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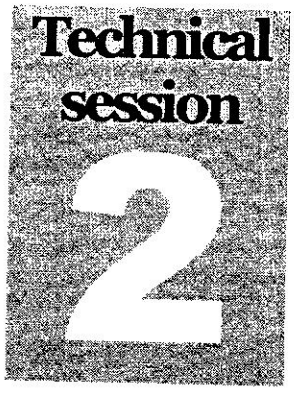

New disease of sugarcane – Yellow Leaf Disease (YLD) & its management

Technical Session – II : New disease of sugarcane – Yellow Leaf Disease (YLD) & its management

Status of Yellow Leaf Disease occurrence in sugarcane, its impact on sugarcane yield and management

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ABSTRACT

Yellow leaf disease (YLD) caused by sugarcane yellow leaf virus is recorded in almost all the sugarcane growing countries in the world. The disease was observed during 1999 in the country for the first time and later its wide spread occurrence throughout country was found. In popular varieties, the disease intensity varied from 0 to 100 % and the disease incidence varied from 0 to 75.0% under field conditions. The expression of symptoms under field conditions was 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 certain cultivars. YLD infected sugarcane plants recorded lesser photosynthetic activity and reduced mobilization of photosynthates from the leaves to sink, there by reducing the sucrose accumulation in the affected stalks. Planting of setts from virus infected canes has been found as the chief source of disease introduction in the field and secondary transmission of the disease through aphid vectors has been reported to varying extent. Detailed studies based on serological (ELISA) and molecular techniques (RT-PCR) conducted at the institute have confirmed the association of sugarcane yellow leaf virus (SCYLV) with the disease. Among the different management approaches, clean seed programme is found to be the most effective to control the disease. Elimination of the virus through meristem culture has been demonstrated to purify the virus infected planting material. Hence a healthy seed nursery programme is suggested to manage the disease for the sustainable sugarcane production.

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 Argentina, Australia, Brazil, Colombia, Cuba, Hawaii, West Indies, Florida, Texas, Mauritius, Reunion, South Africa and other countries. YLD is the name 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 reliable serological and molecular diagnostic techniques, SCYLV was found to be widespread in most sugarcane producing countries. Symptoms of YLD have been attributed to many causes, both biotic and abiotic, but the biotic causes are associated with infection by a *Luteovirus* or Phytoplasmas in all the sugarcane-growing countries. The paper reviews the disease scenario, symptoms, associated pathogens, impact on sugarcane yield and physiological changes, diagnostic techniques and management strategies.

SYMPTOMATOLOGY AND ETIOLOGY

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 discoloration may spread laterally to adjoining laminar region parallel to midrib up to a distance of 2.0 cm. Reddish discoloration 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, symptoms of necrosis of discolored laminar region from leaf tip to bottom along the mid rib and subsequent drying of entire leaf is noticed. The sugarcane varieties showing mild symptoms of midrib yellowing usually record normal cane growth. In severely infected clumps with yellowing of mid rib along with laminar region, cane thickness and stalk height are significantly affected. Severe infection of the disease leads to shortening of internodes in the top. This effect culminates in bunching of leaves at the top. Usually such infection results in drying of entire clumps. Usually the expression of the symptoms will be more severe in ratoon crops than plant crop.

Before the clear-cut establishment of etiology and epidemiology, it was been reported that part of YLD symptom expression could be related to other biotic or abiotic factors such as water logging, drought and cool winters (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 clearly influence symptom expression. It is speculated that the symptom expression is elicited by assimilate backup in the stalks and that the fluctuation of symptom expression is caused by the growth rhythm of mature sugarcane stalks (Lehrer and Komor, 2008).

