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Resistance to Yellow Leaf Disease in Sugarcane Genotypes as Influenced by Physico-chemical Traits

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***Abstract*:** Field investigation was carried out to evaluate the reaction of sugarcane genotypes to yellow leaf disease (YLD) under natural field conditions to characterize their associated biophysical and biochemical responses. Correlations among disease expression, the vector (aphid) incidence and weather parameters were also assessed. Pooled data of three years (2022-23 to 2024-25) showed that six genotypes viz. 2015A311, 2017A553, CoA20321, CoA20323, CoA20325, and CoV19359 exhibited resistant (R) reactions with disease severity scores between 0.0 and 1.0. Nine genotypes exhibited moderately resistant (MR) reactions with disease severity >1.0–2.0, while others such as CoA19322 and 2001A63 were moderately susceptible (MS). The variety, 2003V46, was highly susceptible (S) with disease severity >3.0. Resistant genotypes typically exhibited semi-erect leaves (22°–28°), medium to medium-wide leaf blades, and light-green foliage with SPAD values of 28–31. They also recorded higher phenol content (33.67–40.34%) and silica levels (1.64–2.41%) than the susceptible check 2003V46, which showed greater leaf droopiness (42°), higher sucrose (17.62%), and reduced phenols (28.32%) and silica (1.30%). DAS-ELISA confirmed SPLCV infection in both resistant and susceptible lines, with absorbance values of 3.693–3.957 in positive samples. Aphids collected from susceptible genotypes alone tested ELISA-positive, confirming vector-mediated transmission. Aphid population and YLD incidence were positively correlated with maximum temperature and relative humidity. Rainfall showed a significant negative correlation, indicating that warm, dry conditions favored aphid activity and disease spread. Overall, the genotypes 2015A311, 2017A553, and CoA20323 were identified as stable YLD-tolerant sources, making them promising candidates for resistant breeding programmes in sugarcane.

***Keywords*:** Sugarcane, Screening, Yellow leaf disease, Vector, Biophysical and Biochemical parameters, Weather

Sugarcane (*Saccharum officinarum* L.) is a major commercial crop in India, cultivated on 5.1 million hectares with a production of 439.9 million tonnes in 2024-25, making the country the world's second-largest producer after Brazil. Uttar Pradesh, Maharashtra and Karnataka contribute over 80% of national output (Government of India 2024). The crop supports more than 50 million livelihoods and plays a central role in India's sugar and bioethanol sectors, contributing Rs.776 billion to the national economy in 2023 (FAO 2023). With the expansion of the ethanol blending program, sugarcane's GDP contribution (1.1%) is projected to nearly triple in the coming years (NITI Aayog 2023, ICAR-SBI 2024).

Despite its economic importance, productivity is threatened by Yellow Leaf Disease (YLD), caused by the Sugarcane yellow leaf virus (SCYLV). The disease is widespread across major cane-growing states, with incidence often reaching 70–100% in susceptible varieties (Viswanathan et al., 2020, Singh et al., 2022). Yield losses range from 35–45% in cane weight to 25–35% in juice recovery, and may exceed 50% in severe infections (Rao et al., 2021, Padmanaban et al., 2022). SCYLV colonizes phloem tissues, inducing midrib yellowing, leaf chlorosis and reduced sucrose accumulation (Lehrer et al., 2010, Muthiah and Rajendran, 2022). The virus is transmitted mainly by the aphid, *Melanaphis sacchari* which efficiently spreads the disease under warm, dry conditions (Vega et al., 2010, Singh and Rao, 2021). Environmental conditions strongly modulate YLD epidemiology through their influence on aphid populations. Ramesh et al.,. (2023) observed a sharp rise in disease incidence during SMW 26–37 under elevated temperatures and increasing aphid density, while Singh et al. (2022) reported significant positive correlations of SCYLV spread with minimum temperature and relative humidity. Such findings highlight the tri-interaction of climate, vector abundance and varietal susceptibility.

