SUGARCANE: Ratoon stunting and Grassy shoot

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Summary
Sugarcane is the major commercial cash crop grown in tropical and subtropical regions of the country for sugar and bio energy production. Among the various biotic factors severely affecting sugarcane production and productivity, non-fungal diseases viz., ratoon stunting disease (RSD) and grassy shoot disease (GSD) caused by ratoon stunting bacterium and grassy shoot phytoplasma respectively were responsible for the varietal degeneration/decline with other viral pathogens. These non-fungal diseases are affecting the crop in all the growth stages and pose a potential challenge to sugarcane cultivation due to their systemic colonization in the cane and transmission through seed cane. In severe cases, the cane production and productivity are affected up to the extent of more than 50% in plant crop and much more in ratoon crop. Currently, aerated steam therapy and pathogen elimination through meristem tissue culture based seedling production are the only available management techniques to prevent spread of these diseases into new areas. Despite the fast pace of research in pathogen diagnosis and genome organization through recent next generation technologies, the research on management of these diseases under field conditions still remain grey and pose challenges to sugarcane pathologists.

Keywords: Leifsonia xyli subsp. xyli, grassy shoot disease, varietal degeneration, diagnostics, disease management

Introduction
Sugarcane, Saccharum officinarum L. belonging to the family Poaceae, is a commercial crop used to produce sugar in many tropical and sub-tropical countries. About 80% of the world’s sugar is obtained from sugarcane (Saccharum spp.) while 20% comes from sugar beet (Beta vulgaris). It is one of the most important agro-industrial crops of India next to the textile industry. India is the top sugarcane cultivating country in the world in term of total area. However, the average yield per unit area is very low. Hence, India ranks second in production after Brazil, with 341,200 TMT tons and India’s share in world’s sugarcane production was 15% in 2013-14 (Anon 2014). The sugarcane crop is attacked by a number of pests and diseases, responsible for about 15-20%, yield loss (Rott et al. 2000). The disease contributes about 10-15% production decline (Viswanathan and Rao 2011). Among these, ratoon stunting and grassy shoot
diseases are major limiting factor. In this chapter, information regarding the etiology, occurrence and economic importance, symptoms, disease cycle, and management measures are discussed in detail.

**Ratoon Stunting**

Ratoon stunting disease (RSD) is caused by *Leifsonia xyli* subsp. *xyli* (Lxx). Initially, it was suspected as a physiological disorder in Australia and later it was established as a bacterial disease. It has been known in India for many decades however, only limited research works have been carried out till date. Due to RSD, yield losses ranging from 5-30% have been reported in countries like South Africa and the USA (Bailey and McFarlane 1999; Dean and Davis 1990). In India, it has been reported as severely affecting the cane productivity in Karnataka state in the tropical region.

![Fig 1: RSD affected variety shows a stunted growth and degeneration in the field](image)

Based on the studies of ICAR-SBI., Coimbatore during the year 2000-01, the RSD infection had caused drastic reduction in the overall growth parameters of the cane viz. cane thickness (3.2 to 1.6 cm), height (2.9 m to 1.6 m), number of internodes (27.6 to 14.5) and single cane weight (1.64 to 0.34 kg) in severely affected variety Co 419 at Mandya region, Karnataka. The difference in cane thickness was reported to be around 10 mm between apparently healthy and diseased canes in most of the varieties (Viswanathan 2001a) (Fig. 1)

**Symptoms**

The RSD could be clearly recognized based on external and internal symptoms of the affected cane clumps. The diseased clump usually display stunted growth, reduced tillering, thin stalks with shortened internodes and yellowish foliage. Stunting in the field may not be uniform and the diseased fields expressed a characteristic ‘up and down’ appearance when all plants were infected. The disease severity would be more in ratoon than in plant crop hence; it was named as ‘Ratoon stunting disease’ (Fig 2A). Sett germination, growth and yield was reduced considerably compared to healthy due to production of thinner and shorter stalks rather than a reduction in the total number of canes. However, it had not shown any much abnormality in the root system of the
infected plant other than the general reduction of root mass. The internal characteristic symptom of the disease includes reddish brown or pale red discoloration at the lower portion of the nodal regions in severe cases it extended to the entire nodal regions along the vascular bundles, appearance of yellowish to reddish brown dots, commas or short lines when viewed by longitudinal splitting (Fig. 2B & C)

**Fig. 2: RSD symptoms:** Disease affected canes show severe stunting, degeneration with shortened internodes (A) RSD mild symptoms – reddish streaks on the nodal region (B) and RSD severe symptoms (C) – entire nodal region turn reddish (right) healthy canes remain normal (left).

