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Research Paper

Major plant characteristics associated with resistant sugarcane hybrids to *Deltocephalus menoni*, the vector of Sugarcane White Leaf Disease

K.M.G. Chanchala[1]*, V.K.A.S.M. Wanasinghe[1], L. Nugaliyadde[2], W.R.G. Witharama[1], K.S. Hemachandra[3]

1 Sugarcane Research Institute, Uda Walawe, Sri Lanka
2 Sri Lanka Institute of Agriculture, Peradeniya, Sri Lanka.
3 Faculty of Agriculture, University of Peradeniya, Sri Lanka
* Corresponding Author: gayanikoonara@gmail.com https://orcid.org/0000-0002-8433-9372

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Abstract: Sugarcane is affected by a wide range of insects causing more than ten percent yield loss. *Deltocephalus menoni* (Hemiptera: Cicadellidae, Subfamily: Deltocephalinae) is one of the sap-sucking insects serves as the vector of Sugarcane White Leaf Disease (WLD) in Sri Lanka. WLD is a major threat to sugarcane cultivation resulting severe reduction of cane yield, juice volume, and pure obtainable cane sugar (POCS) while increasing fiber in canes. Effective management of WLD vector is important to limit the spread. This study aims to develop an approach to screen sugarcane varieties for resistance against *D. menoni* to manage WLD in sugarcane. Principal Component Analysis (PCA) was used to identify key vector-related traits associated with varietal resistance. Aggregation, amount of feeding and adult longevity were identified as indicators for assessing resistance. Plant morphological traits such as number of leaves, leaf length, and distance between leaves contributed to resistance differentiation among varieties. In addition, PCA of leaf colour, epicuticle wax, arrangement of trichomes, stomata, leaf nutrition, moisture and chlorophyll content also can be used to confirm the resistance status. Resistant sugarcane varieties were characterized by short leaves, lower leaf angle, and thin leaves with higher distance between leaves, leaves with higher red and green colour composition, a higher level of epicuticle wax, shorter trichomes, and thin stomata, a smaller number of thin vascular bundles, thick phloem fiber layer, and a higher distance from the epidermis to vascular bundle. By leveraging these traits, breeders can develop sugarcane varieties with enhanced resistance to *D. menoni* and WLD, contributing to sustainable pest and disease management in sugarcane cultivation.

Keywords: *Deltocephalus menoni*, Plant characteristics, Resistant, Sugarcane White Leaf Disease, Vector.

Introduction

Resistant cultivars are used as one of the pest control approaches in Integrated Pest Management (IPM) Programs. It is generally compatible with other methods of insect control (Khan and Saxena, 1998) and it a more practical approach to leafhopper management in sugarcane. Use of resistant varieties is a more practical approach to the management of *D. menoni* within the overall approach of White Leaf Disease (WLD) management. Developing plants that are either resistant to the phytoplasma or deter vector feeding is the first line of defense in WLD management.

Plant breeding is mostly directed towards developing varieties resistant to disease rather than to the vectors but, several attempts in breeding for vector resistance have also gained success (Martin, 1983; Heinrichs, 1986).

According to Agarwal (1969) and Sanghera and Kumar (2018) making clones resistant to insects is a continuous and time consuming process; however, it may be worth considering this approach in a heterozygous polyploid plant like sugarcane. Breeding of resistance to insects in sugarcane is comparatively difficult due to its complex genome and the inheritance of polygenic traits (White *et al*., 2010). Some of this resistance is based on morphological characteristics (Dicke, 1998). Pest populations are influenced negatively or positively by physiomorphic leaf characteristics (Krips *et al*., 1999) such as, leaf area, leaf hair density, length of leaf and leaf thickness. These characteristics influence the searching capability of the insect (Croft *et al*., 1999; Cedola *et al.,* 2001). As an example, leaf colour is one of the major factors that affects the approach and arrest during the process of insect colonization (Painter, 1951). The colour of leaf lamina also affects the level of host defense, herbivore abundance, and the performance of insects *viz*., larval abundance, growth, performance, survival, pupal mass, adult feeding and fecundity (Courtney and Kibot, 1990; Honek, 1993; Benrey *et al*., 1997; Gripenberg *et al*., 2010). Epicuticular waxes (EW) are generally play an important role in protecting aerial parts against the damages caused by biotic and abiotic stresses (Wójcicka, 2013; Yin *et al.,* 2011; Zhang *et al.*, 2007). Trichome of plant is important component to resist against herbivorous insects (Levin, 1973; Southwood, 1986; Karban and Baldwin, 1997; Traw and Dawson, 2002). They act as physical and chemical deterrents to insect activities (Esau, 1977). Trichomes interfere with insect colonisation, entrapment, physiological incompatibility and/or lethality (Duffey, 1986). Trichome density also affects the interaction between insect herbivores and natural enemies. Further, it affects tolerance to abiotic stress.