Different grades of leaf yellowing are observed under field conditions. Usually the yellowing starts from the midrib and proceeds successively to the leaf blade and eventually lead to complete drying leaf lamia. This progression of yellowing is different from yellowing during senescence (caused by age or by nutrient shortage) where the yellowing starts from the outer side of leaf blades. However, YLD-like leaf yellowing is

also observed when major leaf veins were mechanically interrupted, for example when the midrib of a leaf break because of strong wind or when a partial cut of the stalk occurs. Also severe infestation by *Sipha flava*, an aphid which does not transmit SCYLV (Schenck & Lehrer, 2000), caused local yellowing at the feeding site. Care has to be taken to distinguish YLD symptoms from these other yellowing events during monitoring. However, the strong yellow leaf symptoms and the 'bunchy top' morphology of the green leaf top, when the leaves emerge very closely to each other because of the shortened internodes is not observed after mechanical or insect-caused damage.

ASSOCIATED PATHOGEN(S)

In the middle of the 1990s, small icosahedral particles were found in a symptomatic plant (Anon, 1995) which is transmitted by sugarcane aphid, *Melanaphis sacchari*. This emerging virus has been named as sugarcane yellow leaf virus, with the acronym SCYLV (Schenck *et al.*, 1997; Vega *et al.*, 1997). The complete genome of SCYLV has been sequenced and characterized. It is 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 Pcleroviruses. Nucleotide sequence similarities suggest that at least two independent recombination's 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). Although SCYLV shares genomic properties with members of the genera *Polerovirus* and *Luteovirus* (Borg *et al.*, 1999), it has recently been assigned to the genus *Polerovirus* of the family *Luteoviridae* by the International Committee on Taxonomy of Viruses on the basis of its striking similarities to the 5' half of the *Polerovirus* genome (D'Arcy *et al.*, 2005; Smith and Barker, 1999). The function of the peptide encoded by ORF0 may be linked to expression of symptoms (Van der Wilk, 1997), and more recently has been shown to be a suppressor of RNA silencing. ORFs 1 and 2 are translated together and code for a multifunctional peptide and an RNA-dependent RNA polymerase (RdRp), respectively. The peptide sequence encoded by ORF1 includes sequence motifs of both a serine proteinase and a putative genome-linked viral protein (VPg). ORF3 codes for the coat protein and ORF4 for a movement protein, whereas the peptide encoded by ORF5 is a read-through protein. This latter protein is produced via a translational read-through of the peptide encoded by ORF3 and might be linked to virus transmission by aphids. Using reverse transcription-polymerase chain reaction (RT-PCR) with specific primer pairs the virus genome was amplified and characterized in different countries. Four genotypes viz. BRA, CUB, PER and REU of SCYLV were distinguished (Abu Ahmad *et al.*, 2006a,b). Recently, 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.

There is strong evidence that phytoplasmas 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.

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 (Lockhart *et al.*, 1996, 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).

The sugarcane diseases caused by phytoplasmas include white leaf (ScWL) and grassy shoot (ScGS) in Southeast Asia are transmitted by leafhopper, *Matsumuratettix hiroglyphicus*, 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). Recently a delphacid planthopper, *Saccharosydne saccharivora* was reported to transmit phytoplasma associated with YLD of sugarcane (Arocha *et al.*, 2005) and this vector was also found in India (CAB International, 2001).

SPREAD

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, Comstock *et al.*, 2001, Lehrer *et al.*, 2001). 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 YLD was recorded 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 (Table 2).

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 precedes slowly and sporadically, in the range of a few meters 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 survey 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. Further, it was found that the disease incidence was more severe in ratoons and in poorly maintained fields (Table 1).

EPIDEMIOLOGY

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 (humus) soil have low symptom expression. However, overall symptom outbreak could not be correlated either to ambient air temperature or nutrient shortage. They also found occurrence of yellow leaf symptoms during four peaks in the infected cultivars, at 200, 350, 500 and 600 days after planting. One reason for the symptom fluctuations may lie in the yearly seasons although seasonal differences in Hawaii are small.

