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Status of yellow leaf resistance in sugarcane germplasm and parental clones at Sugarcane Breeding Institute, India

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Abstract

Yellow leaf (YL) caused by *Sugarcane yellow leaf virus* (SCYLV) was first reported in India during 1999 and in recent years it has attained epidemic proportions, seriously affecting sugarcane production in the country. In the absence of disease resistant varieties for cultivation, virus-free tissue culture seedlings derived through meristem culture are recommended to manage yellow leaf in sugarcane. Further, host resistance has not been exploited due to lack of information on resistance to the disease in the germplasm and the parents. Sugarcane Breeding Institute (SBI), Coimbatore houses one of the largest collections of sugarcane germplasm and hybrid collections. We have conducted detailed surveys on YL symptom incidence and severity for the last five seasons in the germplasm resources totalling ~ 4066 genotypes/varieties maintained by the Institute at Coimbatore and its research centres, Agali, Kannur (Kerala) and Karnal (Haryana). Both YL symptom incidence and severity on a '0'-'5' grade system were recorded in the sugarcane germplasm resources. Among the different centres / collections Agali centre recorded more severity to YL followed by National Hybridization Garden (NHG), National Active Germplasm (NAG) and 'Co' canes. However, *Saccharum* spp. clones maintained at Kannur recorded low YL symptom incidence and least severity for the disease symptoms. Overall, the study indicated that most of the parents used for breeding and hybridization were affected by YL to varying severities. High incidence of vector population and constitution of varietal / parental materials are suspected for the high disease incidence and intensity in the two collections. We have identified 463 resistant sources in the hybrid clones and 773 in *Saccharum* spp. Detailed surveys for the YL symptom incidence /intensity for the first time in a large varietal collections and germplasm of sugarcane have identified resistant sources for YL. The outcome of the study lays foundation for developing YL resistance in sugarcane progenies in the country.

Keywords

Sugarcane, Yellow leaf, SCYLV, Disease severity, Incidence, YL-resistance

Introduction

Yellow leaf (YL) caused by *Sugarcane yellow leaf virus* (SCYLV) was first reported in Hawaii in the late 1980s (Schenck, 1990) and later occurrence of the disease has been reported through in all the sugarcane growing regions worldwide (Lockhart and Cronje 2000; Komar et al. 2010). The disease was first reported in India during 1999 and over the years, the disease has attained epidemic status in most of the sugarcane growing regions (Viswanathan, 2002). The disease results in complete yellowing of leaf midrib, yellow laminar discolouration on both sides of the mid rib and in severe cases necrosis of the leaf lamina from the leaf tip spreading downwards along the midrib. In highly susceptible varieties, affected plants show a bushy appearance of leaves at crown due to internode shortening during maturity stage under Indian conditions. Unrestricted movement of virus-infected seed-canes and repeated use of infected seedcane resulted in severe outbreak of the disease in different states in the country. Such fields show stunted growth accompanied by extensive foliage drying (Fig. 1). Globally during the last 10-12 years the YL disease severity and its impact on sugarcane productivity were reported from almost all the sugarcane growing countries. The disease severely reduced cane growth in terms of number of stalks per stool, cane weight and total biomass, juice yield and sugar yield in different countries (Izaguirre-Mayoral et al. 2002, Lehrer et al. 2007, 2009; Rott et al. 2008). Recent studies of Viswanathan et al. (2014) clearly established that SCYLV infection causes 42.9 to 38.9 % reductions in plant growth during grand growth stage and a loss of 34.15 to 30.26 % in juice yield during crop maturity stage in susceptible cultivars under tropical conditions of the country.

Present epidemic status of YL situation in the country warrants strategic immediate management approaches to sustain sugarcane productivity (Viswanathan, 2013). Going for YL resistant varieties will be a sustainable approach. However status of YL resistance in our germplasm and parents used in hybridization is not clearly known. Sugarcane Breeding Institute (SBI) houses one of the two world repositories of sugarcane germplasm. In addition, it plays a dominant role in breeding sugarcane varieties in India through National Hybridization Garden (NHG), where ~ 600 parental clones are maintained and used by sugarcane breeders of ~25 centres from different parts of the country. The institute maintains more than 3,500 sugarcane germplasm clones as part of the World Sugarcane Germplasm Collections at Kannur, Kerala and ~1,500 hybrids developed during the past 100 years at Coimbatore (Nair, 2008). Earlier studies of Viswanathan (2002) revealed varying incidences of YL in varietal collections

from 36.36 to 69.50 % at Coimbatore. Subsequently it was found that ratoon stunting disease infection favour YL severity and such combined infections leads to varietal degeneration (Viswanathan, 2004). Initially association of *Sugarcane yellow leaf virus* (SCYLV) with the disease was established (Viswanathan, 2002, 2004) and subsequently occurrence of three genotypes of the virus in the country was recognized (Viswanathan et al., 2008). Recently studies established complete genome of the virus SCYLV-IND from India (Chinnaraja et al., 2013). However status of YL symptom incidences and disease severity in sugarcane germplasm were not assessed in India. Hence, we have developed a YL disease scoring system with 0-5 grades to assess YL severity and resistance among the germplasm collection under tropical conditions. The new scoring system was used to assess the status of YL in different germplasm clones, parental population and hybrid cultivars to identify resistant sources to YL for future breeding for YL resistance.

Materials and Methods

Disease severity grades of 0 to 5 were newly created based on the nature of leaf symptoms, bunching of leaves in the top and overall crop growth in the tropical climatic conditions in India (Table 1a, Fig 2). Surveys were conducted for YL incidence in germplasm collections of Sugarcane Breeding Institute, Coimbatore ($11^0$0'N, $76^0$54'E) and its three research centres located at Agali ($11^0$10'N, $76^0$41'E), Kannur ($11^0$53'N, $75^0$22'E) (Kerala) and Karnal ($29^0$42'N, $76^0$59'E) (Haryana). At Coimbatore, varietal collection/germplasm resources viz, National Hybridization Garden (654 parents), Arrowing plot (238 parents), 'Co' canes plot (1623 varieties), National Active Germplasm (NAG) (164 varieties) and DUS collections (Distinctness, Uniformity and Stability; 181 varieties) were surveyed for YL symptoms. At Agali, 'Co' canes plot (208 varieties), Co-allied plot (347 varieties), *Saccharum* spp and germplasm collection (234 genotypes) and DUS (217 varieties) were surveyed. At Kannur 'Co' canes plot (1028 varieties), Foreign hybrids (612 genotypes), IA (Indo-American hybrids) clones (130 genotypes), *Saccharum officinarum* (759), *S. robustum* (145), *S. barberi* (42) and *S. sinence* (30) were surveyed. Similarly, at Karnal germplasm collections (222), exotic clones (127), inter-specific hybrids (ISH) (36), inter-generic hybrids (IGH) (77) and inbred and other progenies (29) were surveyed for YL symptoms and recorded the disease incidence and severity.