Since the sugars, amino acids, and other organic metabolites are translocated *via* phloem and xylem, the vascular system has become a target for sap-feeding insects (Brodbeck *et al.,* 1993; Gündüz and Douglas, 2009), especially for phytophagous hemipterans. Therefore, the configuration of the fibro-vascular bundle of plants plays an essential role in the interactions between plants and other organisms, both directly and obliquely.

Nutritional quality of plant tissue affects host-plant selection by phytophagous insects (Bernays and Chapman, 1994). Nutrients include primary macronutrients (nitrogen, phosphorus, and potassium) and secondary macronutrients (calcium, magnesium, and sulphur). Relative availability of various nutrients in the diet affects the growth and fitness of herbivores (Boswell *et al.,* 2008). Leaf chlorophyll and moisture percentage also highly affect the insect performance and their preference for the host plant (Murugesan and Kavitha, 2010).

In Sri Lanka, screening of the sugarcane germplasm for WLD resistance is continuing using a conventional screening method which is a lengthy process. Little work has been done on the resistance or tolerance of sugarcane crop for the WLD vector, *D. menoni;* therefore, this study was conducted with the objective of identifying the level of WLD infection due to secondary transmission by vector in relation to the plant morphological, anatomical and nutritional characteristics, such as leaf lamina colour, level of leaf lamina epicuticle wax, leaf trichome, stomata, configuration of leaf fibro-vascular bundle, leaf nutrition, moisture and chlorophyll content on sugarcane hybrids and wild accessions (*Saccharum spontaneum L.* and *Erianthus arundinaceus*).

Materials and Methods

Study Location and Design

This study was conducted at the Entomology Laboratory of the Sugarcane Research Institute (SRI), Uda Walawe, Sri Lanka. Available information on WLD intensity in the natural environment was considered to select the test varieties/accessions for the study. Sugarcane varieties Co 775, SL 83 06, SL 92 5588, SL 96 128, SL 96 328, SL 97 1442, SLT 4921, SLC 2009 01, and the wild accessions SLC 92 95 (*Saccharum spontaneum L*.) and SLC 92 77 (*Erianthus arundinaceus*) were used. In this study, levels of WLD infection of selected varieties/accessions by *D. menoni* under natural conditions and physio-morphic characteristic of the sugarcane plants were measured to determine the major plant characteristics which influence the level of WLD infection by the vector.

Determining level of WLD infection of selected varieties/accessions by *D. menoni* under natural conditions

Sugarcane plants, obtained from a mother-plant nursery established with hot water treated seed cane (hot-water treatment at 54 °C for 50 min), were used in the experiment. A field infested with *D. menoni* was selected for the trial establishment. Three-budded setts from each variety were obtained and three 5 m length rows from each

sugarcane accession were established in a randomized complete block design (RCBD) with three replicates. The trial was maintained under the recommended agronomic practices (SRI, 2004) and availability of *D. menoni* in the trial was confirmed by monitoring population throughout plant crop stage using a sweep net. After ratooning, WLD-infected clumps were visually detected by considering the appearance of the test plants *viz.,* diffused, proliferated tillering, chlorotic leaves, yellowing of fully-developed leaves of the mature canes and soft texture of symptomatic leaves (Chandrasena *et al.,* 2003; Senevirathne, 2008). The incidence of WLD infection by *D. menoni* was calculated as follows;

$$\text{WLD infection (\%)} = \frac{\text{Number of WLD symptomatic clumps}}{\text{Total number of clumps}} \text{ x } 100$$

Determining the variation of plant characteristics of sugarcane varieties/accessions that have variable resistance to WLD

The TVD from the top of each plant at four-month age was used to measure leaf characteristics and 30 replicates were maintained for each character.

a. physiomorphic plant characteristics

Leaf length (cm): The leaf length was measured from the junction between the leaf blade and the leaf sheath to the tip of the leaf blade using a measuring tape and expressed in centimeters.