IMPACT OF YLD

The incidence of SCYLV in commercial fields can reach up to 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 components (% Brix, % Sucrose, % Fiber and % Purity) 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 leaf components 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. Similar results were found for other members of the *Luteoviridae* family: *Potato leaf roll virus* (PLRV) on potato and *Barley yellow dwarf virus* (BYDV) on barley can cause severe yield losses, and the reaction to these viruses varied according to cultivars. 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 (Table 3).

PHYSIOLOGICAL AND GROWTH ANALYSES

Cytopathology

Diseased plants exhibit the abnormal phloem development which is consistent with diseases caused by luteoviruses and poleroviruses (Vega *et al.*, 1997). Ultra structural analyses of Izaguirre-Mayoral *et al.* (2002) in leaf tissues of symptomatic plants indicated the presence of virus particles only in the cytoplasm of companion cells of the phloem. In symptomatic plants, all the companion cells had nuclei containing masses of electron-dense material, and a high number of lipid droplets were detected in the surrounding cytoplasm. A distortion of the ultra structure of mitochondria, severe disruptions in the outer membrane of the nuclei and a proliferation on the endoplasmic reticulum were also observed. The bundle-sheath chloroplasts contained a high number of glucan grains. However they found none of these cytopathic changes in the companion cells of asymptomatic plants, in spite of the high density of virus particles in the cytoplasm. Fontaniella *et al.* (2003) observed ultra structural changes in diseased sugarcane cv Cuba 120-78 plants. They found abaxial epidermis of diseased leaves showed a large amount of adhered superficial bodies, which partially occluded some stomata. Bundle sheath cells surrounding the bottom of phloem of diseased leaves were separated from the conducting tissues by a large layer of an amorphous matrix similar to wax.

Physiology

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). Young plants may suffer less because the short distance between source and sink results in a steep assimilate gradient. In long stalks the assimilates may accumulate due to the lower pressure gradient in the phloem. This assimilate backup in lodging sugarcane may be further enhanced by lower temperature. It was found previously that cooling has a strong effect on long distance transport of assimilates, stronger than on photosynthesis or sugar synthesis, with the result that the sugar content in the leaves increased at low temperature (Ebrahim *et al.*, 1998). 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 (Table 4). 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).

Vasconcelos *et al.* (2007) observed significant increases in Brix and consequently sucrose content, respectively, 14.6% and 42.9%, in stalks of infected plants. Thus, sucrose

transport downward to roots could be reduced if the root system is damaged, leading to sucrose over accumulation in the stalks. They found that SCYLV infected plants had an average root dry weight decrease of 43% compared to uninfected plants, which was probably related to reductions in the metabolic transport via phloem from leaves to roots. However, the proportion of roots at each depth was not affected by SCYLV infection, and its vertical architecture distribution was preserved

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 starch and sucrose concentration of all leaves in the green leaf top was higher in the SCYLV-infected plants, both symptomatic and non-symptomatic, than in the virus-free plants. The highest levels were found in the non-symptomatic plants followed by the symptomatic plants and then the virus-free controls. The changes of total carbohydrate content by SCYLV infection and/or symptoms were due to increases in sucrose and starch, hexose's 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.

Overall when compared with asymptomatic plants, the symptomatic plants showed: (a) a marked reduction in the area of the leaf and internodes; (b) a high accumulation of total reducing sugars (TRS), glucans and a-amino-N in the leaf blade and of TRS in the corresponding leaf sheath; (c) a decrease in the chlorophyll, phosphorus and nitrogen content in the leaf; (d) the disappearance of the leaf diurnal fluctuations in TRS accumulation and export as well as the daily oscillations of TRS and glucans between dawn and dusk; and (e) major ultra structural alterations in the companion cells of the phloem, including the accumulation of SCYLV particles in the cytoplasm. In asymptomatic plants, none of the growth and physiological alterations described above is observed.