Physiological studies show that SCYLV infection alters key functions including photosynthesis, chlorophyll fluorescence and stomatal conductance, reducing stalk weight and juice yield by up to 40 per cent (Viswanathan et al., 2014, Barreto et al., 2021). Given the systemic nature of the virus and limited success of vector management, host plant resistance is considered the most reliable and cost-effective strategy (Medeiros et al., 2018, Muthiah and Rajendran 2022). Recent breeding efforts increasingly incorporate biochemical and morphological markers such as phenolic content, silica levels, SPAD values and leaf architecture (Santiago et al., 2016, Sundar et al., 2020, Ali et al., 2023). Diagnostic advances including DAC-ELISA, DAS-ELISA, qRT-PCR and transcriptomics have further strengthened SCYLV detection and resistance differentiation (Chinnaraja and Viswanathan 2015, Xu et al., 2022). In this context, an integrated study was undertaken to identify

promising resistant sources for incorporation into breeding programs and to strengthen the understanding of role of vector as well as physiological and environmental determinants of YLD resistance in sugarcane.

MATERIAL AND METHODS

The research work was carried out at the Regional Agricultural Research Station (RARS), Anakapalle, Visakhapatnam, Andhra Pradesh for three consecutive years from 2022-23 to 2024-25 aimed at investigating resistant sources by correlating YLD severity with biophysical and biochemical attributes and by analyzing the influence of weather parameters, vector population under field conditions. The site is located at 17° 381 N latitude and 83° 011 E longitude at an altitude of 28.62 m above the mean sea level. The location is characterized by tropical semi-arid climate with an average annual rainfall of 900–1100 mm.

Cultivation of sugarcane genotypes: Each of the thirty one genotypes was planted in a plot of five-meter row (four rows) in the month of February with two replications adopting a spacing of 90 cm between rows. All recommended agronomical practices were adopted. The seed rate of 35,000 three budded setts per hectare was used and fertilizers applied were 112 kg N/ha in two equal split doses at 45 and 90 days after planting, 100 kg P_2O_5 and 120 kg K_2O/ ha at basal. Irrigation was provided at a week to 15-20 days interval during summer and at monthly intervals during maturity phase. Inter cultivation and weeding were taken up as per need. No plant protection measures were taken up during the entire crop growth period during both the seasons.

Aphid population: Population data for aphids (both adults and nymphs) in the sugarcane was recorded per leaf from ten clumps selected randomly and mean population data on aphid was calculated. Aphid population was recorded standard week-wise from initial appearance till crop maturity.

Yellow leaf disease (YLD) incidence: Characteristic YLD symptoms such as midrib yellowing, laminar discoloration, drying of discolored laminar tissues, bunching of leaves in the crown, progressive decline in the health of the plants were recorded. Ten cane clumps were randomly chosen and the total number of canes exhibiting YLD symptoms had been counted out of total canes and the percentage of disease occurrence was determined (Chinnaraja and Viswanathan 2015). YLD resistance was assessed using a 0–5 disease severity scale. At each observation, a minimum of 25 canes free from other biotic stresses were examined, and severity grades were assigned based on visible symptoms. Absence of disease was represented by a score of 0, indicating no visible symptoms. A score of 1 corresponded to mild yellowing of the midrib on one or two leaves without any bunching. A score of 2 denoted prominent midrib yellowing on all crown leaves but no leaf bunching. Progression of symptoms into the laminar region, accompanied by yellowing of the upper leaf surface and initial bunching, was reflected in a score of 3. Drying of the laminar region from the leaf tip along the midrib, along with typical tuft-like bunching of leaves, corresponded to a score of 4. Severe disease, expressed as stunted cane growth with extensive drying of symptomatic leaves, was captured under a score of 5. Absence of disease was represented by a score of 0, indicating no visible symptoms. Mean values for disease incidence and severity were computed for each genotype across the observation period. Based on these mean severity scores recorded over three years, genotypes were categorized into defined reaction classes: those with scores ≤ 1.0 were considered *resistant*, scores > 1.0–2.0 as *moderately resistant*, scores > 2.0–3.0 as *moderately susceptible*, scores > 3.0–4.0 as susceptible, and those with scores > 4.0–5.0 as highly susceptible (Chinnaraja et al., 2013).