Laminar thickness (µm): A thin cross-section from the middle portion of the leaf was made and placed on a clean glass slide. Width was measured from the upper epidermis to the lower epidermis using Olympus biological microscope (model Cx31) under 100xmagnification.

Leaf angle: The angle between the leaf blade and the stem was measured using a protractor.

Distance between leaves (cm): the distances between two adjacent leaves starting from the top leaf to next five leaves were measured and average was calculated.

Spine density: The number of spines per 1cm^2 on the leaf sheath (just below the junction between leaf blade and leaf sheath) was counted using a hand lens.

Internodal length: The internodal length of the cane was measured from the bottom to top of the cane using a measuring tape and expressed in centimeters.

b. leaf lamina colour

The TVD from the top of each plant was used for measuring leaf color. Leaf images were captured from the adaxial leaf surface in middle region of leaves. The images were taken using a digital camera (Nikon 100, Tokyo, Japan) according to the Chanchala *et al,* (2020). Both RGB (Red-Green-Blue) and HSB (Hue-Saturation-Brightness) values were measured to describe leaf color. Values of RGB channels were read using Adobe Photoshop CS 6 software according to the Hasanuzzaman *et al,* (2016). The ratios of R: B, B:G, B:R, r = R :(R+G+B), g = G :(R+G+B) and b= B :(R+G+B) were also recorded for the analysis.

c. leaf lamina wax

Twenty-five plants from each variety were kept in an insect-proof screen house and maintained under the recommended agronomic practices (SRI, 2004). At the age of four months, 10-20 third leaves of the plants were collected and the middle portions of the leaves were taken for wax extraction. Extraction was done according to Chanchala *et al.* (2020).

d. leaf trichome and stomata densities

Measuring density of trichome on unit area of sugarcane leaf (cm2)

At four-month of age the TVD of the plants (30) were collected and the middle portion of the leaf was taken for the study. A thin film of a white adhesive was applied onto the lower side of the leaf blade (3cm sections from the middle of the leaf was used) and the bottom was kept touching water to avoid the desiccation and kept until the glue layer gets dried. Then properly dried glue film was removed from the leaf piece and placed on a clean slide for trichome and stomata observations. Trichome density, length and stomata density and width (15 mm^2 area) and were observed under Olympus biological microscope: model Cx31 under 10x magnifications.

e. configuration of leaf fibro-vascular bundle

The TVD leaves of four-month-old plants were selected for the histological assessment. Tissue samples were collected and processed to obtain measurements *viz.,* thickness of the abaxial epidermis, and distance from abaxial epidermis to vascular bundle, thickness of the phloem fibre

layer, area of the phloem, width of the vascular bundle, and number of vascular bundles available within 1.5 mm width of the leaf lamina according to Chanchala *et al,* (2021). These measurements were taken for all three types of vascular bundles, namely large, medium and small in the leaf laminar.

f. leaf nutrition

TVD of the four-month-old sugarcane plants of the test varieties were used for the study. Leaf samples were digested using wet digestion method with H_2O_2 / (Se) H_2SO_4 -1N HCl. Leaf N, P, and K were analyzed using UV spectrophotometer and Ca and Mg by Atomic absorption spectrophotometer (AAS 6300), respectively.

g. moisture content

The TVD of each selected sugarcane varieties/ accessions was collected and 15 cm-long tissue samples were excised from the point midway along the length of the blade. Ten grams (W_1) of fresh leaves were taken and kept in a drying oven at 65 ^oC, for 72 hours. After drying, samples were weighed and placed back in the oven at the same temperature for another six hours. Leaves were taken out from the oven and kept in a desiccator for 10 minutes and weighed (W_2). When a constant weight was achieved, the moisture content (%) were calculated, according to the following formulae: $(W_1 – W_2)/ W_1 \times 100$.

h. chlorophyll content (spad reading)

Chlorophyll content was measured using a SPAD chlorophyll meter (SPAD 502 Plus) in the TVD of the four-month-old sugarcane plants of the selected varieties. Chlorophyll content of the middle portion of the leaf blade (2 x 3 mm2 area) was measured and to minimize the effect of sunlight to the measurements, the chlorophyll meter was shaded with the body. SPAD readings were recorded for each variety/ accession.

