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RESEARCH ARTICLE

Identification of sugarcane yellow leaf virus resistance in *Saccharum* parental hybrids in India

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Abstract
Yellow leaf disease (YLD) is one of the important viral diseases in sugarcane. It is caused by Sugarcane yellow leaf virus (ScYLV), a positive sense single stranded RNA virus primarily transmits through infected setts and secondarily transmits through aphid *Melanaphis sacchari.* Earlier, it was identified as a minor disease in India, but in recent years it attained the epidemic status with the disease incidences from 30 to 50 % in plant crop and more than 70% in ratoon crops which significantly affected the crop productivity. At present, it is being effectively managed through tissue culture derived virus free planting materials. However, after transplantation of TC plants in the field, it succumbed to the virus and made difficulties to sustain the high yield and high sugar varieties in field for long time. Hence, the present study was taken up to identify the yellow leaf (YL) resistant lines from the large pool of parental and commercial hybrids. During the year 2015-21, YLD incidence, ScYLV resistant (apparent) and susceptible lines were identified based on natural screening in parental population maintained at National Hybridization Garden, ICAR-SBI, Coimbatore. The highest YLD incidence of 24.20% was observed with least 75.63% resistance during the year 2021 followed by 23.70% incidence with 76.33% resistance in 2019. In contrast, the least YLD incidence of 7.90% with high resistance of 92.09% was observed during the year 2017. During last seven years, YLD incidence was observed in the ranges of 7.90% to 24.20% in the parental population. Based on seven years of field phenotyping, 105 apparently resistant lines were identified in the study which could be used for YL resistant breeding programme in the future.

Keywords: YLD incidence; Field screening; ScYLV resistant lines

Introduction

Sugarcane is one of the most important commercially grown field crops. Viral diseases are the major biotic constraints in sugarcane production. Yellow leaf disease (YLD), mosaic and leaf fleck are the important viral diseases. YLD is caused by *Sugarcane yellow leaf virus* (ScYLV) *(Polerovirus, Luteoviridae)* (Scagliusi and Lockhart 2000; Smith et al. 2000; Singh et al. 2009); mosaic is caused by *Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus (Potyvirus, Potyviridae)* and *Sugarcane streak mosaic virus (Poacevirus, Potyviridae)* (Grisham 2000; Viswanathan et al. 2007); leaf fleck is caused by *Sugarcane bacilliform virus* (Badnavirus, Caulimoviridae). Amongst, YLD is considered as a major threat to sugarcane cultivation worldwide (ElSayed et al. 2015; Viswanathan et al. 2016). It was first reported in the year 1989 in Hamakua (Hawaii) on the variety H65-0782 (Schenck 1990) and subsequently from the United States (Comstock et al. 1994), Africa

(Bailey et al. 1996), Brazil (Vega et al. 1997), India (Viswanathan et al. 1999), China (Wang et al. 2003), and other countries on the basis of visual symptoms, electron microscopic observations of virus particles, and serological reactions. ScYLV is primarily transmitted through infected setts, and secondary transmission from one plant to other through insect vector *Melanaphis sacchari* (Chinnaraja and Viswanathan 2015).

The disease is characterized by intense midrib yellowing on the abaxial surface, lateral spread of yellow discolouration to the leaf lamina followed by tissue necrosis from the leaf tip to downwards along the midrib. In most susceptible varieties, typical yellowing of midribs and laminar region is noticed on upper surface of the leaves. Severe infection of the disease leads to shortening of internodes on the top (Fig. 1a,1b). Usually symptoms appear/visible during 6-8 months stage of the crop in the field and symptom expression would be severe in ratoon crops than plant crop (Viswantahan 2002; Viswanathan et al. 2012). Although it was identified as a minor disease in India over the years it attained epidemic status with incidences ranged from 30 to 50 % in plant crop and more than 70% in ratoon crops and significantly affects crop productivity. In India, the symptomatic plants had shown 38.9% - 42.3% reduction in plant growth attributes and 34.15% reduction in juice yield in the susceptible varieties like CoPant 84211, Co 86032, and CoC 671 compared with the disease-free plants (Viswanathan et al. 2014). Although several serological and molecular diagnostics have been developed for ScYLV across the countries, the RT-PCR is being widely used for diagnosis and characterization of ScYLV (Chinnaraja et al. 2014). In India, it is being effectively managed through tissue culture derived virus free planting materials. However, after the field release it gets quickly succumbed to the virus, which makes us to understand the importance of deployment of resistant variety. Although several advancements have been made on viral disease management right from tissue culture (Viswanathan et al. 2012), transgenics (Gilbert et al. 2009) , and up to the recent genome editing approaches, the conventional method of resistance breeding should go hand in hand to sustain the results. In order to identify the YLD resistant material, the present study was taken up from the large pool of parental lines and commercial hybrids.

