



Impact of the viruses associated with mosaic and yellow leaf disease on varietal degeneration in sugarcane

K. Bagyalakshmi · R. Viswanathan · V. Ravichandran

Received: 21 March 2019 / Accepted: 27 June 2019 / Published online: 4 July 2019
© Springer Nature B.V. 2019

Abstract The viruses *Sugarcane mosaic virus* (SCMV) and *Sugarcane streak mosaic virus* (SCSMV) causing mosaic disease and *Sugarcane yellow leaf virus* (SCYLV) causing yellow leaf disease (YLD) are synergistically associated with varietal degeneration of sugarcane in India. The objectives of the study were to understand the degree of degeneration in the sugarcane cultivar CoJ 64 due to the mixed infection of the three viruses. The study was also aimed to quantify the threshold limit of viruses for the plant to express the symptoms. Symptomatic plants exhibited significant reductions in germination and growth as compared to the asymptomatic plants and maintained poor vigour. Such plants recorded significant reductions in different physiological parameters such as photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll fluorescence ratio and leaf chlorophyll content. At the time of harvest a drastic reduction in the juice yield by 30.13–36.04% was found. Diagnosis by qRT-PCR revealed that both asymptomatic and the symptomatic plants

showed mixed infections of all the three viruses. The copy numbers in the asymptomatic plants were detected between 10^2 and 10^3 for all the three viruses whereas, the copy numbers in the symptomatic plants were in the range of 10^4 and 10^6 for all the three viruses. The present study established a threshold limit of three RNA viruses causing varietal degeneration in sugarcane and this information will be used while selecting new varieties to avoid varietal degeneration under field conditions.

Keywords Sugarcane · Varietal degeneration · SCSMV · SCMV · SCYLV · qRT-PCR assay

Introduction

Sugarcane (*Saccharum* spp.) is a major commercial/plantation crop cultivated in many parts of the world, especially in the tropics and subtropics. India ranks second in sugarcane production after Brazil and first in sugar production (www.fao.org/corp/statistics/en). Globally, the crop meets ~70% of sugar requirement and this also emerges as an alternate source for bioenergy since ethanol from sugarcane is used as automobile bio-fuel in Brazil and other countries. In India, sugarcane is cultivated in around 5.2 mha ([url-3http://www.vsisugar.com/india/statistics/world_indiasugar.html](http://www.vsisugar.com/india/statistics/world_indiasugar.html)). Many pathogens belonging to fungi, bacteria, viruses and phytoplasma infect sugarcane thereby affecting its growth and metabolism, which in turn adversely affect the crop yield. Vegetative propagation favours systemic accumulation of

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12600-019-00747-w>) contains supplementary material, which is available to authorized users.

K. Bagyalakshmi · R. Viswanathan (✉)
Division of Crop Protection, Indian Council of Agricultural
Research - Sugarcane Breeding Institute, Coimbatore 641007,
India
e-mail: rasaviswanathan@yahoo.co.in

V. Ravichandran
Department of Crop Physiology, Tamil Nadu Agricultural
University, Coimbatore 641003, India

different pathogens in sugarcane (Viswanathan and Rao 2011). Especially, the viruses increase their titre in seed canes without exhibiting any symptoms and this leads to loss of vigour and degeneration in elite varieties of sugarcane (Viswanathan 2016). The two viruses *Sugarcane mosaic virus* (SCMV) a member of the genus *Potyvirus* and *Sugarcane streak mosaic virus* (SCSMV) a member of the genus *Poacevirus* causing mosaic disease belong to the family *Potyviridae* whereas *Sugarcane yellow leaf virus* (SCYLV) a member of the genus *Polerovirus*; family *Luteoviridae* causing yellow leaf disease (YLD) seriously affect sugarcane production and productivity in India (Viswanathan and Rao 2011; Viswanathan et al. 2014, 2018).

Varietal degeneration due to mosaic is a serious concern that results in near extinction of elite cultivars from cultivation as witnessed during the recent decades to the erstwhile ruling varieties like Co 740 and CoC 671 in the tropical (Viswanathan and Balamuralikrishnan 2005) and CoLk 8102, CoPant 90,223, and CoS 767 in the subtropical (Singh et al. 2003) regions in India. Mosaic disease is the first epidemic among the viral diseases to be reported in sugarcane and affects elite cultivars across the world deteriorating the crop yield (Gonçalves et al. 2012). Although, Hema et al. (1999) reported association of SCSMV alone with the disease, detailed studies of Viswanathan et al. (2007) established that mosaic in India is caused by both SCMV and SCSMV, together or separately. Occurrence of YLD was first reported in India during 1999, detailed symptoms of the disease, virus diagnosis and its impact to sugarcane growth were reported later (Viswanathan 2002). Recently, impact of SCYLV on sugarcane growth, yield and photosynthetic efficiency was established (Viswanathan et al. 2014). Further, YLD occurrences were reported from all over the country and susceptible cultivars in commercial fields recorded up to 100% (Viswanathan 2018). Serious impact of SCYLV infection to sugarcane growth and economical yield were reported worldwide (Lehrer et al. 2007; Rassaby et al. 2003; El Sayed et al. 2015).

The symptoms caused by mosaic is characterized by the appearance of blotchy chlorotic patches on leaves, ranging from light green to yellow and dark green patches. The symptoms are more conspicuous in young plants and gradually disappear while ageing (Balamuralikrishnan et al. 2003); however during severe infections without symptom recovery all the leaves exhibit pale green or yellow canopy with symptoms close

to systemic yellowing. Thereby it reduces the plant vigour and affecting plant growth significantly. Whereas the SCYLV infection is characterized by intense yellowing of midribs on the abaxial surface, lateral spread of yellow discolouration to the leaf lamina followed by necrosis of discoloured tissues from the leaf tip spreading downwards along the midrib and a bushy appearance of the crown of the plant due to shortening of internodes (Chinnaraja and Viswanathan 2015; Viswanathan 2012).

Worldwide, sugarcane is mainly cultivated through vegetative propagation, ultimately leading to carry over of diseases over successive generations once they are infected. Such transmission of pathogens over several generations will eventually increase their titre within crops leading to varietal degeneration (Viswanathan 2016). Mosaic disease unlike YLD can be transmitted through mechanical inoculation which may result in rapid transfer of the virus from infected to healthy plants. Widespread occurrence of YLD and mosaic in India and their role in varietal degeneration along with *Leifsonia xyli* subsp. *xyli*, bacterium associated with ratoon stunting disease (RSD) has been established (Viswanathan 2016; Viswanathan et al. 2017a, b). Further, extensive variation in different virus population was observed in the country (Viswanathan et al. 2008a, b, 2009) and such variable population restricts spread of a variety from tropical to subtropical region and vice versa. This is being witnessed at Sugarcane Breeding Institute, Coimbatore, where hundreds of parental clones from subtropical region are maintained at National Hybridization Garden (NHG), a national facility to make biparental crossing and varietal development. Here many subtropical varieties used as parents quickly succumb to viruses, degenerate and fail to flower in few years. Hence, there was a necessity for a detailed investigation to assess the viruses associated with such degeneration, impact of the viruses on cane growth, yield and photosynthetic efficiency in the severely degenerated plants in comparison with asymptomatic plants. Also, when a sugarcane crop is degenerated due to the influence of three important viruses like SCSMV, SCMV and SCYLV, quantifying their virus titre would help to draw the threshold level for degeneration which has not yet been done so far in sugarcane. Hence an initiative was taken to quantify the viral load simultaneously for three viruses in a single plant by quantitative RT-PCR which would identify the threshold limit required for such degeneration.