Data analysis

All data collected on WLD incidence, insect performance parameters, physicomorphic leaf characteristics, nutritional parameters, moisture content, chlorophyll content, and other measured variables were subjected to analysis of variance (ANOVA) using SAS software version 9.0. Prior to analysis, count and percentage data were square-root transformed where necessary to satisfy the assumptions of normality and homogeneity of variance. Statistical analyses were performed using the transformed data, while means presented in tables and figures are reported in their original scale for ease of interpretation. Means were compared using Duncan's Multiple Range Test (DMRT) at the 0.05 probability level.

Principal Component Analysis (PCA) was conducted using SAS software to identify plant traits associated with WLD infection and vector performance. Data were standardized before analysis, and components with eigenvalues >1.0 were retained for interpretation. Variable loadings were used to determine the contribution of individual traits to the observed variation among varieties/accessions**.**

Results and Discussion

Level of WLD infection of selected varieties/accessions by *D. menoni* under natural conditions

The rate of WLD infection was significantly higher in variety/accession SL 97 1442, followed by SL 96 128 and CO 775, while WLD infection was not observed in variety SLC 92 77 (Table 1) under the moderate pressure of *D. menoni* and WLD inoculum in the surrounding area.

The variation of plant characteristics of sugarcane varieties/accessions that have variable resistance to WLD

a. physico-morphic plant characteristics of sugarcane varieties/accessions

Variations of the physico-morphic plant characteristics among sugarcane varieties are presented in Table 1. The highest leaf angles were recorded on variety SLC 2009 01 (9.99 ± 0.37º) and Co 775 (9.00 ± 0.01º), while, the lowest leaf angles were recorded on accessions SLC 92 55 (5.25 ± 0.13º) and SLC 92 77 (5.10 ± 0.41º).

The highest distance between leaves was recorded in accession SLC 92 77 (9.01 ± 0.72cm) and the lowest distance between leaves was recorded in variety SL 92 5588 (3.47 ± 0.00cm). Varieties SL 8306, SL 92 5588, and SLT 4921 associated with decreased leaf angles with increased distance between two leaves caused leaves in the canopy having an erect and spacious structure.

The highest spine densities were recorded on variety SL 97 1442 (81.89 ± 0.12) and accession SLC 92 77 (81.38 ± 0.25). The lowest spine density was recorded on variety SL 83 06 (0.3 ± 0.31cm) and no spines was found on SL 92 5588.

Varieties with the highest internodal lengths were SL 96 128 (9.5 ± 0.43cm) and SL 97 1442 (9.125 ± 0.18cm). The lowest internodal length was recorded on accession SLC 92 95 (7.43 ± 1.29cm). Accession SLC 92 77 had the highest rind hardness (33.051 ±

0.00cm) and variety SLC 2009 01 was with the lowest rind hardness (10.103 ± 0.007cm).

Table 1. Variation of physico-morphic plant characteristics under natural environment