The disease surveys were conducted during five planting seasons of 2009-10, 2010-11, 2011-12, 2012-13 and 2013-14 at 10 to 11^{th} month in all the collections except Kannur and

Karnal where the surveys were conducted during 2011-12 and 2013-14 planting season, respectively. During the surveys characteristic YL symptoms such as midrib yellowing, laminar discolouration, drying of discoloured laminar tissues, bunching of leaves in the crown, progressive decline in the health of the plants were recorded. To assess YL symptom incidence, entire population in the plot was taken into account. To assess disease severity, a minimum of 10 canes were subjected for observation using the 0-5 severity grade (Table 1a) and genotype reactions to the disease were recorded as resistant (R), moderately resistant (MR), moderately susceptible, susceptible (S) and highly susceptible (HS) based on the new scoring system (Table 1b). While recording data, infestations of borer pests, rodent/termite infestations or mechanical injury were excluded and only YL affected canes were subjected for disease symptom incidences and severity.

After analysing the disease symptom incidence and severity, the parents/germplasm resources from Coimbatore, Agali and Karnal were grouped based on their origin under 14 different states in the country such as Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Tamil Nadu, Uttarakhand and Uttar Pradesh. Further, 'Co' canes (Coimbatore varieties), ISH (Interspecific hybrids), IGH (Inter generic hybrids), Natal Coimbatore (South Africa-Coimbatore) and IA (Indo-American hybrids) were separately presented. At world germplasm collection, Kannur, *Saccharum* spp were grouped as, *S. officinarum*, *S. barberi*, *S. robustum* and *S. sinence*. *Saccharum* hybrids at Kannur were grouped based on their origin like Australia, Barbados, Brazil, Colombia, Fiji, Mauritius, Puerto Rico, Taiwan and USA.

Results

YL incidence in sugarcane varieties/germplasm

In total, 4066 genotypes were surveyed for YL incidence across different germplasm/hybrid varieties maintained at Coimbatore, Agali, Kannur and Karnal. Among them 1867 (45.9 %) genotypes were found to be free from YL symptoms and 2199 genotypes were found infected with varying levels of the disease symptoms. When the infected genotypes were compared among themselves for the levels of disease incidence, ~ 48 % of them showed very high levels of disease symptom incidence ie 81-100 %. About 26 % of the genotypes exhibited very low disease symptom of less than 20 % and 14 % recorded disease symptom incidence of 21-40 %. Rest of the genotypes showed disease symptom incidences of 41-80 % (Table 2). Comparison

of disease incidence based on the source/ origin of the genotypes revealed that hybrid genotypes recorded more disease symptom incidences than *Saccharum* spp.

At Kannur, *S. officinarum* constituted the majority of *Saccharum* spp collections and among them *S. officinarum* exhibited more disease incidences as compared to *S. robustum* and *S. sinence*. However, only 166 of 759 *S. officinarum* clones exhibited the disease. Among the hybrid genotypes, those originated from Australia, Barbados, Brazil, Fiji, Mauritius, Puerto Rico, Taiwan and USA had less disease incidences than the Indian hybrids. Among the Indian hybrids, 'Co' varieties and ISH clones developed by the institute constituted major chunk of the materials followed by IA clones, varieties from Uttar Pradesh and Andhra Pradesh. Almost all the genotypes from Assam, Haryana, Kerala, Madhya Pradesh, Maharashtra and Odisha recorded varying levels of YL symptoms. Nearly 74 % of 1430 'Co' canes developed by the SBI showed the disease and among them 40% recorded 81-100 % disease in the fields. Similarly ~ 55 % of interspecific hybrids (ISH) developed by the Institute were found to be YL affected. However, 107 of 111 Indo-American (IA) hybrid genotypes remained free from the disease (Supplementary Table 1). Overall, it was found that most of the infected clones except (IA) had high degree of disease symptom spread in the population.

Disease severity

The infected plants were assigned 1-5 grades based on the severity of the YL symptoms and the disease severity in the sugarcane genotypes / varieties was rated on a 0-5 scoring system. Overall, it was found that most of the infected genotypes exhibited severity grades 2 or 3 and only ~7 and 3 % of them recorded grades 4 and 5, respectively. This finding indicated that disease severity levels were moderate in the infected genotypes and similar trend was observed in both the hybrid and *Saccharum* spp genotypes. However, infected genotypes from Australia, Barbados, Brazil, Colombia, Fiji, Mauritius, Puerto Rico and Taiwan recorded grades of 1 and 2, only indicating that the disease severity was comparatively less. When genotypes originated from different states in India were compared for YL severity it was found that genotypes of Tamil Nadu, Andhra Pradesh, Punjab and 'Co' canes recorded the most severe grade '5' in 20, 17, 7 and 3 % of the infected genotypes, respectively (Table 2).

YL incidence and severity of germplasm collection/resources

A total 6482 varieties/genotypes were maintained in different germplasm resources at Coimbatore, Agali, Kannur and Karnal with duplications among the centres and resources.

Overall, about 42 % of them were infected with SCYLV. Each germplasm resources had a different constitution of clones with respect to origin, purpose for which they are maintained and longevity in the collection. Among the different resources, NHG at Coimbatore with 654 genotypes had 87 % disease symptom incidence and this was followed by 80 % in Agali having 1006 genotypes, germplasm (63 %) and exotic clones (54.3 %) at Karnal, NAG at Coimbatore (43 %), hybrid varieties maintained at Kannur (32.8 %), Arrowing Plot at Coimbatore (22.7 %) and *Saccharum* spp in Kannur (13.3 %). Disease incidences across the germplasm resources indicated that Agali centre recorded more severe YL symptom incidences of 81-100 % and which was followed by NAG, NHG and 'Co' cane plot at Coimbatore (Table 3). The same genotypes maintained in 'Co' cane plots at Coimbatore and Kannur recorded almost similar disease incidence of ~ 30 %. This reflected similar behaviour of the genotypes to YL disease in both the locations. However for disease severity we found location specific expression in the genotypes. For e.g. the genotypes maintained at Agali recorded high severity grades of 4 and 5 in ~ 8 % of the genotypes and the 'Co' cane plot maintained at Coimbatore recorded such severity only in ~ 3 % of the genotypes. However, the same 'Co' canes maintained at Kannur did not show high severity indicating a location-specific disease expression in the genotypes.