Materials and methods

Field experiments/ sugarcane varieties used

Seed canes from the cv CoJ 64 asymptomatic without mosaic and YLD and exhibiting normal growth brought from Sugarcane Research Station, Kapurthala, Punjab State and disease affected canes maintained at the Institute with both the diseases were planted in eight rows of 6 m each in the Plant Pathology farm of Sugarcane Breeding Institute, Coimbatore, India. The crops were raised in the experimental field by following package of practices recommended for a tropical sugarcane. Physiological and growth parameters were recorded during three planting seasons viz., 2013–14, 2014–15, and 2015–16. Planting was done during February during each season and the crop was harvested after 360 days. Asymptomatic and disease affected plants for the trials were derived from the respective set of mother plants for different seasons separately maintained under greenhouse conditions. In addition to the field trials, the same experiment was conducted under pit method of planting where ~10 setts were planted in each field pit [0.6 × 0.6 X 0.3 M (LxBxH)] to have a very close observation on disease symptoms. For each treatment four pits were maintained and crop husbandry procedures were similar to the field trials.

Physiological parameters in symptomatic and asymptomatic sugarcane

The physiological parameters viz., photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), chlorophyll meter (SPAD) value and chlorophyll fluorescence ratio (F_V/F_M) of asymptomatic and mosaic affected plants were recorded in the field trials during the grand growth (240–250 days) stage. Ten plants were randomly chosen during the different planting seasons to record observations on individual parameters.

Gas exchange parameters

Gas exchange parameters viz., photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (E) were recorded in the trials using a LI-6400XT Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA). All the leaf parameters were recorded during 11.00 to 11.45 AM with leaf temperatures of 33–35 °C and photosynthetically active radiation (PAR) values of

1497–1586 $\mu\text{mol Photons m}^{-2} \text{s}^{-1}$. Leaf number 2 in the crown was selected for these analyses (First totally visible dewlap in the crown is considered as leaf number 1).

Chlorophyll meter (SPAD-502) readings

Leaf chlorophyll contents were recorded using a SPAD-502 Chlorophyll Meter (SPAD-502 Konica Minolta Co. Ltd., Japan). Measurements (SPAD values) were recorded at 11.00 to 11.45 AM with temperatures of 33–35 °C in the adaxial surface of the middle laminar region in leaf number 2 of the crown. In addition, leaf chlorophyll was also recorded in leaf numbers 0 to 4.

Chlorophyll fluorescence ratio

Chlorophyll fluorescence of the leaves was measured using Chlorophyll fluorometer OS-30p (Opti-Sciences, Hudson, USA). Using dark adaptation clips provided with the instrument, the middle portion of the leaf laminar region was closed with clip shutters. After five minutes, leaf clip shutters were withdrawn exposing the dark adapted site to a saturating excitation light source provided by a 660 nm solid state source and saturating at 3000 $\mu\text{E m}^{-2} \text{s}^{-1}$. The parameters recorded were initial fluorescence (F_0), maximum fluorescence (F_M), variable fluorescence (F_V) and the ratio of variable to maximal fluorescence (F_V/F_M). The ratio F_V/F_M is used to assess the quantum efficiency for photochemistry of PSII (Krause and Weis 1991).

Growth and yield parameters

The growth parameters viz., germination and number of tillers and yield parameters viz., cane height, number of internodes, internodal length, stalk girth and weight were recorded during different growth stages and harvest. Internode length was computed by using the formula; cane height /number of internodes. Stalk girth was measured in the middle region of the cane using Mitutoyo Absolute Digimatic Caliper (Japan). The cane yield parameters were measured with 10 numbers of plants collected randomly from the rows at the time of harvest during the three seasons.

To assess the impact of the diseases on cane juice quality parameters, 10 canes were drawn from each plot during harvest at 360 days. The essential juice quality parameters of brix, sucrose (pol), purity and commercial cane sugar (CCS) per cent were recorded (Wagih et al.

2004). First, harvested canes were weighed using a DS 215 Hanging Scale (Essae, India) followed by juice extraction using three roller power operated Standard Type-1 sugarcane crushers (Industrial Machinery Agency, India) and juice weight was recorded. Later, juice was filtered into a clean 500 ml bottle using filter cloths. Brix was measured using a Brix hydrometer spindle (Thomson Instruments, India) in a measuring cylinder. Three gm of lead subacetate ($\text{Pb}_3(\text{OH})_4(\text{CH}_3\text{COO})_2$) was added to 100 ml of juice to provide an optically clear solution and sucrose content of the solution was measured in a Saccharomat Touch polarimeter (SCHMIDT HAENSCH, Germany). Purity of the juice was calculated using the formula $\text{sucrose \%}/\text{brix} \times 100$. CCS % was calculated by applying the formula $(\text{sucrose \%} \times 1.022) - (\text{brix \%} \times 0.292)$.

In addition, weight of each internode and their respective juice quantity were recorded between asymptomatic and symptomatic plants. Ten plants were randomly taken for the comparison from each plot. Each internode was cut off from the stalks using a billhook and numbered from the bottom. Weight of each internode was measured using a DS-852 weighing scale (Essae, India). Internodes were crushed separately in a hydraulic sugarcane press OL18 (Pinette Emidecau Industries, France) and juice weight was recorded.

Statistical analysis

The experimental data were statistically analyzed using student's *t* test. *P*-values <0.05 were considered statistically significant.

Viral diagnostic assays

Reverse transcription (RT) – PCR assay

Asymptomatic and disease-affected leaf samples were collected from three different clumps of three different plots in the field. Total RNA was extracted from the leaf samples using TRI reagent (Sigma, USA). The RNA (1 µg) was reverse transcribed to cDNA using RevertAid H minus First Strand cDNA Synthesis Kit (MBI Fermentas, USA), following the manufacturer's protocol in a thermocycler (Master cycler gradient, Eppendorf, Germany). PCR was carried out with three primer sets SCSMV- 690F (5'-GGATCCGGACAAGGAACGCAGCCAC-3') & SCSMV-690R (5'-AGATCTTCTGRTCTCGCCGACATTTCC-3') (Viswanathan et al. 2008a); SCMV-

380F (5'-GGTNTGGTGYATBGARAATGGTTGCTCACC-3') & SCMV-380R (5'-CTGRGTCTCGCCGACATTTCCGTCAG-3') (Viswanathan et al. 2018); SCYLV 615F (5'-GGATCCATGAATACGGGGCGCTAACCGYYCAC-3') & SCYLV 615R (5'-AGATCTGTGTTGGGGRAGCGTGCYATACC-3') (Viswanathan et al. 2008b) targeting 690, 380, and 615 bp of their respective coat protein genes. The reaction was performed with 2 µl cDNA, 2.5 µl of 10X PCR buffer containing 15 mM MgCl_2 , 0.5 µl of 10 mM dNTP mix, 40 nmol each of forward and reverse primers, 1 unit of *Taq* polymerase (Merck, India), and sterile milliQ water to the final volume of 25 µl. The PCR programme consisted of an initial denaturation at 94 °C for 4 min, following 30 cycles of 94 °C for 1 min, 65 °C for 1 min, 72 °C for 45 s with a final extension for 10 min at 72 °C. Amplicons were electrophoresed on 1.5% agarose gels, stained with ethidium bromide and visualized.