Variety	Level of disease infection (%)	Leaf Characteristics				Plant Characteristics		
		Leaf length (cm)	Number of leaves (#)	Leaf Angle (º)	Distance Between Leaves (cm)	Spine Density (#/ unit area)	Internodal Length (cm)	Rind Hardness
Co775	4.80 ± 0.09 ^{a}	120.80 ± 0.12^{bc}	08.64 ± 0.05^{b}	9.00 ± 0.10^{b}	4.30 ± 0.38 ^{cd}	18.46 ± 0.24^{c}	7.84 ± 0.54 ^{abc}	21.81 ± 0.27^{de}
SL8306	0.78 ± 1.73^{d}	087.82 ± 0.02 ^{e}	09.78 ± 0.04^{e}	7.38 ± 0.54^{cd}	7.18 ± 0.10 ^{b}	00.30 ± 0.31^{g}	8.15 ± 0.49 ^{abc}	22.61 ± 0.78 ^{de}
SL925588	0.35 ± 1.73^{de}	113.93 ± 0.29^{d}	08.07 ± 0.08^{h}	7.14 ± 0.19^{de}	3.47 ± 0.00 ^{d}	00.00 ± 0.23^{g}	7.74 ± 0.66 ^{abc}	21.24 ± 0.35 ^{e}
SL96128	4.86 ± 0.09 ^{a}	125.41 ± 0.18^{b}	09.47 ± 0.14^{ef}	8.28 ± 0.14^{bc}	4.91 ± 0.189 ^{c}	04.72 ± 1.24^{f}	9.50 ± 0.43^{a}	21.43 ± 0.28^{de}
SL96 328	2.42 ± 0.03^{c}	118.41 ± 0.12^{cd}	09.80 ± 0.42^{e}	7.05 ± 0.35^{d}	4.50 ± 0.68 ^{cd}	11.35 ± 0.16^{d}	8.94 ± 1.28^{ab}	23.75 ± 0.05^{d}
SL971442	5.26 ± 0.2 ^{a}	126.50 ± 0.07^{b}	09.07 ± 0.52^{fg}	8.29 ± 0.20^{bc}	5.11 ± 0.30^{c}	81.89 ± 0.12^{a}	9.123 ± 0.1 ^{ab}	23.30 ± 0.66 ^{de}
SLT4921	3.88 ± 0.31^{ab}	133.79 ± 0.00^{a}	08.00 ± 0.23^{h}	6.25 ± 0.40^{e}	5.06 ± 0.33^{c}	23.77 ± 0.45^{b}	8.36 ± 0.75 ^{abc}	22.92 ± 0.93^{de}
SLC 2009 01	0.93 ± 1.73^{d}	117.29 ± 0.13^{cd}	10.40 ± 0.42^{b}	9.99 ± 0.37^{a}	4.21 ± 0.32^{cd}	06.35 ± 0.25^{e}	7.87 ± 0.60 ^{abc}	19.10 ± 0.70^{f}
SLC 92 95	1.02 ± 1.01^{cd}	112.60 ± 0.08^{d}	13.96 ± 0.05^{a}	5.25 ± 0.13^{f}	7.51 ± 0.02^{b}	00.49 ± 0.06^{g}	7.44 ± 1.29^{bc}	28.22 ± 0.60^{c}
SLC 92 77	0.00^{e}	135.95 ± 0.17^{a}	11.08 ± 0.13^{b}	5.10 ± 0.41 ^{f}	9.01 ± 0.72^{a}	81.38 ± 0.25^{a}	8.23 ± 2.16^{abc}	33.05 ± 0.00^{b}

Note: Within a column means followed by the same letters are not significantly different at 5% probability level

b. leaf lamina colour

Leaf lamina colour parameters red (R), green (G), blue (B), hue (H), saturation (S), and brightness (B) showed significant variation among the tested varieties/accessions (Table 2). Based on mean separation, the varieties/accessions were grouped into distinct statistical classes. For the red (R) colour component, SLC 92 77, SLC 92 59, and SL 83 06 were grouped together and exhibited significantly higher values than the other varieties/accessions. For the green (G) colour component, SLC 92 77 and SLC 92 59 were grouped together and recorded significantly higher values compared with the remaining varieties/accessions.

c. leaf lamina wax

The amount of wax extracted from leaf lamina varied significantly ($F_{11, 18}= 7.95$, $p<0.05$) among the tested sugarcane varieties. The higher level of the leaf lamina epicuticular wax per unit area of leaves was extracted from the varieties/ accessions SL 83 06 and SL 92 5588. In contrast, low levels of leaf lamina epicuticular wax on unit area of sugarcane leaves were observed in the SL 96 128 and Co 775.

d. leaf trichome and stomata

Trichome density ($F_{29, 60}= 2.98$, $p<0.05$) and the length ($F_{29, 60}= 3.82$, $p<0.05$) of trichomes were significantly varied with the sugarcane varieties on both the leaf blade ($F_{29, 60}= 2.90$, $p<0.05$) and the midrib ($F_{29, 60}= 3.61.98$, $p<0.05$). On leaf blade, the highest number of trichomes were recorded on accession SL 92 95 (160.52/15mm^2) and the lowest number of trichomes were recorded on accession SL 92 77 (7.67/15mm^2). The highest and the lowest mean trichome length were recorded on variety SL 96 128 (136.31 µm) and accession SL 92 77 (24.44 µm) respectively.

The number of stomata on the midrib, width of the stomata on midrib, and leaf were significantly varied in sugarcane varieties/ accessions. Number of stomata on leaf in sugarcane varieties/ accessions was not significantly different. Accession SL 92 77 had the highest (344) mean number of stomata and variety Co 775 was having highest mean width of stomata (174.15) on leaf blade. Variety Co 775 had the highest mean number of stomata (498.56) and variety SLT 4921 had the highest mean width (144.61) of stomata on midrib. The lowest mean width of stomata (30.42) on the leaf was recorded on variety SL 8306.