Resistant sources to YL

The clones consistently recorded with scores '0.0-1.0' in all the collections during the surveys were categorised as resistant (R) to YL based on low severity of sympotms. Among the hybrids, maximum of 481 'Co' cane varieties were found to be resistant to YL disease. This group was followed by 113 ISH, 17 Uttar Pradesh, six each of Bihar and Gujarat, eight of Andhra Pradesh, 4 each of Haryana and Uttarakhand and 3 each of Punjab and Tamil Nadu. In case of *Saccharum* spp, 609 *S. officinarum*, 33 *S. barberi*, 129 *S. robustum* and 31 *S. sinence* were found to be resistant (Table 4a). Among the foreign hybrids, 11 of USA, 5 of Barbados, 3 each of Mauritius and Puerto Rico and 2 each of Australia and Colombia were found to be resistant (Table 4b).

Susceptible genotypes

The genotypes in the germplasm collections, constantly exhibiting severe scores between 3.1 and 5.0 were collectively categorized as susceptible to the disease. Among the hybrids, maximum of 117 'Co' cane varieties were found to be S/HS followed by 35 Andhra Pradesh, 13 Tamil Nadu, 11 Uttar Pradesh, 5 Punjab, 4 Maharashtra, 3 Uttarakhand and 2 each of Assam, Bihar, Gujarat, Madhya Pradesh and ISH varieties/genotypes. In *Saccharum* spp, *6 S.*

officinarum, one each of *S. robustum* and *S. sinence* were susceptible. In case of foreign hybrids two each of Barbados and USA were susceptible (Table 5).

Discussion

After the first report of the disease from Hawaii (Schenk et al. 1990) suspected occurrence of disease was reported from many countries during 1990s (Lockhart and Cronje, 2000). Viswanathan (2002) reported occurrence of the disease from India during 1999 and subsequently he reported detailed symptomatology of the disease, severity and impact of the disease on sugarcane growth. This was the first detailed report on YL disease from India and then onwards detailed studies were conducted on disease epidemiology, diagnosis, variation in SCYLV and the disease management (Viswanathan et al. 2008, 2009, 2012a, 2014; Chinnaraja et al. 2013, 2014a). YL affected plants exhibit prominent midrib and laminar yellowing, extensive necrosis of discoloured lamina and midrib from leaf tip downwards along the midrib, different degrees of bunching of the crown etc (Viswanathan, 2002). It was also established that combined infection of YL and ratoon stunting disease (RSD) caused more damages than their separate infections in sugarcane varieties (Viswanathan, 2004). Recently, Viswanathan (2012a, 2013) reported "varietal degeneration" in sugarcane due to infections of pathogens causing YL, RSD and mosaic and this phenomenon is attributed to poor performances in older varieties. The situation reveals that SCYLV infection adversely affects cane growth either alone or in combination with other pathogens. This can be addressed by virus elimination through meristem culture and developing healthy materials in sugar factories (Viswanathan, 2012b). However, for the sustainable management of the disease, host resistance needs to be given importance hence detailed studies were taken up on the status of YL disease in the germplasm/parents maintained by the institute at different centres and to identify YL resistant sources.

All the germplasm repositories are maintained by replanting the clones every year at Coimbatore and other centres. The new crop may acquire the virus transmitted through setts or through sugarcane aphid *Melanaphis sacchari* mediated secondary transmission. Results of the study clearly revealed that sugarcane fields at Coimbatore and Agali are heavily infested by viruliferous aphids and they may spread the virus across the fields. Using the new scoring system of 0-5, the clones could be grouped as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible with respective scores of 0.0-1.0, 1.1-2.0, 2.1-3.0, 3.1-4.0 and 4.1-5.0. Based on the symptom appearance in Hawaii, Lehrer and Komar

(2008) previously created a grading system of 0-6 for YL-affected sugarcane plants. However, leaf drying associated with YL was not applied in this grading system and under Indian situations, YL severity resulted in lamina drying more frequently. This phenotypic expression of YL was consistently found in most of the varieties/genotypes when expressing severe symptoms under field conditions as well as in germplasm collections (Fig. 1). Hence, Lehrer and Komar (2008) scoring system was not adapted. During the repeated surveys and documentation we found there is overlapping of grades 4 and 5 in the clones, hence the scores between 3.1 and 5.0 were categorized as susceptible clones. Depending on the prevailing environment, the clone may behave as S or HS and we found frequently such occurrences in different clones across centres or fields in the same location.

Among the germplasm/parental sources, NHG at Coimbatore and Agali centre recorded maximum YL symptom incidences of 87.0 and 80.0 %, respectively. In both the locations more than 25 % of the genotypes showed higher YL symptom incidences in the range of 81-100 %. In the world collection at Kannur, maximum YL recorded in hybrid population than the *Saccharum* spp. Also most of the genotypes/varieties in Kannur show less than 20 % disease incidences as compared to other collections, In case of disease severity also Agali centre recorded proportionately more HS varieties/genotypes, whereas in the Arrowing plot and NAG at Coimbatore and Kannur centre most of the infected genotypes/varieties showed only grade 1 or 2.