qRT-PCR assay for detecting the threshold limit

Standard curves were obtained for the three viruses SCMV, SCSMV and SCYLV using pTZ57R/T plasmid vectors ligated with the coat protein gene fragments of respective viruses. Plasmid copy numbers were calculated using online dsDNA copy number calculator provided by URI Genomics & Sequencing Center. Based on calculated copy numbers, plasmids were serially diluted in 1:10 fold from 3×10^2 to 3×10^7 copies per microliter. Real time primers were designed for the qRT-PCR assay for the three viruses using the software BioEdit version 7.0.4.1 (Hall 1999) targeting the coat protein region ligated into the plasmids given in the Table 1. The leaf samples from asymptomatic and symptomatic plants of the cv CoJ 64 were assessed for the virus titre. RNA (300 ng) isolated from the leaf samples were reverse transcribed to double stranded cDNA using RevertAid H minus First Strand cDNA Synthesis Kit, primed with 40 nmol of oligo (dT) for SCSMV and SCMV and 40 nmol of SCYLV-R615 for SCYLV by following the manufacturers protocol (MBI, Fermentas, USA) in a thermocycler (Mastercycler gradient, Eppendorf, Germany). Real-time quantitative PCR was performed with three replications of each sample with 700 ng of double stranded cDNA template, 12.5 µl of SYBR® Green JumpStart *Taq* ReadyMix (Sigma, India) and 40 nM of SCSMV, SCMV and SCYLV RT-Primers (Table 1) in a 25 µl reaction volume in StepOnePlus Real-Time PCR System (Applied

Table 1 Coat protein gene specific primers used in qRT-PCR assays for three RNA viruses infecting sugarcane

S. No	Virus	Primer name	Sequence 5'→3'	Amplicon size
1	SCMV	SCMV-RT-For SCMV-RT-Rev	GGACAATGATGGATGGAGAHGARCA GCGCTAAGCTATAGTCGGTGAGA	128 bp
2	SCSMV	SCSMV-RT-For SCSMV-RT-Rev	GGTTCAGTTCCTCGGTTTCGTAGC TCTGCTGGTGAGAGGATGTTTAC	199 bp
3	SCYL	YLS-RT-For YLS-RT-Rev	CGTCAAGAGGAACGCCAAGAAAGTC GACGAATTGCTCTGCTAGGCTCGA	132 bp

Biosystems, USA). PCR conditions were 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Melt curve was performed at 60 °C to assure that a homogenous amplification product was produced. The leaves of –2 to 6 positions from both asymptomatic and symptomatic plants were analysed for virus titre assays during two seasons.

Results

Morphological parameters

The average total number of tillers recorded for three seasons in the field trials were in the range of 47.87–49.87 per row and 29.87–31.75 in the asymptomatic and symptomatic plots, respectively, with a percentage reduction of 33.33–40% in the disease-affected rows. However, in the pit trials, the average total number of tillers in asymptomatic and disease-affected plots were in the range of 20.5–23.75 and 14.73–15.0 per pit, respectively with a percentage reduction of 26.80–35.86% in the latter. The average number of millable canes recorded in the field trials for three seasons were in the range of 35.71–38.12 and 24.23–26.5 per row in the asymptomatic and disease-affected plants, respectively, with a percentage reduction of 25.72–33.11% in the latter. Whereas, in the pit trials, the number of millable canes for both asymptomatic and affected plants were in the range of 14.25–16.0 and 9.25–10.0 per pit, respectively, with a percentage reduction of 30.0–38.5% in the latter (Supplementary Table 1).

Plant growth parameters

The mean cane height of asymptomatic plants at the time of harvest measured ~117.2, 165 and 175 cm,

respectively in the three seasons recorded, whereas the height of the disease-affected plants were 98.5, 147 and 135 cm, with significant reductions of 15.95, 10.09, 22.85%, respectively (Table 2). The leaf length and leaf width also showed significant reductions in the disease-affected plants during the three seasons (Data not shown). The number of internodes were in the range of 19–36 in the asymptomatic canes whereas in the disease-affected canes it ranged from 18 to 34 with significant reduction of 5.2–24%. The stalk girth was 1.90–2.4 cm in the asymptomatic canes, whereas it was only 1.72–2.0 cm girth in the disease-affected canes with significant reductions of 16.6–9.5%. The asymptomatic plants recorded single cane weight of 1.8–2.23 kg whereas in the disease-affected plants, it was in the range of 1.31–1.51 kg, with significant reductions of 27.2–32.28%, respectively during the three seasons (Table 2). The canopy of the virus infected leaves displayed a yellowish green with mosaic and yellow leaf symptoms as compared to healthy leaves in the asymptomatic plants during grand growth phases. The leaves 1 to 5 showed a progressive increase in mosaic symptoms and yellow blotches in the symptomatic plants as compared to symptom-free leaves of asymptomatic plants. The youngest leaves in the whorl exhibited typical mosaic symptoms of dark green patches with chlorotic streaks in the symptomatic plants. Overall, severity of mosaic and YLD led to poor crop stand in the field as well as in the pits showing a clear degeneration in the symptomatic plants (Fig. 1, Supplementary Fig. 1a, b, c).

Impact on physiological parameters

Chlorophyll content measured as SPAD values in the asymptomatic leaves was in the range of 36.24–37.8, whereas in the disease-affected plants

Table 2 Crop growth and cane yield parameters recorded in asymptomatic and symptomatic sugarcane cv CoJ 64 at the time of harvest (360 days) during different seasons

Sl No	Cane yield parameters	2013–14		2014–15		2015–16	
		Asymptomatic plants	Symptomatic plants	Asymptomatic plants	Symptomatic plants	Asymptomatic plants	Symptomatic plants
1	Height (cm)	117.2±4.9	98.5±4.7***	165±3.4	147±3.14***	175±2.4	135±2.6***
2	No. of internodes	19±2.2	18±4.4*	36±3.1	34±3.7*	25±3.1	19±4.3***
3	Internode length	8.5±4.5	7.3±3.67*	6±4.1	5±2.45*	9.4±1.2	7.2±2.10*
4	Girth	1.90±0.12	1.72±0.35*	2.2±0.29	2.0±0.41*	2.4±0.32	2.0±0.24***
5	Single cane weight (kg)	1.8±0.4	1.31±0.62***	2.23±0.74	1.51±0.72**	1.9±0.31	1.21±0.61***
	% reduction		16.0		10.1		22.9
	% reduction		5.2		5.5		24.0
	% reduction		14.1		16.7		23.4
	% reduction		9.5		9.1		16.6
	% reduction		27.2		32.3		36.3

Data of three planting seasons (\pm SD) in eight rows (40 plants/ row)

