, but has a small measurable effect on leaf and phloem and cause adverse effects on sugarcane physiology.Due to that YLD causes significant losses in sugarcane yield. Further, the disease is responsible for varietal degeneration in popular varieties and such degeneration is rapid and severe when other non-fungal pathogens infect sugarcane together as compared to their separate infections. The YLD occurrence to epidemic levels in different states is

a serious concern for sugar industry and due to that longevity of the affected varieties in the field is threatened.Apical meristem culture combined with molecular diagnosis has been proved to be effective in producing virus free plantlets and a method of choice to eliminate SCYLV from infected mother plants. Now some of the sugar industries have initiated sugarcane multiplication through tissue culture. Further, the recent developments in sugarcane multiplication through single bud or bud-chip seedlings should take advantage of virus-free tissue culture derived seedlings to multiply and distribute disease-free quality planting materials to the growers. Establishing disease-free nursery chains of sugarcane varieties across the country would facilitate healthy sugarcane in the field and varietal vigour will also be maintained in the long run. We offer virus-indexing service to tissue culture production units in the country. This effort would lay foundation to create seed nursery chains in different regions to produce quality planting materials. Ultimately these efforts would increase sugarcane productivity and sustain sugarcane cultivation in the country.

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**Table 1: Indexing of mother clones/*in vitro* stock cultures for virus indexing in sugarcane at SBI, Coimbatore**

| Year | Tissue culture laboratory | No of batch samples | SCYLV | | SCMV | | SCSMV | | GSD-phytoplasma | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve |
| 2009-10 | EID Parry, TN; Rajshree Sugars, TN | 185 | 179 | 6 | - | - | - | - | 33 | 143 |
| 2010-11 | VSI, Pune; EID Parry, TN; Rajshree Sugars, TN | 378 | 270 | 108 | 13 | 7 | 19 | 1 | 17 | 166 |
| 2011-12 | VSI, Pune; EID Parry, TN; Rajshree Sugars, TN. | 181 | 107 | 74 | 3 | 36 | 3 | 33 | 5 | 111 |
| 2012-13 | VSI, Pune; EID Parry, TN; Rajshree Sugars,TN; KEMROCK Agritech Pvt Ltd, Gujarat;KCP Sugars, AP;C.G. Bhakta Inst., Bardoli, Gujarat | 280 | 257 | 123 | 52 | 208 | 40 | 220 | 34 | 251 |
| 2013-14 | Rajshree Sugars, TN; C.G. Bhakta Inst., Bardoli, Gujarat; Lokmangal Organic R&D, Wadala, Solapur, MS, Navabharath Ventures Ltd, Samalkot, AP, Sarvaraya sugars, Chelluru, AP, EID Parry Ltd, Pugalur, TN | 427 | 150 | 277 | 49 | 336 | 47 | 338 | 68 | 287 |
| 2014-15 | Rajshree Sugars, TN; Navabharath Ventures Limited, Samalkot, AP; Sarvaraya sugars, Chelluru, AP; Harinagar Sugar Mills, Harinagar, Bihar | 288 | 87 | 201 | 26 | 256 | 14 | 268 | 24 | 258 |
| **Grand Total** | | 1739 | 1050 | 789 | 143 | 843 | 123 | 860 | 181 | 1216 |

*Note : (-) Not tested; Additionally SCMV and SCSMV were tested for few batches.