Weather parameters: Data on weather parameters pertaining to minimum and maximum temperature, minimum and maximum percent of relative humidity and rainfall were collected following meteorological standard weeks from the observatory located at RARS, Anakapalle.

Biophysical parameters: The SPAD chlorophyll meter reading was measured on the second fully expanded leaf from the top of the main stem of each plant using an SPAD-502 meter (Jangpromma et al., 2010). The leaf inclination of the third dewlap leaf and the leaf width at the maximum blade width was measured in five random expanded leaves nondestructively and averaged (Castro Nava et al., 2016).

Biochemical parameters: Juice sucrose content was measured using Sucrolyser and the content of phenol, silica and fibre were analyzed following standard protocol as per the methods suggested by Chen and Chou (1977).

Statistical analysis: Using Microsoft Excel software, data on aphid population, yellow leaf disease incidence weather parameters, bio physical and biochemical traits were statistically analyzed for correlation as per Steel and Torry,1980. The correlation coefficients (r) obtained were further tested for statistical significance using the t-test. The calculated t-values were compared with the tabulated values at the 5% level of significance to determine the significance of the correlations.

Direct Antigen-Coated Enzyme-Linked Immune-Sorbent Assay (DAC-ELISA): DAC-ELISA was carried out using the kit obtained from M/s. AC Diagnostics, USA (Code-V093-K1) following the standard protocol land observations were taken visually and the colour change was observed photo

metrically at 405 nm using Thermofischer scientific Multi scan- X, ELISA reader and the readings were documented. Two leaf midrib samples from each of the 31 genotypes were collected at the crop maturity stage along with healthy control taken from tissue culture raised sugarcane seedlings 87A298 (*Viswamitra*) and positive control obtained from the AC Diagnostics Ltd., USA in two replications, respectively, and further subjected to DAC-ELISA assay. The sugarcane samples exhibited various symptoms including mild to prominent yellowing of the leaf mid-ribs spreading laterally across the leaf lamina with shortened internodes and leaf-tip necrosis were collected and stored at −80 °C for further detection. DAC-ELISA was performed to detect the association of ScYLV with the YLD of sugarcane samples collected from the experiment (Clark and Bar-Joseph 1984). The assay was performed in 96 well polystyrene microtiter plates (Costar, Sigma, USA). 96 well plates were coated with diseased leaf-midrib extracts diluted 1:4 (w/v) in coating buffer contained 15 mM sodium carbonate, 35 mM sodium bicarbonate, and 2% polyvinylpyrrolidone (PVP-40) with pH 9.6 and incubated at 37 °C for 1 h. After three subsequent washings with PBS-T buffer contained 136 mM NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl, 8 mM Na_2HPO_4, 0.05% Tween-20, with adjusted pH 7.4, these plates were further blocked with 2% bovine serum albumin (BSA) for 1 h at 37 °C. After three repeated washings with PBS-T, specific antiserum against the coat protein (CP) of sugarcane yellow leaf virus (ScYLV) obtained from the AC Diagnostics Ltd., USA diluted (1:1000) with PBS-TPO contained PBS-T with 2% PVP-40 and 0.2% ovalbumin was loaded to the wells of ELISA plate and incubated at 37 °C for 1 h followed by three washing with PBS-T. Goat anti-rabbit IgG-AP conjugate (Sigma-Aldrich, St. Louis, USA at a dilution of 1:30,000 in PBS-TPO) was added and incubated at 37 °C for 1h. Finally, the plates were washed thrice with PBS-T and para-nitrophenyl phosphate (pNPP) substrate (at 0.5 mg/ml pNPP dissolved in 9.7% diethanolamine buffer, pH 9.6) was added. The OD values at 405 nm were measured by ELISA reader (Thermo Scientific, Multiscan) after 1 h of substrate incubation at 37 °C. DAC-ELISA test results were treated as positive if the absorbance value (OD 405) is more than 0.626 i.e., more than two times the OD 405 value of negative control (OD405 = 0.313), whereas, as negative if absorbance value is less than that value.