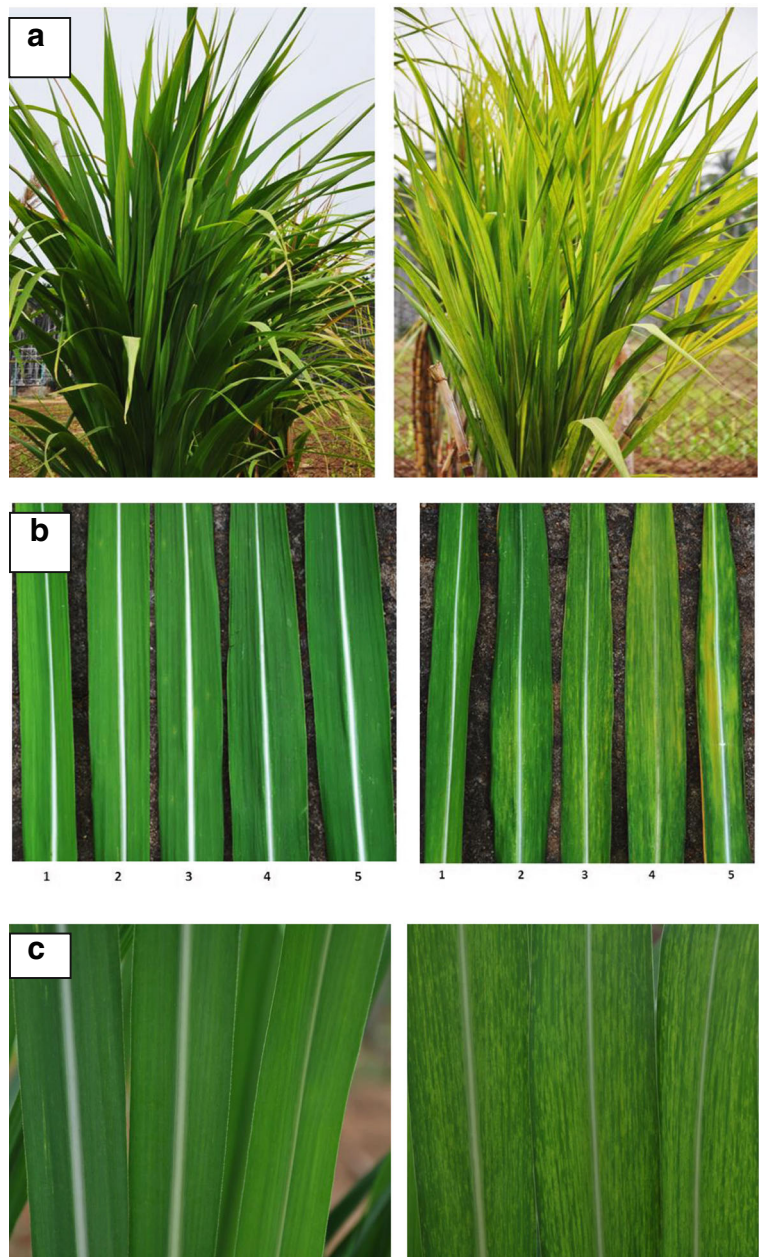
*** $P < 0.05$, ** $P < 0.01$, * $P < 0.1$ and ns-not significant. Data recorded on 10 plants from each row in each of the three planting seasons

the values were in the range of 22.17–24.83 leading to significant reductions of ~34.31–38.82% due to viruses' infection. The chlorophyll fluorescence ratio was in the range of 0.81–0.91 (F_V/F_M) in the asymptomatic plants as compared to 0.58–0.62 (F_V/F_M) in the disease-affected plants with a reduction of 28.48–31.57% due to virus infections. During the formative stage of the crop, significant differences were observed between asymptomatic and symptomatic plants for all the physiological/growth parameters. Also, during the grand growth stage, most of the parameters were recorded with significant differences. In case of gas exchange parameters, photosynthetic rate in the asymptomatic plants ranged from 34.7 to 35.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, whereas, the values in the affected plants it ranged from 17.5 to 19.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ with a reduction of 45.4–49.3% during the two seasons. In case of stomatal conductance, a value of 0.19 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ was recorded in the asymptomatic plants in both the seasons whereas in the affected plants the mean values ranged from 0.08–0.10 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ with a reductions of 47.48–54.7%. The value of transpiration rate was 4.75–5.62 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in the asymptomatic plants, whereas in the affected plants, the values observed were in the range of 2.72–3.37 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ with 40.03–42.52% reductions (Table 3, Supplementary Fig. 2, 3).

Impact on sugarcane juice quality parameters

Symptomatic canes have shown a significant reduction of ~30.13–36.14% in juice yield as compared to asymptomatic canes during harvest. Likewise, symptomatic canes also showed reduction in juice quality parameters viz., Brix, sucrose %, purity % and CCS %. The respective reductions for Brix, sucrose %, Purity % and CCS% in the disease-affected canes were 6.8–8.4, 13.43–16.2, 5.48–6.97 and 15.49–16.10% (Table 4). Similarly, the juice yield calculated from the internodes showed significant reductions in the symptomatic plants when compared to asymptomatic plants. The internodes from the virus-infected canes recorded an overall reduction of 31.72% in juice yield. The impact of virus infection in the symptomatic canes showed a clear reduction in juice yield particularly in the bottom internodes, the reductions were very high (data not shown).

Fig. 1 Variation in leaf canopy and disease symptoms in asymptomatic and symptomatic plants of sugarcane cv CoJ 64 in the trials. **a.** The canopy of the virus infected leaves show yellowish green with mosaic and yellow leaf disease symptoms (Right) as compared to healthy leaves in the asymptomatic plants (Left) by 180 days. **b.** Progressive increase in mosaic symptoms and yellow blotches in the leaves 1 to 5 of symptomatic plants (Right) as compared to almost healthy leaves of asymptomatic plants (Left). **c.** Close-up symptoms of young leaf in the whorl exhibiting typical mosaic symptoms of dark green patches with chlorotic streaks in the symptomatic plants (Right) as compared to normal leaves in asymptomatic plants (Left)



RT-PCR assays for viruses for the identification of mixed infections

In the RT-PCR assays, prominent amplicons for the three viruses SCSMV, SCMV and SCYLV at 690, 381 and 615 bp, respectively were amplified from both asymptomatic and symptomatic plants (Supplementary Fig. 4a, b, c). qRT-PCR assays were standardized using the coat protein specific primers with the amplicon sizes

of 128, 199 and 132 for SCSMV, SCMV and SCYLV, respectively (Results not shown). 25S rRNA was used as an endogenous control that amplified at 156 bp (Supplementary Fig. 4d).

qRT-PCR assay for the quantification of virus titres

Standard curves were obtained using the C_t values for known titre of diluted plasmids for all the three viruses

Table 3 Physiological parameters recorded in asymptomatic and symptomatic sugarcane cv CoJ 64 at the time of harvest (360 days) during different seasons

Sl No	Physiological parameters	2013–14			2014–15		
		Asymptomatic plants	Symptomatic plants	% reduction	Asymptomatic plants	Symptomatic plants	% reduction
1	Chlorophyll (SPAD-502 unit)	37.8 ± 2.4	24.83 ± 2.5***	34.31	36.24 ± 2.1	22.17 ± 2.6***	38.82
2	Chlorophyll fluorescence (F _v /F _M)	0.912 ± 0.14	0.624 ± 0.11***	31.57	0.811 ± 0.12	0.58 ± 0.24*	28.48
3	Photosynthetic rate (A) (μmol CO ₂ m ⁻² s ⁻¹)	34.7 ± 3.1	17.5 ± 1.6***	49.3	35.2 ± 1.2	19.2 ± 2.3***	45.4
4	Stomatal conductance (gs) (mol H ₂ O m ⁻² s ⁻¹)	0.19 ± 0.02	0.10 ± 0.02***	47.48	0.19 ± 0.04	0.08 ± 0.01***	54.7
5	Transpiration rate (E) (mmol H ₂ O m ⁻² s ⁻¹)	4.75 ± 2.2	2.72 ± 4.8 ns	42.52	5.62 ± 3.4	3.37 ± 1.7**	40.03

Temperature 33–35 °C; Mean of 10 plants (±SD). Plants from 8 rows each planted with asymptomatic and symptomatic plants were recorded during two planting seasons

*** $P < 0.05$, ** $P < 0.01$, * < 0.1 and ns-not significant

(Supplementary Table 2). A linear line with an equation of $y = -3.2988x + 39.847$ and a correlation coefficient of $R^2 = 0.9921$ for SCSMV, $y = -3.2842x + 40.943$ and $R^2 = 0.99$ for SCMV and $y = -3.1442x + 37.588$ and