Materials and Methods

Natural field screening of YLD based on phenotypic symptoms was done at National Hybridization Garden (NHG), ICAR-SBI, Coimbatore for the last seven years from 2015-21. For screening, all the characteristic symptoms of the disease such as midrib yellowing, laminar discolouration, drying of leaves from tip to downwards, and bunching of leaves in the crown were recorded at 15 days intervals from August to December every year. All the canes present in 6m length of rows were evaluated by following the 0-5 scale (Table 1) and accordingly, they were categorized as resistant with a score of 0-1 (R); moderately resistant with a score of 1.1-2 (MR); moderately susceptible with a score of 2.1-3(MS), susceptible with a score of 3.1-4 (S) and highly susceptible with a score of 4.1-5 (HS) (Viswanathan et al. 2016) and the canes with borer/ rodent/termite infestations were excluded. All the plants were screened during the grand growth to maturity phase of the crop for a period of seven years and the leaf samples from asymptomatic and symptomatic were collected during the year 2018-19 and 2019-20, stored at -80 °C for further processing. Total RNA was extracted, cDNA synthesized from all the collected symptomatic and asymptomatic samples for further RT-PCR and qRT-PCR analysis.

Table 1: Yellow leaf disease scoring grades and severity scale

Disease grade	Description
0	No symptom of the disease
1	Mild yellowing of midrib in one or two leaves, no sign of typical bunching of leaves caused by YLD
2	Prominent yellowing of midrib on all the leaves in the crown. No bunching of leaves
3	Progress of midrib yellowing to laminar region in the whorl, yellowing on the upper leaf surface, and bunching of leaves
4	Drying of laminar region from leaf tip downwards along the midrib, typical bunching of leaves as a tuft
5	Stunted growth of the cane combined with drying of symptomatic leaves

Figure 1a. Severe YLD symptom showing midrib yellowing on the abaxial leaf surface

Results and Discussion

During the year 2015-21, YLD incidence, ScYLV resistant (apparent) and susceptible lines were

Figure 1b: Field view of healthy (left) and YLD affected plants (right)

identified in parental population maintained at NHG, Coimbatore. During the year 2021, out of 431 entries 10.44%, 11.26% and 2.5% were MR, MS and S & HS respectively. The highest YLD incidence of 24.20% was observed with least 75.63% resistance during the year 2021 followed by 23.70% incidence with 76.33% resistance in 2019. In contrast, the least YL incidence of 7.90% with high resistance of 92.09% was observed during the year 2017 (Table 2). In the seven years duration, YLD incidence was observed in the ranges of 7.90% to 24.20% in the parental population (Fig. 2) such a wide variance in the

natural incidence may be due to the climatic influence in the expression of symptoms. The least incidence of 7.90% and the negligible percent of HS lines in the year 2017 may be due to the combined effect of healthy planting materials selection and climatic parameters.

During the year 2015, out of 611 entries, 7.85%, 1.96% and 1.36% were MR, MS, S&HS respectively; in the year 2016, out of 629 entries, 1.74%, 8.10% and 7.63% were MR, MS, S, &HS respectively; in the year 2017, of the 607 parental lines 5.43%, 1.81% and 0.65% were identified as MR, MS, S&HS respectively, of that LG 641, CoJ 82, CoS 01268, and CoPb 1181 were identified as highly susceptible with score of 3-5. During the year 2018, out of 595 entries, 5.21%, 7.9% and 5.6% were MR, MS and S&HS respectively. In the year 2019, out of 617 entries, 11.18%, 10.53% and 2% were MR, MS and S&HS respectively; in the year 2020, out of 424 entries, 1.65%, 9.43% and 3.30% were MR, MS and S&HS respectively of that CoH 76, LG 14482, CoP 9301, LG 07482, CoA 13321, Co 91010, LG 99001, LG 06810, LG 05493, Co 86011, CoV 92102, CoJ 85, CoSnk 15102, and LG 07595 were identified as highly susceptible with score of 3-5.