The present study revealed that majority of the parental clones maintained at NHG and Arrowing plot used for hybridization had severe SCYLV infections (Table 7). It may be due to the location and constitution of parental population. A higher proportion of disease susceptible varieties combined with higher vector activity may be congenial for disease spread. Further, collections at NHG and Agali have diverse hybrid parents brought from different states, probably would have brought varying virus populations from different regions. The new virus variants would have moved horizontally in the populations along with other viruses and caused varietal degeneration. Further, germplasm collections at Kannur are maintained free from mosaic for more than five decades and probably increased severity of YL disease at Coimbatore and Agali may be due to combined infections of viruses causing mosaic and YL diseases (Viswanathan, personal observation). Prevalence of severe mosaic infections at NHG and Agali would be a triggering force for more SCYLV infections and its severity. Earlier studies of Viswanathan and Karuppaiah (2010) and Viswanathan et al. (2013) also revealed infection of mosaic causing viruses in most of the varieties maintained in NHG. Previous studies of

Viswanathan (2004) established that elimination of RSD bacterium through heat treatment reduced YL incidences in certain varieties harbouring both pathogens and improved crop stand. Additionally, relatively higher aphid population at NHG may be another favourable factor for the disease spread. Studies conducted at SBI revealed that *M. sacchari* population varied greatly among the sugarcane genotypes in NHG and other collections. Varieties such as Co 8371, CoS 96268, CoH 110, CoLk 9229, Co 93020, CoJ 83536 and Co 62174 recorded an average *M. sacchari* of 45, 42, 61, 43, 60, 49 and 47 per plant, respectively. Some of the susceptible varieties like CoTl 85441, Co 86010 and CoC 85061 recorded up to 128 aphids per plant (Viswanathan, unpublished). However, further studies are required on the seasonal prevalence of aphid in different varietal collections to directly relate vector population to disease severity. In the routine varietal development programme it has been established that within 4 to 5 years the new progenies acquire SCYLV and exhibit severe grades under Indian situation (Viswanathan, unpublished). This situation depicts that under natural conditions prevailing at Coimbatore, secondary transmission of the virus takes place very rapidly in the virus free progenies and it is possible to select YL resistant types among the progenies. Hence, the 0-5 scoring system to screen sugarcane varieties/genotypes for YL-resistance may be appropriate. However, further research work on artificial screening of sugarcane progenies for YL resistance through aphid vector needs priority.

Surveys for YL in other countries such as Florida, USA recorded 89 % incidence, 90% of cultivars in Ecuador, Guatemala and Honduras, 98% of stalks in Reunion, 73% of cultivars in Colombia and 62% of stalks in the central valley of Costa Rica (Comstock et al. 1999; 2002, Rassaby et al. 2004, Victoria et al. 2005, Moreira et al. 2006). Mean YL incidence in Guadeloupe was 6.4%, and it increased to 11.2 % in the first ratoon crop and it ranged from 0 to 21% according to cultivar and geographical location (Edon-Jock et al. 2007). A comparison of CL (Clewiston) and CP (Canal Point) germplasm in 1970-1989 series established that CL has more resistance to SCYLV than CP germplasm (Comstock and Milligan 2007). In Thailand, around 300 germplasm cultivars tested for SCYLV and found infection in 27 % of them. Imports from Fiji to Thailand exhibited a lower proportion of infected cultivars (17%) than cultivars from Canal Point, Florida, USA and Kantalai, Sri Lanka (84-100%) (Lehrer et al. 2008). The worldwide distribution of YL in different countries may be facilitated through germplasm exchange and it depended very much on whether the imported germplasm was susceptible to and infected by SCYLV as observed by Lehrer et al. (2008).

Incidence of SCYLV in world collection of sugarcane and related grasses at Miami, Florida revealed 7.0% in *S. spontaneum*, 75.8% in *S. officinarum* and *S. robustum*, *S. sinence* and *S. barberi* with 62.5, 46.2 and 13.6%, respectively (Comstock et al. 2001). Schenck and Lehrer (2000) tested varietal collections at Hawaii against SCYLV using tissue-blot and found that more positive clones of SCYLV in *S. officinarum* and *S. sinence* than *S. robustum* and *S. spontaneum*. They also found that relatives of sugarcane, *Miscanthus* and *Erianthus* were not infected. Comstock et al. (2001) reported that a cross between *S. officinarum* susceptible to SCYLV and *S. spontaneum* (resistant) yielded a high proportion of progenies which remained free from SCYLV for more than 10 years. However, they found only 30 % of selected commercial hybrid lines in Hawaii and Florida were resistant and suggested that the selection processes in the breeding programme were probably against SCYLV-resistance. Recently, Komar (2011) found two thirds of the commercial hybrids and noble canes maintained at Hawaii were infected with SCYLV and were classified as susceptible whereas it was reverse in case of *S. spontaneum* and *Erianthus arundinaceous*. Further he found the pedigree list of registered commercial varieties showed that 80 % of cultivars were SCYLV susceptible. A cross between a resistant *S. robustum* and susceptible *S. officinarum* produced 85 % resistant progenies indicating that SCYLV resistance is a dominant trait. Through RT-PCR and RT-qPCR, Zhu et al. (2010) reported that resistant cultivars to SCYLV contain at least 100 fold lower virus titer than susceptible cultivars.

Long range of SCYLV transmission occurs through infected seed canes and secondary spread of SCYLV in the field is mediated by aphid transmissions in a persistent manner (Rassaby et al. 2004). Our earlier studies revealed occurrence of three SCYLV genotypes from India, of which CUB' was the major genotype. It is possible that the virus population would have moved reciprocally from India to other countries or vice versa through seed canes (Viswanathan et al. 2008). Studies of Akbar et al (2010) indicated that sugarcane cultivars vary in their level of resistance to *M. sacchari* in Louisiana. Their antibodies tests revealed that life history parameters such as duration of reproductive period and fecundity of the aphid were negatively affected on HoCP 91-555, resistant to SCYLV as compared to the susceptible L97-128. Chinnaraja et al. (2014) through quantity PCR assays recently found that SCYLV titre progressively increased in virus free plants after inoculation feeding of viruliferous aphids from 7 to 300 days. However, further studies are required to relate YL resistance to aphid resistance under Coimbatore conditions. This will also throw more light on nature of resistance in sugarcane varieties to SCYLV, aphid and both. In another study, the transmission of SCYLV

through *M. sacchari* was confirmed and progress in virus titre in the inoculated plants was quantified in RT-qPCR. In that, the number of SCYLV copies 7 days after aphid inoculation was recorded with a maximum of 22.3 x 10^3 and it gradually increased to the maximum to 4.78 x 10^6 by 300 days (Chinnaraja et al. 2014). Since YL resistance level in various parental clones is low, major focus should be addressed on developing YL-resistance stocks among the parental populations. Although many genotypes of *Saccharum* spp. appeared to be resistant to YL they cannot be directly used to transfer YL resistance. However, ISH (inter-specific hybrid) clones with YL resistance can be utilized to develop YL resistant progenies through a focussed breeding programme. Further, using virus derived genes for developing transgenic lines has proved success in different crops. In sugarcane also transgenic lines developed with untranslatable coat protein gene of SCYLV reduces 10^3 fold virus titres than non-transformed (Zhu et al. 2011)