$R^2 = 0.9852$ for SCYLV were obtained. Using the equations of the standard curves (Supplementary Fig. 5a, b, c) obtained, their respective copy numbers in the samples were assessed using the respective equations given below:

Equation for obtaining copy number of SCSMV = $10^{(Ct \text{ sample} - 39.847)/(-3.2988)}$,
 Equation for obtaining copy number of SCMV = $10^{(Ct \text{ sample} - 40.93)/(-3.2842)}$ and
 Equation for obtaining copy number of SCYLV = $10^{(Ct \text{ sample} - 37.588)/(-3.1442)}$

In the asymptomatic leaves, for SCSMV, the leaf position -2 had the Ct value 24.1 and 29.11 for the first and second seasons, respectively. The Ct value gradually increased in the leaf positions 4, 5 and 6 (Tables 5 and 6). The leaf position 1 had highest copy number for all the leaves taken for the study with 2.3×10^3 in the first season and 9.6×10^3 in the second season. For SCMV, the leaf position -2 had the Ct value range of 27.19–28.44 in two seasons. As in SCSMV, gradual increase in the Ct values was recorded in the leaf positions 4, 5 and 6 for SCMV. The highest copy numbers were observed in leaf position -1 for the first year of ~14,738 whereas, for the second year the copy number was highest in the leaf position 1 with 50,234 for SCMV. For SCYLV, the highest copy number was observed in -1 leaf and 1st leaf positions with 410.865 and 1738, respectively in the two seasons (Tables 5 and 6). Melt curve analysis at 60 °C resulted in a single peak for all the three replicates of the positive samples and the negative samples were recorded with no peaks (Data not shown).

In the virus infected leaves, the qRT-PCR assay for SCSMV recorded the highest copy number of about

1.3×10^6 and 3.5×10^5 in the leaf position 1 during the first and second seasons, respectively. Similarly for SCMV, the copy numbers were highest in the same leaf position of 1.6×10^6 and 8.8×10^5 for the two seasons. For SCYLV, the copy numbers were lesser when compared to SCSMV and SCMV and which were in the range of 3.4 – 3.6×10^4 . The copy numbers were comparatively higher in the leaf position 1 when compared to the other leaf positions for SCYLV as in the cases of SCSMV and SCMV. The sixth leaf positions showed very less copy numbers (Tables 7 and 8).

Young leaves in the symptomatic as well as asymptomatic plants had shown high titre for all the three viruses. However, the symptomatic leaves recorded many folds in higher virus titre than the respective leaves of asymptomatic plants. Among the two seasons of qRT-PCR assays, only during 2013–14 the leaves of symptomatic plants recorded huge differences for virus titre than the asymptomatic plants. For SCSMV, the leaf number 2 recorded as high as 800 fold in higher virus titre, for SCMV the leaf number 1 recorded ~1642 fold higher virus titre and for SCYLV leaf number -1

Table 4 Juice yield and quality parameters recorded in asymptomatic and symptomatic sugarcane cv CoJ 64 at the time of harvest (360 days) during different seasons

Sl No	Cane quality parameters	2013–14			2014–15			2015–16		
		Asymptomatic plants	Symptomatic plants	% reduction	Asymptomatic plants	Symptomatic plants	% reduction	Asymptomatic plants	Symptomatic plants	% reduction
1	Juice weight/single cane weight	0.86 ± 0.3	0.55 ± 0.2***	36.04	0.73 ± 0.24	0.51 ± 0.81 ns	30.13	0.83 ± 0.31	0.53 ± 0.67 ns	36.14
2	Brix	21.4 ± 0.21	19.9 ± 0.8 ns	7.0	21.2 ± 0.37	19.4 ± 0.4***	8.4	22.2 ± 0.64	20.7 ± 0.1***	6.8
3	Sucrose%	21.28 ± 0.16	18.24 ± 0.14***	14.28	20.76 ± 0.1	17.97 ± 0.61***	13.43	21.75 ± 0.72	18.22 ± 0.95***	16.2
4	Purity%	96.27 ± 0.7	90.25 ± 0.61***	6.25	97.05 ± 0.18	91.73 ± 0.27***	5.48	97.25 ± 0.23	90.47 ± 0.66***	6.97
5	CCS%	15.34 ± 0.9	12.87 ± 0.21***	16.10	14.97 ± 0.62	12.65 ± 0.3***	15.49	16.24 ± 0.63	13.66 ± 0.17***	15.88

Data of three planting seasons (\pm SD) from eight rows (40 plants/ row)

*** $P < 0.05$, ** $P < 0.01$, * $P < 0.1$ and ns-not significant. Data recorded on 10 plants from each row in each of the three planting seasons

recorded 291 fold higher virus titre. During the 2014–15 season, the fold changes in virus load of the symptomatic plant leaves were lesser for all the three viruses probably indicating additional infection of the viruses in asymptomatic plants or increase in virus titre due to virus multiplication.

Discussion

Mosaic disease in sugarcane is considered to be a serious disease in different parts of the world (Grisham 2000; Koike and Gillespie 1989). Similarly, severe occurrences of YLD were recorded in India and most of the sugarcane growing countries (El Sayed et al. 2015; Viswanathan 2002). Studies were further focussed more on associated viruses, their genome characterization and developing diagnostics in India (Viswanathan et al. 2008a, b, 2009, 2010; Viswanathan and Karuppaiah 2010). With a clarity on associated viruses, the present study were done to assess their impact on varietal degeneration in the cv CoJ 64, which was one of the most popular sugarcane varieties of subtropical India. Although the variety succumbed to red rot in the 1990s, it is being used as a potential parent at NHG, Coimbatore, where more than 600 parental clones are maintained every year for the national breeding programme (Nair 2011). During its maintenance at Coimbatore for many years it succumbed to infection by different viruses and degenerated (Viswanathan unpublished). In this study, we found that SCSMV has the highest virus titre, may be the primary pathogen to infect the crop, followed by SCMV and SCYLV in the cv CoJ 64. This finding further strengthens the previous report of Viswanathan and Karuppaiah (2010) that SCSMV is predominant among the three viruses where almost 93.44% of varieties were infected with SCSMV, 31.15% with SCMV and 27.87% with SCYLV and SCSMV has been found as a major causative virus of sugarcane mosaic.

In this study, a gradual increase in mosaic pattern and severity was clearly observed with the advancement of crop age (Supplementary Fig. 1a). The infection pattern from the time of planting till the harvest showed a gradual progress in symptom severities indicating disease progression reaching a maximum. The leaves of the asymptomatic plants were very broad and fully expanded, whereas in mosaic affected plants, leaves became narrow and erect facing upwards with uneven expansion (Fig. 1). The chlorophyll content in the symptomatic

Table 5 Ct mean and their corresponding copy numbers of SCSMV, SCMV and SCYLV target genes obtained from asymptomatic samples of the cv CoJ 64 in relative standard curve method of qRT-PCR collected during the 2013–14 season