**Epidemiology**

The pathogen enters through wounds or cut ends of the cane during the field operations whereby it systemically colonizes the xylem vessels. It remains viable and infectious for several months apparently in left over crop debris, stubbles and soil. The severity of the disease gets aggravated in moisture stress drought conditions in summer and during severe water logging conditions in the field. Besides, cultivation of older varieties for many years in a region was reported as a major reason for the build-up of bacterial titre in the cane stalks which led to varietal degeneration. The rate of disease severity depends on the varietal susceptibility. Also mono-cropping of sugarcane with single variety for longer period of time, poorly maintained ratoon fields and multiple ratoon were reported as favouring the disease spread in Cauvery basin in Karnataka state (Viswanathan 2001). The RSD bacterium was reported as transmitted through setts taken from the diseased plants and also by mechanical inoculation with diffusates extracted from the infected plant stalks. Also, it spread through cane cutting knives and cutter planter machines during harvest. As on date, the pathogen has been reported only in sugarcane with no other alternate hosts.

**Detection and diagnosis**

ELISA based serological assays were standardized to detect Lxx in the suspected sugarcane varieties (Viswanathan 1996) with bacterial ooze/ diffusates from the infected stalks as antigen. Further studies on varying symptom expression in different sugarcane varieties revealed that the varieties with prominent streaks at the nodes and the older varieties recorded high titre for
Lxx and the asymptomatic plants were also positive to the bacterial infection with low titre. The varieties like Co 421, Co 997, Q 28 and CP 52-68 were identified as indicator varieties to Lxx as they were consistently positive to the RSD (Viswanathan 1997 a). During the year 2001, the RSD affected cane materials collected from the Mandya region of Karnataka viz. Co 92020, Co 99005, Co 99010, Co 99014, CoC 671, 93 R5 and CoM 88121 exhibiting severe stunting had high titres for Lxx whereas, the cvs Co 8371, Co 62175, Co 86032, Co 86249, Co 92005, Co 93020, CoC 90063 and CoC 98061 exhibiting no stunting also tested positive for RSD in ELISA. Although the cvs Co 62175, Co 86249, Co 92005, CoC 90063 and CoC 98061 were free from RSD symptoms and stunting they also tested positive to RSD (Viswanathan 2002, 2004a). After ELISA, tissue blot immunoassay (TBIA) was developed for the detection of the RSD pathogen in sugarcane. The suspected cane stalks were blotted directly on to nitrocellulose membranes and infection of different vascular bundles was detected using the polyclonal antiserum. In these studies, infected vascular bundles showed characteristic color development on the membranes to alkaline phosphate substrate and the bacterium-free bundles were remained colourless indicating that TBIA a useful technique to detect RSD infected seed canes under field conditions (Viswanathan and Balamuralikrishnan 2004a).

Recently, three serological techniques viz., ELISA, DBIA and tissue blot were compared for their sensitivity to detect Lxx in sugarcane. In ELISA, 41 of 47 test samples were found to be positive, whereas in DBIA 17 of 32 test samples were positive and in TBIA all but one of 11 samples were positive. When a set of 11 samples were simultaneously compared for their sensitivity it was found that ELISA was found to be most sensitive followed by TBIA and DBIA. Eleven, ten and seven were positive to the bacterial infection by ELISA, TBIA and DBIA, respectively. Later, PCR assay was standardized to detect the bacterium from the xylem sap (Viswanathan, unpublished).

**Combined infections of RSD and YLD**

The sugarcane materials collected from the Mandya region of Karnataka viz., cvs. Co 99005, Co 99010, Co 99014, Co 92020, 93R5 and CoM 88121 had high titre for both Lxx and Sugarcane yellow leaf virus (SCYLV). Observations on sugarcane growth in the trials indicated that the cultivars positive to either one or both were stunted severely. Samples from the previous season clearly established that those clones from Karnataka with significant stunting were infected with high populations of Lxx and the same cultivars in Tamil Nadu were free from the pathogen. The results also revealed that those clones with high Lxx populations had severe symptoms of YLD. Although ELISA assays were not conducted at the time to confirm the causal agent of YLD, later studies showed that RSD infected sugarcane clones had high titre for SCYLV. These studies suggest that stunting and poor performance of sugarcane clones in different trials as well as in the fields in Karnataka state (Viswanathan 2001a; Viswanathan 2004b) were primarily due to their susceptibility to RSD and YLD. Subsequent studies of Viswanathan (2002) clearly demonstrated that RSD infection in sugarcane favours building up of SCYLV titre in different sugarcane cultivars and elimination of RSD bacterium in setts through heat treatment reduces SCYLV titre. Combined infections of two pathogens are adversely affecting the cane and sugar yield further.
Management
The impact of RSD on sugarcane had resulted in varietal degeneration in many older varieties. The growing severity of RSD warranted careful seed selection and proper monitoring of the disease in the field. To address the disease detailed studies were conducted at SBI and the results revealed that meristem culture and/or aerated steam therapy at 50 °C for 1 hr was highly effective in eliminating RSD bacterium from the infected seed canes. This procedure has resulted in rejuvenation of old varieties such as Co 419 and Co 740 in Peninsular India, where they were totally degenerated due to severe RSD. Better crop stand was observed in many locations in Peninsular India where RSD was severe, whenever these methods were followed which either partially or completely eliminated the pathogen (Rao and Viswanathan 2004).