Present study has revealed the spread of YL symptoms across the sugarcane germplasm and parents used in hybridization programmes. Based on this study 463 genotypes/parents originated from different states of India were identified as resistant to YL. Similarly, 773 genotypes of *Saccharum* spp showing resistance against YL were identified. This suggests that the YL resistant parents in the hybridization blocks can be effectively utilized to develop YL-resistant progenies. This strategy would lead to developing YL-resistant varieties for commercial cultivation and sustain sugarcane productivity in the country in the future. However, elimination of virus through meristem culture to produce virus free planting materials is to be followed till YL-resistant varieties are deployed in the field.

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Table 1a YL disease severity grades developed to assess the disease severity under tropical India

YL disease severity grade	Overall symptoms on sugarcane
0	Healthy plant with green leaves
1	Matured leaves showing mild midrib yellowing
2	Young leaves with mild midrib yellowing and matured leaves showing initial laminar discoloration and tip drying
3	Young leaves with bright midrib yellowing and matured leaves showing extensive laminar discoloration with increased leaf drying. Internode shortening leads to the bunchy top.
4	Young leaf with initial tip drying, matured leaves with ~ 50% of leaf area drying and bunchy top.
5	Plant showed stunted growth with completely dried matured leaves and young leaves with ~ 50% of leaf area drying.

Table 1b YL disease rating scale

Score	Disease reaction
0.0-1.0	Resistant
1.1-2.0	Moderately resistant
2.1-3.0	Moderately susceptible
3.1-4.0	Susceptible
4.1-5.0	Highly susceptible

Table 2 Status of YL incidence and disease severity in germplasm accessions in various collections maintained at Coimbatore, Agali and Kannur by Sugarcane Breeding Institute, Coimbatore**.**

Source of origin	Number of clones	Asymptomatic	Infected	Level of disease incidence (%)					Disease severity grade				
				< 20	21-40	41-60	61-80	81-100	1	2	3	4	5
Andhra Pradesh	106	12	94	9	7	7	4	67	7	17	33	21	16
Assam	19	1	18	1	4	0	0	13	0	2	11	4	1
Bihar	49	17	32	12	2	2	0	16	6	11	11	4	0
Gujarat	22	2	20	6	1	2	0	11	4	9	5	2	0
Haryana	47	3	44	17	4	1	2	20	16	10	17	1	0
ISH	262	117	145	41	26	24	5	49	26	60	47	12	0
IGH	117	77	40	9	26	5	0	0	22	16	2	0	0
Karnataka	7	2	5	3	1	1	0	0	0	2	3	0	0
Kerala	13	0	13	3	1	0	0	9	0	4	7	2	0
Madhya Pradesh	19	0	19	1	2	1	0	15	1	9	7	2	0
Maharashtra	19	1	18	0	0	0	0	18	0	5	11	2	0
Odisha	3	0	3	3	0	0	0	0	0	3	0	0	0
Punjab	50	8	42	5	10	1	0	26	6	12	16	5	3
Tamil Nadu	46	2	44	7	3	0	0	34	3	7	14	11	9
Uttarakhand	33	7	26	5	8	2	1	10	6	6	10	3	1
Uttar Pradesh	211	46	165	31	41	17	7	69	29	57	71	8	0
‘Co’ canes	1430	377	1053	188	147	113	24	581	132	418	400	71	32
Inbreds and other progenies	29	25	4	1	1	2	0	0	2	2	0	0	0
Natal Coimbatore (NCo)	7	3	4	4	0	0	0	0	0	2	2	0	0
Indo-American (IA clones)	111	107	4	3	0	0	0	1	1	3	0	0	0

Saccharum spp*													
S. officinarum	759	593	166	74	8	8	4	72	29	69	53	6	9
S. barberi	42	32	10	6	0	0	0	4	1	8	1	0	0
S. robustum	145	125	21	18	0	0	0	3	5	13	2	0	1
S. sinense	30	24	6	4	2	0	0	0	2	4	0	0	0
Foreign hybrids**													
Australia	33	17	16	3	4	3	0	6	2	8	5	1	0
Barbados	77	49	28	21	2	0	0	5	5	14	4	4	1
Brazil	10	5	5	4	0	1	0	0	0	4	1	0	0
Colombia	11	2	9	7	0	0	0	2	2	4	3	0	0
Fiji	32	17	15	14	0	1	0	0	0	14	1	0	0
Mauritius	18	8	10	8	1	0	0	1	3	7	0	0	0
Puerto rico	57	39	18	12	1	0	0	5	3	12	3	0	0
Taiwan	15	10	5	2	0	0	0	3	0	4	1	0	0
USA	237	140	97	54	8	8	1	26	14	54	26	2	1
Total	4066	1867	2199	576	310	199	48	1066	327	870	767	161	74

*-Kannur and Agali Clones. **-Kannur clones, Kerala.

Table 3 YL disease incidence and severity across different resources of germplasm collections.

Germplasm collection / resources	Coimbatore				Agali	World Germplasm Collections at Kannur			Karnal				
	NHG	NAG	Arrowing plot	'Co' canes plot		'Co' canes plot	*Saccharum* spp	Hybrids	Germplasm	Exotic	ISH	IGH	Inbred and other progenies
Total number	654	345	238	1623	1006	1028	976	612	222	127	36	117	29
Infected	569	151	66	455	809	311	130	201	140	69	19	40	4
Disease incidence (%)													
0 (Healthy)	85	194	172	1168	197	717	846	411	82	58	17	77	25
< 20	242	54	33	125	184	215	104	176	31	14	9	9	1
21-40	90	11	7	97	122	26	3	3	70	35	2	26	1
41-60	45	9	4	85	102	33	0	5	22	19	7	5	2
61-80	14	2	4	36	32	16	0	0	8	1	0	0	0
81-100	178	75	18	112	369	21	23	17	9	0	1	0	0
Disease severity (grade)													
1	72	61	6	50	139	95	32	40	69	39	11	22	2
2	265	57	41	214	306	194	72	135	46	28	6	16	2
3	212	30	19	135	279	22	24	26	4	1	1	2	0
4	20	3	0	24	60	0	2	0	21	1	1	0	0
5	0	0	0	32	25	0	0	0	0	0	0	0	0