Leaf position	25SrRNA	SCSMV	No of target genes	SCMV	No of target genes	SCYLV	No of target genes
-2	18.4 ± 0.61	29.11 ± 0.17	1798 ± 520	28.44 ± 0.11	6,354 ± 547	30.4 ± 0.83	195 ± 74
-1	16.74 ± 0.43	26.41 ± 0.36	11,839 ± 2514	27.24 ± 0.34	14,738 ± 1467	28.73 ± 0.82	657 ± 54
1	16.32 ± 0.72	25.4 ± 0.52	23,956 ± 3245	28.67 ± 0.85	5,408 ± 412	29.37 ± 0.22	411 ± 50
2	19.48 ± 0.39	28.07 ± 0.19	3716 ± 857	30.72 ± 0.76	1,285 ± 321	28.47 ± 0.74	794 ± 67
3	16.72 ± 0.37	30.99 ± 0.64	482 ± 94	31.69 ± 0.47	651 ± 54	30.9 ± 0.71	134 ± 32
4	16.24 ± 0.44	31.044 ± 0.18	466 ± 75	31.87 ± 0.61	874 ± 26	31.07 ± 0.5	118 ± 21
5	19.4 ± 0.7	32.24 ± 0.45	202 ± 20	31.27 ± 0.22	574 ± 34	31.73 ± 0.51	73 ± 0.7
6	19.99 ± 0.9	32.97 ± 0.49	121 ± 34	33.27 ± 0.22	215 ± 34	32.6 ± 0.62	39 ± 0.8

25SrRNA was used as a reference gene for normalization

plants is not synthesized in the same rate as in asymptomatic plants or some amount of chlorophyll is destroyed as a consequence of infection (Srivastava et al. 2010). In this study, the severity of mosaic infection turned the leaves from green to yellow blotches after 5 to 6 months probably due to a drastic reduction in chlorophyll synthesis rate in the symptomatic plants (Fig. 1). In the asymptomatic plants, the mosaic expression was not phenotypically observed and the plants looked healthy. It is clear that the viruses causing mosaic and YLD alter the physiological processes such as chlorophyll synthesis and photosynthesis in sugarcane.

As sugarcane cultivation is done routinely using setts (vegetative stem cuttings), the viruses in the mother setts accumulate more titre in the next generations and subsequently passes on to more number of generations thereby affecting the physiological efficiency, productivity and yield. Due to YLD, ~60% of yield losses in commercial cv PR692176 were reported in Venezuela (Izaguirre Mayoral et al. 2002) and similarly, 30%

reduction in number of stalks per clump, 29% reduction in biomass and 26% reduction in sugar yield were reported in the cv H87–4094 in Hawaii (Lehrer et al. 2009). It has been established that YLD severity increases in the ratoon crops and hence yield reductions are comparatively higher in ratoons than the plant crop (Izaguirre Mayoral et al. 2002; Rassaby et al. 2003). In Indonesia, Putra et al. (2014) recorded a reduction in sugar yield of about 19–21% due to SCSMV infection in sugarcane varieties. Viswanathan et al. (2014) found a significant reduction in cane growth, yield and physiological parameters due to SCYLV in different sugarcane varieties. Agnihotri (1996) reported that SCMV causes an appreciable damage in susceptible varieties and even 10–15% yield loss due to the disease is highly significant because of extensive cultivation of the crop. Our previous study established that SCMV infection significantly reduced the net CO₂ assimilation rate during the grand growth period. At harvest, cane stalks from virus-infected plots recorded a significant

Table 6 Ct means and their corresponding copy numbers of SCSMV, SCMV and SCYLV target genes obtained from asymptomatic samples of the cv CoJ 64 in relative standard curve method of qRT-PCR collected during the 2014–15 season

Leaf position	25SrRNA	SCSMV	No of target genes	SCMV	No of target genes	SCYLV	No of target genes
-2	18.2 ± 0.17	24.1 ± 0.18	58,884 ± 1458	27.19 ± 0.21	15,262 ± 1457	28.09 ± 0.24	1,049 ± 412
-1	16.78 ± 0.24	25.2 ± .65	27,542 ± 2416	27.54 ± 0.66	11,942 ± 2165	27.9 ± 0.26	1,206 ± 401
1	16.62 ± 0.36	23.4 ± 0.2	96,761 ± 5681	25.49 ± 0.45	50,234 ± 4987	27.4 ± 0.3	1,739 ± 578
2	19.41 ± 0.21	24.32 ± 0.67	50,816 ± 884	28.47 ± 0.19	6,222 ± 854	29.2 ± 0.95	465 ± 46
3	18.54 ± 0.43	27.27 ± 0.92	6,494 ± 734	29.08 ± 0.7	4,057 ± 735	30.17 ± 0.47	229 ± 26
4	18.63 ± 0.38	30.4 ± 0.61	731 ± 85	30.54 ± 0.99	1,457 ± 617	30.41 ± 0.95	192 ± 25
5	19.4 ± 0.22	30.48 ± 0.46	691 ± 46	30.77 ± 0.70	1,241 ± 649	32.94 ± 0.55	30 ± 0.12
6	16.45 ± 0.35	32.74 ± 0.97	143 ± 37	31.41 ± 0.48	791 ± 57	32.47 ± 0.19	42 ± 0.15

25SrRNA was used as a reference gene for normalization

Table 7 Ct mean and their corresponding copy numbers of SCSMV, SCMV and SCYLV target genes obtained from disease affected samples of the cv CoJ 64 by relative standard curve method of qRT-PCR collected during the 2013–14 season

Leaf position	25SrRNA	SCSMV	No of target genes	SCMV	No of target genes	SCYLV	No of target genes
-2	18.14 ± 0.32	20.62 ± 0.48	673,721 ± 2014	20.47 ± 0.25	1,697,567 ± 5247	22.14 ± 0.51	83,018 ± 6524
-1	16.1 ± 0.19	18.46 ± 0.81	3,042,748 ± 8624	18.56 ± 0.41	6,477,389 ± 5461	20.98 ± 0.84	191,470 ± 3575
1	15.27 ± 0.61	16.31 ± 0.22	13,646,146 ± 9542	18.11 ± 0.65	8,879,735 ± 6243	20.22 ± 0.19	334,057 ± 7520
2	18.1 ± 0.8	18.5 ± 0.34	2,958,966 ± 4235	21.3 ± 0.17	948,642 ± 4301	24.6 ± 0.27	13,514 ± 4581
3	16.33 ± 0.40	23.48 ± 0.32	91,517 ± 2315	23.7 ± 0.99	176,332 ± 3324	26.58 ± 0.94	3,170 ± 621
4	18.46 ± 0.5	23.7 ± 0.47	78,487 ± 574	24.77 ± 0.1	41,309 ± 879	26.1 ± 0.26	4,505 ± 247
5	20.8 ± 0.76	24.59 ± 0.39	42,171 ± 456	27.21 ± 0.27	15,051 ± 674	28.09 ± 0.27	1,049 ± 65
6	16.5 ± 0.31	28.92 ± 0.94	9,147 ± 94	26.02 ± 0.5	5,542 ± 78	29.47 ± 0.5	382 ± 74

25SrRNA was used as a reference gene for normalization

reduction in cane diameter, cane weight and number of internodes and reduced juice quality parameters (Viswanathan and Balamuralikrishnan 2005). Further, Agnihotri (1990) observed that a synergy between SCSMV and RSD also exists and greater losses are incurred when sugarcane is infected with both the pathogens simultaneously than their separate infections. Impact of combined infections of the viruses causing mosaic and YLD and RSD bacterium on varietal degeneration has been well established (Viswanathan 2016).