SUGARCANE GRASSY
Sugarcane grassy shoot (SCGS) is one of the destructive diseases in sugarcane. It is caused by sugarcane grassy shoot phytoplasma belonging to 16SrXI-B subgroup. It was first reported in India during the year 1949 in cv Co 419 near Belapur in the Ahmadnagar district of then Bombay (Maharashtra) (Chona 1958; Chona et al. 1960; Rane and Dakshindas (1962). Later, it was reported in other major sugarcane growing countries such as Thailand, Malaysia, Pakistan, Myanmar, Sri Lanka, Vietnam, China and Sudan (Rishi et al. 1973; Rishi and Chen, 1989; Rao et al. 2008; Wongkaew et al. 1997) and its severity is now been increasing in Asian countries (Nithya et al. 2020). In India the disease has led severe outbreaks in all the major sugarcane growing states like Uttar Pradesh, Bihar, Maharashtra, Karnataka, Andhra Pradesh, Haryana and Tamil Nadu. In case of severe incidence, 5 to 70% loss in plant crop and up to 100% in ratoon crops were reported in CoC 671, Co 86032, CoS 95255, CoS 07250 (Tiwari et al. 2012; Viswanathan et al. 2011). Also, severe reduction in stalk height, stalk girth, and length of internodes were reported as 35%, 15% and 50-60% respectively in ratoon crop (Viswanathan 2000, 2001b). Recently, the cv. CoS 767 was phased out of cultivation due to severe SCGS incidence in one of the major sugarcane growing state U.P (Tiwari et al. 2016; Viswanathan 2016).

Fig. 3: GSD symptoms: Disease affected canes show severe tillering with no millable canes (A), excess of Chlorotic symptoms with stunted growth (B) and severe chlorotic streak symptoms noticed in ratoon crop of variety CoS 767 (C).
The infected plants show grassy appearance with production of long, lanky, chlorotic tillers in numerous numbers along with severe stunting; hence the disease was named by its symptoms as sugarcane grassy shoot disease (Fig. 3). The disease symptoms expression can be seen in all the growth stages of the crop from germination to maturity stage. The raton crops exhibit quick proliferation of chlorotic tillers in huge numbers during raton establishment, and such tillers wither and dry quickly. The infected sett either not produce any millable canes or produce only very few thin canes. The disease was named either as sugarcane grassy shoot (SCGS) and white leaf disease (WLD) in different countries (Marcone 2002).

**Epidemiology**

The disease transmission primarily takes place through infected setts and secondary transmission through insect vectors. The phloem sap feeding leafhopper *Deltocephalus vulgaris*, *Cofanaunimaculata* (Signoret), *Exitianusindicus* (Ross), *Maiestasportica* (Melichar) were identified as vectors of SCGS phytoplasma in Uttar Pradesh (Singh et al. 2002; Srivastava et al. 2006; Tiwari et al. 2016 and 2017).

**Detection and diagnosis**

GSD is caused by *Candidatus* Phytoplasma which is small, wall less prokaryotic, pleomorphic obligate parasites, resides exclusively in the nutrient rich phloem sieve tube elements of plants and spreads systemically through sieve pores. Earlier this pathogen was identified as Mycoplasma like organism (MLO) and now being known as “Phytoplasma” with designation of new genus as ‘*Candidatus* phytoplasma’ (IRPCM 2004) since MLOs resisted all attempts to culture *invitro* in cell-free conditions (Lee et al. 2016).

During 1980’s GSD pathogen was mainly identified based on its visual symptoms; later in 90’s many diagnostic techniques were developed for precise detection of SCGS disease; 4’,6-diamidino-2-phenylindole (DAPI) based staining technique was widely used for localization of phytoplasma in the infected phloem tissues. Also, by using the transmission electron microscope, ultrastructure of the phytoplasma present in the phloem sieve tubes were revealed by hand-made ultrathin sections of stem, leaf and root. The shape of the phytoplasma was round to oval with 200-300 nm in diameter, has trilaminar plasma membrane unit structure on cell wall Under high magnifications, the phytoplasma cell showed the presence of circular DNA strands, ribosomes with small RNA strands in the sieve tube elements, endoplasmic reticulum, fibrils, vacuole, degenerated mitochondria, along with special type of starch grains in the sieve tube elements further, its movement was confirmed through sieve tube pores (Velmurugan, 1987). Later, progress has been made in the serological assays, as varying intensities of chlorosis in the leaves due to some nutritional deficiency symptoms were often mistaken as GSD in the field and preparing ultra-thin sectioning was a tedious time consuming process for electron microscopic sample observations and also for precise detection during seed cane indexing and quarantine (Viswanathan 1994). Various serological assays viz. ELISA, FAT and immuno fluorescent techniques were developed using polyclonal antisera produced against partially purified antigen preparations of SCGS-affected sugarcane plants (Viswanathan 1994, 1997b, 2000). Later, nucleic acid amplification based nested PCR assay was standardized
Viswanathan et al. 2005) for accurate and rapid identification of associated phytoplasma in the diseased plants.