RESULTS AND DISCUSSION

Reaction of sugarcane genotypes to YLD: The pooled field evaluation revealed considerable variability in the response of different sugarcane genotypes to YLD under natural conditions (Table 1). Out of the tested genotypes, six entries viz. 2015A311, 2017A553, CoA20321, CoA20323, CoA20325, and CoV19359, exhibited disease grade scores ranging from 0.0 to 1.0, categorizing them as resistant (R). These genotypes showed negligible symptom expression with mild or no leaf chlorosis, indicating effective field tolerance against YLD infection. Majority of genotypes (18 entries) including CoA20322, CoC20336, CoC20337, CoA20326, CoC20338, CoOr20346, CoC19336, CoV19357, CoV18356, CoOr18346, CoA92081, CoC01061, CoOr03151, CoV18358, CoV19359, CoV92102, Co86249, and Co06030 recorded mean disease grade scores between >1.0 and 2.0, and were categorized as moderately resistant (MR). These varieties exhibited mild yellowing and slight midrib discoloration, suggesting partial tolerance and restricted disease progression. Genotypes such as CoA19322, CoC20339, CoA19321, CoA19322, CoA20325, and 2001A63 displayed mean scores in the >2.0 to 3.0 range, falling into the moderately susceptible (MS) group. These showed prominent leaf yellowing and loss of turgidity, especially in lower leaves. Only one genotype, 2003V46, recorded a score between >3.0 to 4.0, indicating a susceptible (S) reaction. None of the evaluated genotypes were found to be highly susceptible (HS) under natural field conditions. These observations confirm the presence of a wide spectrum of resistance in the breeding material, offering opportunities for further use in resistance breeding programs. The frequency distribution of resistance classes suggests that 32% of genotypes screened were resistant, 58% moderately resistant, and only 10% moderately susceptible, demonstrating encouraging progress in

Table 1. Reaction of different genotypes of sugarcane to yellow leaf disease under natural conditions (Pooled)

Disease grade	Reaction	Genotypes
0.0 - 1.0	R	2015A311, 2017A 553, CoA20321, CoA 20323, CoA 20325, CoV 19359
>1.0 – 2.0	MR	CoA 20322, CoC 20336, CoC 20337, CoA 20326, CoC 20338, CoOr 20346, CoC 19336, CoV 19357, CoV 18356, CoOr 18346, CoA 92081, CoC 01061, CoOr 03151, CoV 18358, CoV 19359, CoV 92102, Co 86249, Co 06030
>2.0 – 3.0	MS	CoA 19322, CoC 20339, CoA 19321, CoA 19322, CoA 20325, 2001A 63
>3.0 – 4.0	S	2003V 46
>4.0 – 5.0	HS	- -

breeding for YLD tolerance. This trend is in line with national varietal evaluations in India, where more than one-third of advanced clones have displayed moderate to high resistance under natural infection conditions (Viswanathan et al., 2020, Sundar et al., 2020). Field-based screening remains an effective approach to identify genotypes with durable resistance, as disease expression is strongly influenced by natural vector pressure and agro-climatic factors (Singh and Viswanathan, 2019). Similar patterns of genotypic variation for YLD resistance have been reported from South America, Australia, and China, where selection of resistant cultivars such as SP78-4764 and Q124 led to significant reductions in YLD incidence (Vega et al., 2010, Lehrer et al., 2010). Resistance is often associated with restricted virus accumulation in phloem tissues and low transmission efficiency by*M. sacchari*, (Rott et al., 2008, Chinnaraja et al., 2013). Studies have shown that resistant genotypes express slower symptom development, reduced viral RNA replication, and delayed aphid acquisition (Comstock and Irey 2017). Such combined physiological and molecular defense responses contribute to field-level stability of YLD resistance across locations and seasons.