In the present study, growth, yield and physiological parameters were significantly reduced in symptomatic plants when compared to the asymptomatic ones due to varietal degeneration. The cv CoJ 64 was healthy, free of mosaic disease and YLD on its arrival from the subtropical state of Punjab, however, during its maintenance at Coimbatore the plants showed systemic mosaic with no symptom recovery. Probably, tropical conditions

prevailing at Coimbatore may be ideal for the variety to pick up different strains of the viruses as reported by Agnihotri (1996). Further, the NHG with diverse genetic background may favour horizontal transmission of the viruses across the clones. We also earlier documented prevalence of enormous variation in different strains of SCSMV and SCSMV infecting different parental clones maintained at NHG (Viswanathan et al. 2008a, 2009). Moreover, the viral strains prevailing in Coimbatore may be virulent that probably causes severe crop damage with high mosaic symptom expression along with YLD. Further observations indicated that the affected canes do not flower due to poor growth and degeneration as compared to 80–90% flowering in the asymptomatic plants (Data not shown). This is another finding that degeneration of the parental clones adversely affects flowering and makes such parental clones unsuitable for hybridization and sugarcane breeding. Furthermore,

Table 8 Ct mean and their corresponding copy numbers of SCSMV, SCMV and SCYLV target genes obtained from disease affected samples of the cv CoJ 64 in relative standard curve method of qRT-PCR collected during the 2014–15 season

Leaf position	25SrRNA	SCSMV	No of target genes	SCMV	No of target genes	SCYLV	No of target genes
-2	16.67 ± 0.34	20.42 ± 24	774,462 ± 4239	18.95 ± 0.66	4,927,198 ± 5634	24.89 ± 0.40	10,864 ± 4018
-1	16.88 ± 0.6	18.63 ± 0.18	2,702,091 ± 5731	18.41 ± 0.47	7,195,649 ± 7461	22.47 ± 0.61	63,925 ± 4687
1	16.92 ± 0.45	17.2 ± 0.52	16,807,379 ± 8724	18.24 ± 0.63	3,547,317 ± 6428	20.1 ± 0.2	363,031 ± 5304
2	16.24 ± 0.37	22.4 ± 0.67	194,446 ± 4137	20.49 ± 0.11	1,673,929 ± 4091	24.9 ± 0.9	10,785 ± 3742
3	16.72 ± 0.40	24.1 ± 0.43	59,368 ± 6324	23 ± 0.71	288,005 ± 6273	26.5 ± 0.42	3,342 ± 743
4	16.85 ± 0.66	24.82 ± 0.91	35,916 ± 2413	23.5 ± 0.29	202,871 ± 548	26.4 ± 0.81	3,595 ± 540
5	16.37 ± 0.72	26.74 ± 0.5	11,353 ± 2314	26.66 ± 0.7	22,131 ± 340	27.01 ± 0.22	2,300 ± 238
6	16.78 ± 0.16	28.41 ± 0.77	6,489 ± 271	28.55 ± 0.21	2,658 ± 348	30.21 ± 0.94	221 ± 20

25SrRNA was used as a reference gene for normalization

even if few flowers emerge in the degenerated canes, the floral organs were malformed and do not set the seed (Hemaprabha, personal communication).

Viswanathan et al. (2014) reported that though asymptomatic plants for YLD were positive for the virus they exhibited a normal crop stand without bunching of leaves in the crown, stunting and reduced leaf growth as compared to symptomatic plants. This suggests that a threshold of virus titre is required for phenotypic expression of disease symptoms and altered physiological changes in the plant. The quantified viral load in the asymptomatic plants was in the ranges of 10^2 – 10^3 copy numbers for all the three viruses in this study. Such plants phenotypically showed no symptom expression on all the leaves. This shows that the plants were able to tolerate virus titre within the threshold limit and probably beyond which the symptoms may be visible or severe. In the symptomatic plants, the copy numbers for the viruses ranged above 10^4 – 10^6 and such plants exhibited systemic yellowing and the canopy colour have totally changed from green to yellowish green with blotches indicating the degeneration of the crop (Fig. 1). Mixed viral infections are common in plants and interactions may occur between viruses within the same host cell. Such viral interactions may be antagonistic or synergistic (Hull 2002). Antagonism usually occurs when the co-infecting viruses are related, resulting in interference (Sakai et al. 1983) or cross-protection (Watts and Dawson 1980). In our situation, a combination of three different viruses led to synergistic effect and caused the variety to degenerate in sugarcane. qRT-PCR assays also clearly revealed that the degenerated cane leaves recorded manifold higher titre for all the three viruses than the asymptomatic plants (Tables 5, 6, 7 and 8). Synergism normally occurs in mixed infections when the pair of viruses involved are unrelated, resulting in more severe disease symptoms than those produced by single infections (Cho et al. 2000).

Cane productivity remained stagnant over the last five to six decades in India. Although newly released varieties were superior for yield and quality, slow degeneration occurs in them due to systemic accumulation of viruses in the canes as evidenced in the cv CoJ 64. Probably, phytoplasmas and RSD bacterium may also be involved in degeneration along with the viruses as reported in case of other varieties (Viswanathan 2016). Varietal degeneration has been recognized only in the recent years in the country because of epidemic occurrences of YLD, molecular characterization of the viruses and development

of molecular diagnostics for the viruses. This causes losses of several million US \$ every year to sugarcane farmers and sugar industry (Viswanathan et al. 2018). Now that impact of varietal degeneration on sugarcane production has been proved and this has to be taken as a serious issue to prevent yield losses in the present ruling varieties from the destructive viral infections. Also management of the viral diseases has to receive momentum in the way of disease-free nurseries to enhance sugarcane productivity in the country and addressing varietal degeneration has to be a part of integrated disease management in sugarcane (Viswanathan 2016; Viswanathan and Rao 2011). Further, the findings revealed that the asymptomatic plants taken for the study was though not free from viruses, was able to tolerate the counter defense exerted by the viral population to a greater extent probably through RNA silencing mechanism and able to protect themselves from systemic infection. Probably in the disease affected plants the viruses would have overcome the host resistance hence it is worthwhile to investigate further on the RNA silencing mechanism adopted by the host and RNA silencing suppressors of the respective viruses to go for a RNAi mediated disease resistance to the major RNA viruses in sugarcane.

Acknowledgements The financial support received from the Department of Biotechnology, New Delhi (BT/PR/4978-AGR-36712-2012) is greatly acknowledged.

Compliance with ethical standards

Conflict of interest The authors declared that there is no conflict of interest.

References

- Agnihotri, V. P. (1990). *Diseases of sugarcane and sugar beet* (pp. 23–72). New Delhi: Oxford & IBH Publishing Co Pvt Ltd.
- Agnihotri, V. P. (1996). Current sugarcane disease scenario and management strategies. *Indian Phytopathology*, 49(2), 109–126.
- Balamuralikrishnan, M., Doraisamy, S., Ganapathy, T., & Viswanathan, R. (2003). *Sugarcane mosaic virus* infection progress in relation to age of sugarcane. *Sugar Tech*, 5(1), 21–24.
- Chinnaraja, C., & Viswanathan, R. (2015). Variability in yellow leaf symptom expression caused by the *Sugarcane yellow leaf virus* and its seasonal influence in sugarcane. *Phytoparasitica*, 43(3), 339–353.
- Cho, J. D., Kim, J. S., Park, H. S., La, Y. J., & Kim, K. S. (2000). Ultrastructural aspects of the mixed infections of watermelon mosaic *Potyvirus* isolated from pumpkin and cucumber green