Diagnosis

Tissue-blot immunoassay (TBIA) is probably the most widely used technique to detect the virus in different countries (Schenck *et al.*, 1997; Comstock *et al.*, 1998; 1999; Rassaby *et al.*, 2003; Chatenet *et al.*, 2001; Victoria *et al.*, 2005). Double antibody sandwich-enzyme linked immunoassay (DAS-ELISA) has also been successfully used to detect the pathogen in infected plant material (Scagliusi and Lockhart, 2000; Viswanathan, 2004; Viswanathan and Balamuralikrishnan, 2004). Reverse transcription-polymerase chain reaction (RT-PCR) is being routinely used to diagnose the presence of a virus in sugarcane with primers specific to the virus. More recently, real-time RT-PCR assays are also developed. 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 symptom less but infected material for many years, until efficient diagnostic tools were available, especially in quarantine. We have standardized RT-PCR technique with 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. 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.

Management

Disease free planting material is the prerequisite for the better crop establishment that will delay the disease development and spread. Since, sugarcane is propagated through vegetative cuttings that carry the virus to the field, supply of disease-free setts forms the basis of disease management in sugarcane. In certain countries, it was found that spread of the viral infection to neighbouring plants in the plantation fields via aphids was relatively slow and in the range of a few metres per year. No indication of long-distance transfer could be seen. This indicates that it may be possible to produce and use virus-free seed cane for planting of high-yielding but YLD-susceptible cultivars. However, SCYLV-infection proceeds at a rate of 20–80 per cent in Florida within 18 months (Comstock and Miller, 2004). Studies in this area of work have to be taken under Indian conditions.

Virus elimination

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. Three methods are currently used to eliminate the virus/phytoplasma from infected planting materials viz., thermotherapy, tissue culture and chemotherapy. Of these methods, 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 (Faccioli and Marani, 1998). 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.

Fitch *et al.* (2001) reported that all plants regenerated from callus derived from meristems or buds, except for two where meristem explants were 1 mm or larger, produced virus-free plants that remained free from SCYLV for at least 4 years. Callus derived from young leaf rolls gave 100 per cent success in the elimination of both SCYLV and SCYP, and no abnormal growth was observed in any of the plants. A likely explanation for the elimination of both pathogens is their localization within the phloem. Lack of connection between the somatic embryos and the phloem limits movement of the virus or phytoplasma. Parmessur *et al.* (2002) tried to eliminate the SCYLV and SCYP by tissue culture from infected sugarcane plants and they found that 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. Although these techniques eliminate the virus, hundred percent elimination of virus is not possible. Previously Chatenet *et al.* (2001) achieved virus elimination of 92 %, however they got only 64 % disease free plantlets. 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. Here leaf roll explants from the stalks were placed on MS0.6 medium (MS salts and vitamins, 20 g/L sucrose, 0.5 g/L casein, 0.6 mg/L 2,4-D, 8 g/L agar pH5.8) (Snyman *et al.*, 2006) for five weeks with fortnightly subculture intervals. Embryo germination occurred on MS0 (MS salts and vitamins, 20 g/L sucrose, 0.5 g/L casein, 8 g/L agar pH 5.8) after a further five weeks. 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, Snyman *et al.* (2007) suggested that donor plants require conventional screening for the presence of known causal agents prior to micro propagation.

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.

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.

Summary

Overall, the investigations conducted during the last 12 years across the countries show 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 and cause adverse effects on sugarcane physiology resulting in yield loss. Although the correlation of symptom expression with infection status is not consistent in certain locations, SCYLV-infection is most likely the cause of symptoms of YLD in many countries. Further studies are being conducted on leaf yellowing in infected sugarcane to reveal the interference of SCYLV on the metabolism of phloem cells.

Also studies conducted in different countries indicate that SCYLV infection, which is a threat for susceptible, high-yielding sugarcane varieties, can be confined relatively easily because of the slow progression of the virus in the field. Thus, with proper seed-field maintenance, it should be possible to plant susceptible cultivars without the danger of YLD problems.