NHG: National Hybridization Garden, NAG: National Active germplasm, ISH: Inter-specific hybrids, IGH: Inter-generic hybrids

Table 4 Sugarcane varieties/germplasm maintained in hybridization blocks and germplasm collections showing resistance to YL with score '0-1'

Origin	Variety/genotype
Andhra Pradesh	CoA 84081, CoA 09321, CoA 98082, 70A5, 70 CoA 5 , 88A162, 93A53, 97A85
Bihar	BO 91, BO 128, BO 139, CoP 9302, CoP 02181, CoP 04182
Gujarat	CoN 05071, CoN 05072, CoN 98133,
Haryana	CoH 1, CoH 102, CoH 114, CoH 7803
Karnataka	CoSnk 03044, CoSnk 03632
ISH	ISH 2, -7, -19, -22, -25, -26, -27, -30, -31, -33, -37, -44, -48, -49, -57, -58, -63, -67, -102, -106, -113, -117, -119, -120, -122-124, -127, -137, -140, -144, -147, -148, -150, -151, -152,-154, -155, -160, -161, -163, -164, -167, -170, -173, -174, -176, -181, -186, -185, -188, -190, -192, -193, -195, -196, -197, -201, -206, -211, -216, -216, -218, -220, -224, -225, -226, -231, -234, -236, -240, -244, -245, -248, -250, -251, -252, -253, -255, -256, -257, -259, -263, -264, -265, -266, -267, -268, -269, -270, -273, -274, -275, -276, -279, -281, -282, -283, -285, -287, -291, -293, -300, -302, -303, -305, -307, -308 -312, -313, -314, -318.
Madhya Pradesh	CoJn 80143
Punjab	CoJ 80, CoJ 89, CoPb 10182
Tamil Nadu	CoC 778, CoC 98061, C 4772, C 84136
Uttarakhand	CoPant 84213, CoPant 92134, CoPant 92227, CoPant 97222
Uttar Pradesh	LG 94164, LG 01014, LG 01170, LG 04604, LG 04605, LG 05460, LG 05480, LG 05810, LG 96029, LG 96115, LG 97154, LG 99001, LG 99017, LG 9902, CoSe 95427, CoSe 96436, UP 40
Co Canes	Co -213, -312, -413, -416, -528, -542, -603, -605, -678, -735, -739, -767, -791, -837, -841, -861, -871, -885, -888, -953, -955, -965, -976, -978, -1034, -1047, -1051, -1054, -1059, -1095, -1104, --1127, -1136, -1186, -1187, -1188, -1189, -1224, -1239, -1251, -1257, -1266, -1282, -1287, -1289, -1295, -1320, -1324, -1328, -1331,-1334, -1343, -62020, -62102, -62134, -62136, -62197, -62223, -62229, -62231, -62232, -62233, -62235, -62236, -62237, -62238, -62243, -62248, -62250, -62251, -62253, -62260, -62261, -62262, -62263, -62264, -62265, -62270, -62271, -62272, -62275, -62279, -62282, -62283, -62287, -62292, -62293, -62296, -62299, -62300, -62301, -62304, -62305, -62307, -62316, -62317, -62318, -62319, -62322, -62323, -62334, -62337, -62346, -62348, -62351, -62354, -62368, -62379, -62381, -62382, -62387, -62390, -62392, -62408, -62410, -62411, -62413, -62414, -62415, -62416, -62421, -62422, -62423, -62424, -6321, -6327, -6329, -6418, -6419, -6420, -6422, -6425, -6516, -6520, -6613, -6707, , -6802, -6803, -6809, -6810, -6812, -6905, -6908, -6909, -6912, -6913, -6914, -7002, -7003, -7004, -7116, -7119, -7203, -7205,-7206, -7208, -7210, -7227, -7303, -7305, -7309, -7310, -7311, -7315, -7317, -7319,-7320, -7324, -7327, -7329, -7330, -7332, -7403, -7404, -7406, -7412, -7414, -7418, -7420, -7421, -7427, -7430, -7431, -7432, -7433, -7435, -7437, -7438, -7440, 7502, -7505, -7506, -7510, -7513,

	-7515, -7519 , -7534, -7535, -7536, -7538, -7539, -7540, -7541, -7542, -7543, -7544, -7545, -7546, -7547, -7603, -7604, -7606, -7607, -7608, -7610, -7614, -7620, -7623, -7632, -7639, -7640, -7641, -7643, -7646, -7651, -7701, -7712, -7715, -7808, -7809, -7810, -7811, -7902, -7904, -7906, -7907, -7916, -8002, -8004, -8009, -8012, -8020, -8022, -8024, -8025, -8101, -8102, -8118, -8120, -8125, -8126, -8127, -8130, -8134, -8137, -8138, -8141, -8144, -8146, -8147, -8148, -8151, -8202, -8203, -8206, -8207, -8214, -8216, -8220, -8222, -8224, -8228, -8232, -8301, -8307, 8346, -8353, -85018, -85020, -85048, -85287, -86016, -86020, -86021, -86024, -86025, -86041, -86042, -86043, -86045, -86046, -86047, -86048, -86058, -86259, -87001, -87003, -87006, -87007, -87008, -87017, -87022, -87024, -87030, -87261, -87264, -88007, -88009, -88011, -88014, -88016, -88019, -88022, -88026, -88043, --89002, -89015, -89024, -89027, -89030, -89037, -88039, -90003, -90005, -90007, -90012, -90014, -90018, -90019, -91001, -91006, -91008, -91011, -91015, -91016, -91020, -92011, -92013, -92014, -92016, -92027, -92033, -93001, -93013, -93014, -93019, -93022, -93027, -94002, -94026, -95009, -95010, -95018, -95023, -95025, -96007, -96011, -97006, -97014, -97016, -98002, -98003, -98004, -98005 -98010, -99001, -99002, -99003, -99005, -99009, -99014, -0104, -0105, -0107, -0108, -0109, -0113, -0114, -0115, -0207, -0216, -0222, -0227, -0228, -0229, -0230, -0233, -0235, -0305, -0313, -0319, -0320, -0323, -0324, -0325, -0328, -0331, -0401, -0407, -0411, -0417, -05010, -05020, -06004, -06013, -06024, -06026, -06031, -07001, -07006, -07007, -07008, -07010, -07013, -07014, -07015, -07016, -07017, -07018, -07019, -07020, -07021, -07022, -07023, -07024, -07025, -07027, -07028, -07031, -07032, -08005, -08006, -08012, -08011, -08013, -08014, -08016, -08017, -08019, -08022, -09003, -09016, -09018, -09019, -2001-09, -2010-02, -2010-03, -2010-06, -2010-08, - 2010-09, -2010-10, -2010-13, - 2010-16, - 2010-20, -2010-22,-2010-23, - 2010-25, -2010-26, -2010-27, -2010-28, -2010-30, -2010-32, -2010-34, -2011-01, -2011-12, -2011-16, -2011-17, -2011-19, -2011-20, - 2011-21, - 2011-22, - 2011-23
Saccharum spp	List not given
India-American clones (IA)	IA 1141
Foreign hybrids*	
Australia	Q 70, Q 83
Barbados	B 35187, B 37161, B 376, B 40175, B 40175, B 41248
Colombia	EPC 38151, EPC 39294
Mauritius	M 213/40, M 72/31, M 73/31
Puerto Rico	PR 1064, PR 1079, PR 1013
USA	CP 44153, CP 521, CP 63359, L 61-67, LF 65-3662, LF 65-4401, H 37-1933, H 50-7209, H 52-663, H 53-263, H 59-3775