The current evaluation thus confirms the presence of diverse resistance gradients among sugarcane genotypes, providing a strong base for incorporating durable resistance into elite breeding populations. The identified resistant lines particularly CoA20323 and 2017A553 also performed well for growth and sucrose traits, underscoring the feasibility of combining disease resistance with productivity in breeding programs (Muthiah and Rajendran, 2022, Singh et al., 2022). The observed differences in disease expression further highlight the role of genetic background, vector ecology, and local environmental conditions in shaping YLD dynamics, warranting continued multi-environment screening for long-term varietal stability.

Biophysical and biochemical traits of resistant genotypes: Distinct differences were observed among the YLD-resistant genotypes in terms of their leaf morphology, chlorophyll content, phenol concentration, juice sucrose, and silica content (Table 2). All six resistant genotypes exhibited a semi-erect leaf orientation with medium to medium-wide leaf blades, facilitating improved aeration and possibly reduced vector colonization. Leaf colour varied from light green (2015A311, CoA20321) to medium green (2017A553, CoA20323, CoA20325, CoV19359), indicating a healthy chlorophyll balance even under YLD pressure. The SPAD readings ranged from 28 (2015A311) to 31 (CoA20325 and CoV19359), while the susceptible check 2003V46 recorded a higher SPAD value (36) corresponding with its darker foliage. Although chlorophyll content was high in 2003V46, the excessive leaf droopiness (420 inclination angle) suggested structural weakness and physiological stress, typical of YLD-susceptible types. Among the resistant entries, phenol content varied between 1.8 and 2.0%, with CoA20323 and CoA20325 recording the highest levels. Phenolic compounds are known to play a defensive role in plant-pathogen interactions, possibly contributing to reduced YLD incidence. The juice sucrose percentage ranged between 35.67% (CoA20325) and 40.34% (CoA20323), indicating that YLD resistance did not adversely affect sugar accumulation. Silica content, an important physical defense trait, was relatively higher in resistant types (1.64–2.41%) compared to the susceptible check (1.30%), suggesting an additional barrier to vector feeding or pathogen entry.

Serological detection of YLD Infection through DAC-ELISA: DAC-ELISA analysis confirmed the presence of Sugarcane yellow leaf virus (SCYLV) in leaf and aphid samples from the tested genotypes (Table 3). The positive leaf samples of resistant genotypes recorded high absorbance index values ranging from 3.543 (CoV19359) to 3.893 (CoA20323), while their corresponding aphid samples showed moderate values (1.623–2.194). The susceptible genotype 2003V46 displayed the highest ELISA index values both in leaves (3.957) and aphids (2.507), indicating heavy

Table 2. Biophysical and Biochemical parameters of genotypes exhibiting YLD resistance under natural conditions

Genotype	Leaf colour		Leaf droopiness		Leaf width		Phenols (%)	Juice sucrose (%)	Silica (%)
	Colour	SPAD	Visual	Inclination angle	Width	Width (mm)			
2015A311	Light green	28	Semi erect	23^{0}	Medium	1.8	38.43	16.10	2.41
2017A553	Medium green	30	Semi erect	25^{0}	Medium-wide	1.9	39.32	16.21	1.83
CoA20321	Light green	29	Semi erect	22^{0}	Medium	1.9	37.24	16.43	2.30
CoA20323	Medium green	30	Semi erect	26^{0}	Medium-wide	2.0	40.34	17.26	1.91
CoA 20325	Medium green	31	Semi erect	24^{0}	Medium	2.0	35.67	16.62	1.86
CoV19359	Medium green	31	Semi erect	28^{0}	Medium-wide	1.9	33.67	16.43	1.64
2003V46	Dark green	36	Droopy	42^{0}	Wide	2.4	28.32	17.62	1.30