- mottle mosaic *Tobamovirus* from watermelon. *The Plant Pathology Journal*, 16(4), 216–221.
- El Sayed, A. I., Komor, E., Boulila, M., Viswanathan, R., & Odero, D. C. (2015). Biology and management of *Sugarcane yellow leaf virus*: An historical overview. *Archives of Virology*, 160(12), 2921–2934.
- Gonçalves, M. C., Pinto, L. R., Souza, S. C., & Landell, M. G. A. (2012). Virus diseases of sugarcane. A constant challenge to sugarcane breeding in Brazil. *Functional Plant Science and Biotechnology*, 6(2), 108–116.
- Grisham, M. P. (2000). Mosaic. In P. Rott, R. A. Bailey, J. C. Comstock, B. J. Croft, & A. S. Saumtally (Eds.), *A Guide to Sugarcane Diseases* (pp. 249–254). Montpellier: La Librairie du CIRAD.
- Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Hema, M., Joseph, J., Gopinath, K., Sreenivasulu, P., & Savithri, H. S. (1999). Molecular characterization and intervirul relationships of a flexuous filamentous virus causing mosaic disease of sugarcane (*Saccharum officinarum* L.) in India. *Archives of Virology*, 144(3), 479–490.
- Hull, R. (2002). *Matthew's plant virology* (4th ed.). London: Academic Press.
- Izaguirre Mayoral, M. L., Carballo, O., Alceste, C., Romano, M., & Nass, H. A. (2002). Physiological performance of asymptomatic and yellow leaf syndrome-affected sugarcane in Venezuela. *Journal of Phytopathology*, 150, 13–19.
- Koike, H., & Gillespie, A. G. (1989). Mosaic. In C. Ricaud, B. T. Egan, A. G. Gillespie, & C. G. Hughes (Eds.), *Disease of sugarcane - major diseases* (pp. 301–322). Amsterdam: Elsevier.
- Krause, G. H., & Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42(1), 313–349.
- Lehrer, A. T., Moore, P. H., & Komor, E. (2007). Impact of *Sugarcane yellow leaf virus* (SCYLV) on the carbohydrate status of sugarcane: Comparison of virus-free plants with symptomatic and asymptomatic virus-infected plants. *Physiological and Molecular Plant Pathology*, 70, 180–188.
- Lehrer, A. T., Wu, K. K., & Komor, E. (2009). Impact of *Sugarcane yellow leaf virus* (SCYLV) on growth and sugar yield of sugarcane. *Journal of General Plant Pathology*, 75(4), 288–296.
- Nair, N. V. (2011). Sugarcane varietal development programmes in India: An overview. *Sugar Tech*, 13(4), 275–280.
- Putra, L. K., Kristini, A., Achadian, E. M. A., & Damayanti, T. A. (2014). *Sugarcane streak mosaic virus* in Indonesia: Distribution, characterisation, yield losses and management approaches. *Sugar Tech*, 16(4), 392–399.
- Rassaby, L., Girard, J. C., Letourmy, P., Chaume, J., Irely, M. S., Lockhart, B. E. L., Kodja, H., & Rott, P. (2003). Impact of *Sugarcane yellow leaf virus* on sugarcane yield and juice quality in Reunion Island. *European Journal of Plant Pathology*, 109(5), 459–466.
- Sakai, F., Dawson, J. R. O., & Watts, J. W. (1983). Interference in infections of tobacco protoplasts with two bromoviruses. *Journal of General Virology*, 64(6), 1347–1354.
- Singh, V., Sinha, O. K., & Kumar, R. (2003). Progressive decline in yield and quality of sugarcane due to *Sugarcane mosaic virus*. *Indian Phytopathology*, 56(4), 500–502.
- Srivastava, M., Gupta, U. P., & Sinha, A. (2010). Viral diseases of leguminous crops. *Journal of Scientific Research*, 54, 135–152.
- Viswanathan, R. (2002). Sugarcane yellow leaf syndrome in India: Incidence and effect on yield parameters. *Sugar Cane International*, 20(5), 17–23.
- Viswanathan, R. (2012). *Sugarcane diseases and their management* (p. 140). Coimbatore: ICAR-Sugarcane Breeding Institute.
- Viswanathan, R. (2016). Varietal degeneration in sugarcane and its management in India. *Sugar Tech*, 18(1), 1–7.
- Viswanathan, R. (2018). Changing scenario of sugarcane diseases in India since introduction of hybrid cane varieties: Path travelled for a century. *Journal of Sugarcane Research*, 8(1), 1–35.
- Viswanathan, R., & Balamuralikrishnan, M. (2005). Impact of mosaic infection on growth and yield of sugarcane. *Sugar Tech*, 7(1), 61–65.
- Viswanathan, R., & Karuppaiah, R. (2010). Distribution pattern of RNA viruses causing mosaic symptoms and yellow leaf in Indian sugarcane varieties. *Sugar Cane International*, 28(5), 202–205.
- Viswanathan, R., & Rao, G. P. (2011). Disease scenario and management of major sugarcane diseases in India. *Sugar Tech*, 13(4), 336–353.
- Viswanathan, R., Balamuralikrishnan, M., & Karuppaiah, R. (2007). Sugarcane mosaic in India: A cause of combined infection of *Sugarcane mosaic virus* and *Sugarcane streak mosaic virus*. *Sugar Cane International*, 25(2), 6–14.
- Viswanathan, R., Balamuralikrishnan, M., & Karuppaiah, R. (2008a). Characterization and genetic diversity of sugarcane streak mosaic virus causing mosaic in sugarcane. *Virus Genes*, 36(3), 553–564.
- Viswanathan, R., Balamuralikrishnan, M., & Karuppaiah, R. (2008b). Identification of three genotypes of *sugarcane yellow leaf virus* causing yellow leaf disease from India and their molecular characterization. *Virus Genes*, 37(3), 368–379.
- Viswanathan, R., Karuppaiah, R., & Balamuralikrishnan, M. (2009). Identification of new variants of SCMV causing sugarcane mosaic in India and assessing their genetic diversity in relation to SCMV type strains. *Virus Genes*, 39(3), 375–386.
- Viswanathan, R., Karuppaiah, R., & Balamuralikrishnan, M. (2010). Detection of three major RNA viruses infecting sugarcane by multiplex reverse transcription polymerase chain reaction (multiplex RT-PCR). *Australasian Plant Pathology*, 39, 79–84.
- Viswanathan, R., Chinnaraja, C., Malathi, P., Gomathi, R., & Rakkiyappan, P. (2014). Impact of *Sugarcane yellow leaf virus* (ScYLV) infection on physiological efficiency and growth parameters of sugarcane under tropical climatic conditions in India. *Acta Physiologiae Plantarum*, 36(7), 1805–1822.
- Viswanathan, R., Nithya, K., Parameswari, B., Jeevalatha, A., & Rao, G. P. (2017a). The current status of *Luteovirus* and *Polerovirus* research in India. In B. Mandal, G. P. Rao, V. Baranwal, & R. Jain (Eds.), *A century of plant virology in India* (pp. 285–305). Singapore: Springer Nature.
- Viswanathan, R., Parameswari, B., Nithya, K., Jeevalatha, A., & Rao, G. P. (2017b). Potyviruses infecting crop plants in India. In B. Mandal, G. P. Rao, V. Baranwal, & R. Jain (Eds.), *A century of plant virology in India* (pp. 361–404). Singapore: Springer Nature.

- Viswanathan, R., Parameswari, B., & Nithya, K. (2018). Molecular characterization of sugarcane viruses and their diagnostics. In R. Prasad, S. S. Gill, & N. Tuteja (Eds.), *Crop improvement through microbial biotechnology* (pp. 175–193). Amsterdam: Elsevier.
- Wagih, M. E., Ala, A., & Musa, Y. (2004). Evaluation of sugarcane varieties for maturity earliness and selection for efficient sugar accumulation. *Sugar Tech*, 6(4), 297–304.
- Watts, J. W., & Dawson, J. R. O. (1980). Double infection of tobacco protoplasts with *Brome mosaic virus* and *Cowpea chlorotic mottle virus*. *Virology*, 105(2), 501–507.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.