*-Collections of Kannur, Kerala

Table 5 Susceptible and highly susceptible varieties/genotypes of sugarcane to YL (Score 3.1 to 5.0) maintained at hybridization blocks and varietal collections

Origin	Variety/genotype
Andhra Pradesh	CoA 01082, CoA 03081, CoA 04081, CoA 7602, CoA 7701, CoA 8013, CoA 8201, CoA 8401, CoA 8402, CoA 88081, CoA 89082, CoA 89085, CoA 90081, CoA 92082, CoA 93081, CoA 93082, CoA 94081, CoA 95081, CoA 96081, CoA 99082, 86 A 146, 97 A 28, CoV 03102, CoV 89101, CoV 92101, CoV 92102, CoV 92103, CoV 94101, CoV 95101, 97 V 97, 83 R 23, 97 R 401, 97 R 383, 98 R 272.
Assam	CoBln 9101, CoBln 9605
Bihar	BO 102, BO 47
Gujarat	CoN 91132, CoSnk 03754
ISH	ISH 108, ISH 242
Kerala	Madhumathi
Madhya Pradesh	CoJaw 70, CoJn 862072
Maharashtra	CoM 7219, CoM 7712, CoM 88121, CoM 9220
Punjab	CoJ 77, CoJ 82191, CoJ 83535, CoJ 84191, CoJ 85
Tamil Nadu	CoC 771, CoC 772, CoC 773, CoC 8001, CoC 85061, CoC 86062, CoC 90063, CoC 92061, CoC 99061, CoC 01061, CoG (SC) 5, CoG 93073, CoSi 776
Co canes	Co 285, -290, -356, -393, -449, -453, -527, -617, -618, -621, -622, -785, -835, -875, -6415, -658, -1007, -1148, -1169, -1253, -1305, -1307, -6709, -6806, -7105, -7114, -7212, -740, -7507, -7527, -7704, -7706, -7805, -7807, -7911, -7914, -7915, -8011, -8013, -8014, -8021, -8104, -8113, -8208, -8213, -8304, -8306, -8308,-8314, -8319, -8322, -8323, -8330, -8336, -8338, -8339, -8342, -8356, -8358, -62033, -85001, -85002, -85004, -85007, -85015, -85019, -85028, -86010, -86011, -86029, -86032, -86082, -86249, -86250, -87009, -87021, -87025, -87044, -87263, -87268, -87269, -87272, -88001, -89023, -89029, -91002, -91003, -91005, -91010, -92020, -93006, -94003, -94008, -94012, -95005, -95007, -95007, -95011, -95017, -97015, -98017, -99004, -99006, -99015,0120, -0124, -0214, -0217, -0225, -0306, -0330, -0424, -05004, -05005, -06007, -2000-15
Uttarakhand	CoPant 84211, CoPant 94215, CoPant 94211
Uttar Pradesh	CoLk 8001, CoLk 97154, LG 95037, CoS 8436, CoS 87231, CoS 88230, CoS 92254, CoS 95255, CoS 96260, CoT 8201, UP 48
S. officinarum	Badila Java, Gungera, Penang, 28 NG 224, 57 NG 77, 57 NG 67str
S. robustum	57 NG 56
S. sinence	Ikhri
Barbados	B 38192, B 43337
USA	CP 52-68, CP 84-1198

Table 6 Parental varieties/genotypes recorded resistance to YL across different germplasm collections of Sugarcane Breeding Institute collections

S.No	Varieties/ genotypes	NHG	NAG	ECC	Agali	Kannur	Karnal
1	BO 147	0	-	-	-	-	1
2	BO 91	0	-	0	0	0	0
3	Co 678		0	0	0	0	-
4	Co 976	0	-	0	-	0	-
5	Co 7527	1	0	1	3	-	-
6	Co 7717	1	0	0	3	-	0
7	Co 87025	0	0	0	3	-	-
8	Co 98003	0	-	0	-	-	-
9	Co 99010	0	-	2	-	0	-
10	Co 0118	0	2	1	-	-	1
11	Co 0237	0	-	1	-	-	0
12	Co 0239	1	2	2	-	-	1
13	Co 0240	1	-	2	-	-	0
14	Co 0331	0	-	0	-	-	1
15	Co 05010	1	-	0	-	-	1
16	Co 06032	0	-	0	-	-	1
17	Co 09021	0	-	-	-	-	1
18	Co 09022	0	-	-	-	-	1
19	CoPant 97222	0	0	-	-	-	0
20	CoA 7602	0	0	1	5	-	-
21	CoH 119	0	0	-	3	-	0
22	CoJ 89	0	0	-	-	-	0
23	CoJaw 270	0	0	-	3	-	-
24	CoLk 8102	0	0	0	4	-	2
25	CoM 7219	0	0	-	3	-	-
26	CoN 05072	0	1	-	0	-	-
27	CoN 85134	0	0	-	3	-	-
28	CoP 9206	1	0	-	-	-	1
29	CoP 9302	0	0	-	-	-	0
30	CoPb 10181	0	-	-	-	-	1
31	CoPb 10182	1	-	-	-	-	1
32	CoPb 10183	1	-	-	-	-	1
33	CoR 8001	0	0	-	3	-	-
34	CoS 109	0	-	-	-	-	1
35	CoSnk 05103	1	0	-	3	-	-
36	CoT 8201	0	0	1	3	-	-
37	ISH 127	0	-	0	-	-	-
38	ISH 135	0	-	0	-	-	-
39	ISH 147	0	-	0	-	-	-