Based on the PCR analysis, SCGS phytoplasma was found to be grouped under ‘Ca P. oryzae’ (16Sr XI group) (Viswanathan et al. 2005, 2011). Subsequently, different set of restriction enzymes were used in the restriction fragment length polymorphism of PCR amplified rDNA region of phytoplasma (16S rDNA, 16S/23S r DNA spacer region and portion of 23S r DNA) to identify and differentiate the phytoplasmas within primary phylogenetic groups and to identify the extent of variations in rDNA regions of SCGS phytoplasma which were showing significant variations in phenotypic symptom expressions collected from different varieties and geographic regions. However, no genotypic variations among the pathogenic isolates (Viswanathan et al. 2011) and the isolates were identified as member of the rice yellow dwarf (RYD) phytoplasma group or 16SrXI-B sub group.

Besides, multidrug resistant two ABC transporter genes were identified from SCGS phytoplasma with an estimated genome segment of 2,362 bp which comprised of two open reading frames with overlapping of 58nt’s and it codes two proteins evbG and evbH which had shown highest similarity with evbG and evbH proteins of ‘CaP. mali’ (73% and 67% respectively). Further, In silico analysis revealed that presence of six transmembrane helices and two different conserved domains (annotated as permease and ATP binding domain) in the complete sequence of evbG (Manimekalai et al. 2014, 2016).

Recent investigation on GSD isolates collected from popular varieties grown in both tropical and sub-tropical places revealed the presence of new sub group other than the existing 16SrXI subgroups viz.,16SrXI-A, B, C, D, E and F based on the insilico virtual RFLP analysis (Nithya et al. 2020). As like viruses, existence of variability among phytoplasma isolates in the highly conserved small ribosomal subunit sequences are seems at rapid at present may be due to high recombination or to encounter the host selection pressure. Reporting or identification of such type of new strains on phytoplasma and its relation with symptom severity is to be investigated in detail in future.

Management

Based on the information generated from the research work and considering the spread of phytoplasma diseases through setts, ICAR-SBI has developed one thermotherapy unit known as Aerated steam therapy (AST) under which three/two/single budded setts are treated at 50°C for 1hr (Edison 1973; Viswanathan 2001b) to eradicate or reduce the pathogen inoculum from seed pieces, further it was established after several field experiments that it doesn’t affect seed bud germination. Subsequently, the advancement of biotechnological methods made possibilities in elimination of viruses from the planting material through apical meristem culture technique for producing disease free planting material (Viswanathan et al. 2011).

In spite of having all these technologies at our hand, the disease spreads taking place across the varieties irrespective of the regions even now due to practical difficulties in disease diagnosis during inter-state seed cane quarantine and also the sugar factories negligence in following the thermotherapy protocols before supplying the planting materials to farmers. In order to minimise the disease spread through planting materials in India, healthy seed material supply
programme was initiated at ICAR-SBI, based on the success in the virus indexing services. It has launched a programme to supply the healthy planting material (seed canes) and apical meristem derived tissue culture seedlings to sugar factories as well as to the interested farmers at affordable prices by considering that developing disease-free nurseries is imperative to sustain productivity of sugarcane and to realize yield potential of popular sugarcane varieties in India.

**Conclusion**

The non-fungal diseases viz., ratoon stunting and grassy shoot diseases along with different viral diseases have been responsible for the elimination of many elite commercial varieties in the past in different epidemics. Further, these non-fungal diseases contribute to decline in their performance due to varietal degeneration. Lack of awareness on seed cane health and ignoring quarantine regulations resulted in introduction of diseases, their epidemics and varietal degeneration in the country. The clones which can resist degeneration need to be identified at early stages of varietal selection. Sugarcane varieties vary in their potential against different diseases and any elite commercial variety may not possess tolerance against all the major diseases. Hence to sustain the productivity in such varieties alternate management strategies need to be developed. Although GSD has created havoc to sugarcane cultivation in the country and we have evolved strategies to manage this disease through meristem culture combined with molecular diagnosis of the virus. Disease surveillance programmes in the country need further strengthening including use of remote sensing approach. This would led to creation of disease maps for non-fungal diseases in sugarcane and this would facilitate developing possible forewarning systems and varietal deployment in a region in the future.

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