Table 3. DAC-ELISA readings of yellow leaf disease infected sugarcane leaves and aphids

Variety	Index values		Leaf sample	Aphid sample
2015A311	+ ve	3.725	1.756	
	- ve	0.735		
2017A553	+ ve	3.693	2.005	
	- ve	0.895		
CoA20321	+ ve	3.757	1.975	
	- ve	0.632		Not applicable
CoA20323	+ ve	3.893	1.623	
	- ve	0.715		
CoA20325	+ ve	3.679	1.735	
	- ve	0.741		
CoV19359	+ ve	3.543	2.194	
	- ve	0.711		
2003V 46	+ ve	3.957	2.507	2.344
	- ve	0.957		

virus accumulation and efficient aphid-mediated transmission. In contrast, negative controls across all genotypes recorded low absorbance values (<1.0), confirming assay reliability. The absence of detectable virus in aphids from the resistant genotypes suggests reduced vector acquisition efficiency or limited virus replication, corroborating the field-level resistance observations. Elevated levels of phenolics and silica are known to contribute to enhanced structural and biochemical defense against SCYLV infection and vector feeding (Santiago et al., 2016, Muthiah and Rajendran, 2022). The juice sucrose percentage remained high (35–40 %), indicating that resistance did not compromise sugar accumulation, a finding consistent with earlier studies linking YLD tolerance and yield stability (Muthiah and Rajendran, 2022).

Correlation between aphid incidence, yellow leaf disease, and weather parameters: Correlation analysis revealed significant interactions between aphid population dynamics, YLD incidence, and prevailing weather factors. Aphid populations exhibited positive correlations with maximum temperature , minimum temperature, and relative humidity I , while a negative correlation was observed with rainfall. Similarly, YLD severity showed positive relationships with minimum temperature, relative humidity II, and maximum temperature, and a negative correlation with rainfall . These findings indicate that warm and humid conditions with low rainfall are conducive to aphid multiplication and YLD spread. Periods of sustained dryness with moderate temperatures likely enhanced vector activity, promoting virus dissemination. Consequently, resistant genotypes maintained stable performance across weather fluctuations. These trends aligning with earlier observations from Brazil and India that identified temperature–humidity interactions as key drivers of YLD epidemics (Vega et al., 2010, Singh and Rao 2021). The stable performance of resistant genotypes across such conditions underscores their resilience and potential suitability for YLD-prone agro-climates. Similar integrative findings have also been reported earlier by Viswanathan et al. (2020) and Singh and Rao (2021), supporting the relevance of these resistant genotypes for deployment in integrated disease management programs.

CONCLUSION

The study demonstrated variability in YLD response among sugarcane genotypes, with a promising proportion exhibiting resistant to moderately resistant reactions under natural field conditions. The resistant lines also displayed favorable biochemical and biophysical traits such as higher phenol and silica content, balanced chlorophyll levels, and stable sucrose accumulation supporting their inherent defensive capacity. ELISA results further confirmed restricted virus accumulation and lower vector acquisition in resistant types. Weather–disease correlations highlighted the role of warm, humid, low-rainfall conditions in accelerating aphid populations and YLD spread, emphasizing the value of climate-resilient resistance. Overall, the genotypes 2015A311, 2017A553, and CoA20323 provide a strong foundation for breeding programs for developing YLD-tolerant, high-yielding cultivars suitable for diverse agro-climatic regions.

AUTHOR'S CONTRIBUTION

R. Saritha conceptualized the study, conducted the

experiments, analyzed the data, and prepared as well as revised the original draft. V. Chandrasekhar helped in experimentation, validation, and data analysis. D. Adilakshmi supplied the genotypes used in the study, while B. Bhavani contributed to the conceptualization. The overall supervision of the work was provided by M. Visalakshi.

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