40	ISH 150	0	-	0	-	-	-
41	ISH 176	0	-	0	0	-	-
42	Co 89010	0	-	0	3	-	-

Table 7 Parental varieties/genotypes exhibiting susceptible reaction to YL across different germplasm collections of Sugarcane Breeding Institute collections

S.No	Variety/genotype	NHG	NAG	Agali	ECC	Kannur	Karnal
1	83R23	2	1	4	-	-	-
2	97R383	3	2	3	-	-	-
3	97R401	3	2	3	-	-	-
4	Co 453	3	0	4	2	2	3
5	Co 617	3	0	4	2	0	-
6	Co 740	3	1	4	2	0	-
7	Co 1148	2	-	4	3	2	4
8	Co 1305	2	0	4	2	-	-
9	Co 1307	3	3	4	1	-	-
10	Co 6415	3	2	3	3	0	-
11	Co 6806	2	0	3	3	1	-
12	Co 8013	2	1	4	2	-	-
13	Co 8208	2	2	4	2	-	-
14	Co 8213	2	-	3	3	-	-
15	Co 8338	2	4	4	3	0	-
16	Co 85002	4	2	4	3	-	-
17	Co 85019	3	2	4	2	-	-
18	Co 86010	4	2	5	4	-	-
19	Co 86011	3	0	4	3	-	-
20	Co 86249	3	0	0	4	-	-
21	Co 86250	4	-	-	3	-	-
22	Co 87025	0	2	5	2	-	-
23	Co 87263	3	2	5	3	-	3
24	Co 87268	0	1	4	0	-	3
25	Co 89003	3	-	1	3	-	2
26	Co 89029	3	3	4	3	-	4
27	Co 91002	3	2	5	3	-	-
28	Co 91010	2	3	4	3	-	-
29	Co 92020	2	2	4	1	-	-
30	Co 94008	3	2	4	3	-	-
31	Co 95005	2	-	3	2	-	-
32	Co 97015	2	2	4	1	-	-
33	Co 99004	3	1	5	2	-	-
34	Co 0124	3	-	-	3	-	4
35	CoA 7602	2	1	5	4	-	-
36	CoA 88081	3	2	4	-	-	-
37	CoA 89085	3	0	5	-	-	-
38	CoA 95081	3	1	4	-	-	-
39	CoBln 9101	4	2	-	-	-	4
40	CoBln 9605	4	3	-	-	-	-

41	CoC 772	3	1	5	-	-	-
42	CoC 8001	3	0	4	-	-	-
43	CoC 85061	2	2	4	2	-	-
44	CoC 86062	3	2	4	-	-	-
45	CoC 90063	3	2	5	2	-	-
46	CoC 92061	3	0	4	3	-	-
47	CoJ 85	2	-	3	-	-	3
48	CoLk 8001	2	-	4	-	-	4
49	CoPant 84211	4	3	-	-	-	4
50	CoS 8436	3	-	3	3	-	3
51	CoT 8201	3	0	4	3	-	-
52	CoV 92103	3	0	4	3	-	-
53	CoV 94101	3	1	4	-	-	-

Supplementary Table 1 List of Indo-American hybrid (IA) clones recorded resistance (Score 0) to YL in germplasm collections of Sugarcane Breeding Institute

Genotype	Variety
Indo-American clones	IA 52, IA 63, IA 110, IA 145, IA 146, IA 133, IA 157, IA 891, IA 993, IA 996, IA 1041, IA 1060, IA 1066, IA 1145, IA 1180, IA 1190, IA 1211, IA 1219, IA 1303, IA 1304, IA 1365, IA 1367, IA 1368, IA 1384, IA 1386, IA 1390, IA 1479, IA 1481, IA 1483, IA 1499, IA 1517, IA 1523, IA 1540, IA 1549, IA 1583, IA 1692, IA 1731, IA 1741, IA 1749, IA 1779, IA 1780, IA 1781, IA 1805, IA 1832, IA 1857, IA 1858, IA 1862, IA 1957, IA 2258, IA 2267, IA 2330, IA 2397, IA 2413, IA 2414, IA 2415, IA 2416, IA 2425, IA 2429, IA 2436, IA 2448, IA 2467, IA 2468, IA 3016, IA 3107, IA 3132, IA 3135, IA 3142, IA 3194, IA 3198, IA 3207, IA 3218, IA 3243, IA 3255, IA 3265, IA 3266, IA 3271, IA 3273, IA 3274, IA 3275, IA 3281, IA 3293, IA 3306, IA 3315, IA 3328, IA 3331, IA 3333, IA 3336, IA 3338, IA 3345, IA 3359, IA 3393, IA 3400, IA 3401, IA 3443, IA 3444, IA 3478, IA 3514, IA 3650, IA 3663, IA 3664, IA 3667, IA 3675, IA 3817, IA 3904, IA 3958, IA 3969, IA 3970, IA 3976, IA 3986, IA 4003, IA 4016, IA 4046.

Fig. 1

A. Field view of severe yellow leaf incidence in tropical India (Variety: Co 86032; Location: Surat District, Gujarat)

B. Close up view of extensive laminar drying in sugarcane due to yellow leaf in Co 86032 in Tamil Nadu

Fig 2 0-5 yellow leaf disease severity grades in sugarcane. Grades 1 to 5 depict the characteristic YL symptoms observed under tropical India during maturity stages of sugarcane. Progressive yellowing of leaf lamina accompanied by bunching of leaves in the top and foliage drying are seen when disease severity increases.