Based on our field phenotyping, about 105 lines were identified as apparently resistant (Table 3)

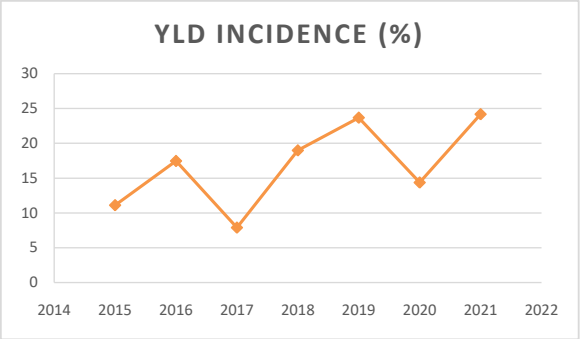

Figure 2. Graphical representation of YLD incidence during 2015-2021 at NHG

of that about 24 lines were identified as would possess the true ScYLV resistance based on our RT-PCR and qRT-PCR analysis (Nithya et al. unpublished). Zambrano et al. (2003) reported that resistance breeding is the most sustainable approach to manage the YLD which could be achieved through massive screening of commercial hybrids to identify and characterize the resistant genes. However, very few countries such as USA, Brazil, France and India have reported the resistant genotypes in the *Saccharum* spp and commercial hybrids based on field screening (Islam et al. 2018; Bourbano et al. 2021; Pimenta et al. 2021; Debibakas et al. 2014; Viswanathan et al. 2016). Hence, identified YL resistant parent lines from this study could be used for future breeding programme to develop YL resistant cultivars.

Table 2: Percent of YLD incidence, susceptible and resistance level at NHG during 2015-2021

Year	Total no. of entries	YLD incidence (%)	Highly susceptible lines (HS) %	Apparently resistant lines (AR) %
2015	611	11.12	1.36	88.87
2016	629	17.48	7.63	82.52
2017	607	7.90	0.65	92.09
2018	595	19.00	5.60	81.00
2019	617	23.70	2.00	76.33
2020	424	14.38	3.30	85.60
2021	431	24.20	2.50	75.63

Table 3: YL resistant lines identified at NHG, Coimbatore (2015-21)

Apparently resistant lines			
CoN 05071	CoSnK 14101	CoA 05323	CoSnk 0361
CoTl 85118	BO 141	C 79218	CoSnk 03632
Co 94005	CoOr 04152	C 81615	BO 91
Co 98014	CoC 22	CoPant 96219	BO 147
CoLk 7901	CoJ 89	CoH 1	CoOr 03152
CoPb 10182	CoBln 94063	CoV 07356	CoP 10182
CoM 6806	CoH 128	CoJ 75	CoN 98133
CoPant 84212	CoC 777	Co 951	Co 8353
Co 8340	CoL 29	51 NG 114	CoP 06436
CoTl 1153	BO 17	57 NG 149	CoS 510
CoH 110	CoLk 97147	28 NG 45	CoN 9220
CoN 95132	CoSe 95427	BO 110	CoA11322
CoJ 80	BO 128	Co 421	CoA 06321
CoM 7704	BO 137	BO 99	CoPant 84214
CoBln 9104	CoLk 8002	CoL 9	CoPant 84213
70A5	CoJ 83	Co 403	CoPb 10183
CoC 775	BO 146	Co 213	CoPant 92227
Co 8341	Co 62175	ISH 176	CoBln 03171
Co 92002	CoPb 11184	Fiji 28	CoBln 05502
CoPant 90223	CoTl 85119	CoJ 65	CoSnk 13104
Co 0116	CoBln 03175	Co 475	CoPant 88220
BO 141	CoH 12	Co 975	CoSnk 03707
CoM 0265	CoPb 13182	CoPb 11182	CoOr 05346
CoN 04131	CoPb 12181	CoPb 11181	CoPant 97222
CoH 104	CoA 09321	PoJ 2878	BO 108
CoN 05072	CoA 14324	CoP 12436	BO 97
CoPb 10181	CoM 9206	BO 128	BO 141

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