Apical meristem culture has been proved to be the most efficient in producing virus free plantlets and a method of choice to eliminate viral pathogens from infected plants. The regenerated sugarcane lines remained virus-free over a period of up to 4 years, whether grown in isolated fields or in the glasshouse. Hence it will be possible to continue the distribution of virus-free seedlings for large scale cultivation.

Improved diagnosis and production of virus-free plants of SCYLV-susceptible cultivars facilitate continuous supply of virus-free planting materials and it would sustain productivity in sugarcane.

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Table 1. Planting of yellow leaf disease infected setts on the disease build up and cane yield (number of millable canes) in the field (2004-05 season)

Sl. No	Variety/Cultivars	Disease incidence (%)		No. of millable canes	
		Healthy	Diseased	Healthy	Diseased
1	Co 658	5.37^{b}	100.00^{a}	28.50^{hi}	25.00^{a}
2	Co 740	9.45^{bc}	87.73^{d-g}	42.50^{d-h}	37.00^{d-i}
3	Co 6511	4.12^{bc}	87.86^{d-g}	45.00^{d-g}	32.50^{e-i}
4	Co 6806	0.00^{a}	93.34^{fg}	33.00^{gh}	31.50^{f-i}
5	Co 6914	0.00^{a}	35.99^{b}	37.50^{e-h}	49.00^{cde}
6	Co 7514	26.99^{cde}	77.06^{cde}	53.50^{bcd}	49.00^{cde}
7	Co 8021	14.46^{bcd}	93.75^{g}	38.00^{e-h}	52.00^{cd}
8	Co 86010	14.59^{bcd}	86.67^{d-g}	41.00^{d-h}	33.00^{e-I}
9	Co 86011	14.73^{bcd}	84.12^{d-g}	51.00^{b-e}	50.50^{cd}
10	Co 86032	53.39^{ef}	92.45^{efg}	37.50^{e-h}	47.50^{c-f}
11	Co 86250	16.67^{bcd}	100.00^{a}	17.50^{i}	12.50^{i}
12	Co 90017	6.50^{bc}	87.79^{d-g}	45.00^{d-g}	41.00^{d-h}
13	Co 93009	8.55^{bc}	87.75^{d-g}	52.50^{b-e}	49.50^{cde}
14	CoC 8001	11.69^{bcd}	81.67^{def}	34.00^{gh}	27.50^{hij}
15	CoC 85061	74.29^{fg}	87.12^{d-g}	43.00^{d-h}	45.50^{d-g}
16	CoC 86062	81.82^{fg}	87.07^{d-g}	52.50^{b-e}	53.50^{cd}
17	CoC 92061	16.34^{bcd}	61.96^{c}	27.50^{hi}	30.50^{f-I}
18	CoC 98061	72.06^{fg}	89.92^{d-g}	29.00^{hi}	49.00^{cde}
19	CoJ 64	88.86^{g}	100.00^{a}	53.50^{bcd}	43.50^{d-h}
20	CoS 687	72.63^{fg}	90.74^{d-g}	62.50^{bc}	52.50^{cd}

21	CoS767	13.81^{bcd}	82.14^{def}	65.00^{b}	63.00^{c}
22	CoS 770	40.45^{de}	100.00^{a}	95.00^{a}	85.50^{b}
23	CoS 802	14.35^{bcd}	92.17^{efg}	50.00^{c-f}	44.50^{d-h}
24	CoV 92101	26.49^{cde}	73.5^{cd}	41.50^{d-h}	32.50^{e-l}
25	CoV 92102	81.67^{fg}	82.96^{d-g}	35.50^{fgh}	28.50^{ghi}
26	57 NG 56	90.95^{g}	100.00^{a}	32.50^{gh}	24.00^{ij}
27	CoC 85061	74.29^{fg}	87.12^{d-g}	28.50^{hi}	25.00^{a}
28	CoC 86062	81.82^{fg}	87.07^{d-g}	42.50^{d-h}	37.00^{d-i}

Means followed by a common letter are not significantly different at the 5 per cent level by DMRT; Disease incidence and NMC data were recorded at 12th month.

Table 2. Incidence of yellow leaf disease of sugarcane in different locations in Tamil Nadu.

Sl. No.	District	Varieties affected	Disease incidence
1	Coimbatore	Co 86032	Moderate to severe
2	Erode	Co 86032	Moderate to severe
3	Kancheepuram	Co 86032	Moderate to severe
4	Villupuram	Co 86032	Moderate to severe
5	Thanjavur	Co 86032	Moderate
		CoV 94101	Moderate
		CoV 92102	Moderate
6	Namakkal	Co 86032	Moderate
7	Sivaganga	Co 86032	Moderate
8	Pudukkottai	Co 86032	Moderate to severe
9	Cuddalore	Co 86032	Moderate to severe

Moderate: 10-25 per cent incidence
Severe : >25 per cent disease incidence

Table 3 Effect of suspected YLD infection on growth in sugarcane varieties.

Variety	Cane diameter at 10th month stage (cm)*	
	Healthy**	YLD infected
Co 775	2.98 ± 0.27	2.59 ± 0.23
CoJ 84191	2.21 ± 0.15	1.99 ± 0.11
CoS 510	2.09 ± 0.16	1.54 ± 0.17
CoS 767	2.18 ± 0.11	1.50 ± 0.15
CoS 8407	2.45 ± 0.24	2.07 ± 0.22
84A125	2.62 ± 0.18	2.35 ± 0.17

*: n=20 canes
**: Asymptomatic canes in the same plot of each variety were taken for comparison

Table 4 Effect of YLD infection on cane juice quality in sugarcane varieties

Variety	HR Brix in different internodes at 10th month*									
	Healthy**					YLD infected				
	3	5	7	9	11	3	5	7	9	11
Co 775	21.87 ± 1.18	22.28 ± 0.93	22.44 ± 0.87	22.36 ± 1.23	22.86 ± 0.86	21.40 ± 2.39	20.96 ± 2.39	20.86 ± 2.37	20.58 ± 2.83	21.34 ± 1.60
CoJ 84191	21.24 ± 0.14	21.16 ± 1.10	21.14 ± 1.14	21.68 ± 1.26	21.76 ± 1.37	19.62 ± 1.65	19.42 ± 1.05	19.86 ± 1.02	20.14 ± 1.21	20.14 ± 1.04
CoS 510	18.16 ± 1.76	18.2± 1.25	18.14 ± 1.65	18.16 ±1.75	18.16 ± 1.77	15.88 ± 2.35	16.08 ± 2.92	16.24 ± 2.13	17.12 ± 2.24	17.30 ± 2.04
CoS 767	19.84 ± 1.67	20.28 ± 2.19	20.56 ± 2.68	20.60 ± 2.42	20.88 ± 2.59	17.04 ± 2.09	17.08 ± 1.86	17.24 ± 2.09	18.40 ± 2.28	18.08 ± 1.88
CoS 8407	20.04 ± 0.97	19.84 ± 1.35	20.32 ± 1.14	20.52 ± 1.05	20.08 ± 1.18	18.86 ± 1.19	19.80 ± 1.37	20.80 ± 1.35	20.70 ± 1.33	20.92 ± 1.80
84A125	23.64 ± 0.54	23.84 ± 0.90	24.0± 0.70	24.00 ± 0.14	23.96 ± 0.64	22.08 ± 1.60	21.92 ± 1.80	22.60 ± 1.12	22.56 ± 1.09	22.40 ± 1.82

*: n = 10 observations

**: Asymptomatic canes in the same plot of each variety were taken for comparison