Table 2. Colour measurement of leaf laminar of TVD of the sugarcane varieties/accession that has variable resistance to WLD

Variety	R	G	B	H^{o}	S (%)	B (%)
SL 83 06	75.33 ± 3.18^{a}	94.50 ± 2.33^{ab}	31.00 ± 2.33^{cde}	75.50 ± 2.33^{b}	0.61 ± 0.04^{ab}	0.37 ± 0.01^{ab}
SL 97 1442	58.00 ± 1.00^{bcd}	75.66 ± 0.88^{de}	23.66d ± 0.88^{e}	80.66 ± 0.88^{ab}	0.69 ± 0.01^{a}	0.29 ± 0.00^{bc}
SL 96 128	53.00 ± 1.15^{cde}	71.00 ± 1.00^{de}	23.00 ± 1.73^{e}	81.33 ± 2.03^{ab}	0.71 ± 0.08^{a}	0.30 ± 0.01^{abc}
SL 92 5588	60.66 ± 4.10^{b}	87.00 ± 0.58 ^{bc}	25.66 ± 0.88^{df}	82.33 ± 1.20^{ab}	0.68 ± 0.01^{ab}	0.32 ± 0.02^{abc}
SL 92 95	80.00 ± 4.61^{a}	97.66 ± 4.37^{a}	55.33 ± 5.69^{a}	85.33 ± 3.38^{a}	0.44 ± 0.04^{c}	0.38 ± 0.07^{a}
SL 96 328	53.00 ± 2.52^{cde}	72.00 ± 1.00^{be}	47.33 ± 3.18^{ab}	86.66 ± 1.67^{a}	0.48 ± 0.09^{bc}	0.35 ± 0.04^{ab}
SLC 2009 01	47.00 ± 1.15^{e}	69.00 ± 1.15^{e}	23.00 ± 0.58^{e}	84.66 ± 2.19^{a}	0.72 ± 0.03^{a}	0.26 ± 0.00^{c}
SLT 4921	77.66 ± 2.33^{b}	99.00 ± 6.03^{a}	37.66 ± 5.46^{bcd}	81.00 ± 2.00^{ab}	0.62 ± 0.05^{abc}	0.39 ± 0.03^{a}
SLC 92 95	64.66 ± 3.48^{b}	80.33 ± 6.69^{cd}	40.66 ± 7.13^{bc}	81.00 ± 1.00^{ab}	0.62 ± 0.02^{ac}	0.38 ± 0.03^{a}
Co 775	51.00 ± 0.58^{de}	69.00 ± 2.89^{e}	21.00 ± 0.58^{e}	82.33 ± 1.15^{ab}	0.53 ± 3.18^{abc}	0.33 ± 0.10^{abc}

Note: In columns, means with the same letters are not significantly different at 5% probability level. Words noted by letters are, red (R), green (G), blue (B), hue (H^{o}), saturation (S) and brightness (B)

e. configuration of leaf fibro-vascular bundle

In each sugarcane accession, three types of vascular bundles were observed *viz.*, large (a), medium (b) and small (c). All the tested varieties were associated with one large vascular bundle per unit area. The highest epidermal width was recorded in SLC 92 77 (11.85 ± 0.35 µm) and variety SL 8306 (11.68 ± 0.01 µm). Width of epidermis was lower in variety SLC 2009 01 (06.94 ± 0.01 µm) and accession SLC 92 95 (06.95 ± 0.08 µm). The highest thickness of the leaf blades was recorded in variety SL 96 128 (328.63 ± 0.74 µm) and SL 96 328 (331.00 ± 1.24 µm). The lowest thickness of the leaf blades was recorded on variety SLT 4921 (110.85 ± 0.15 µm).

Distance from epidermis to vascular parenchyma and epidermis to vascular bundle, thickness of phloem fiber layer, phloem area and width of vascular bundle on the TVD of test sugarcane variety/ accession were varied with the test varieties (Table 12 and 5.13). The highest distance from epidermis to the vascular parenchyma in leaf-large vascular bundle was recorded in variety SL 92 5588 (40.98 ± 0.48 µm) and accession SLC 92 95 (40.26 ± 0.17 µm). The highest distance from epidermis to vascular bundle in leaf-large vascular bundle was found in variety SL 8306 (112.49 ± 0.29 µm). The lowest distance from epidermis to vascular bundle in leaf-large vascular bundle was recorded in variety SL 96 128 (57.63 ± 0.10 µm) and SLC 2009 01 (57.05 ± 0.03 µm). Thickness of phloem fiber layer in leaf-large vascular bundle was high in SL 8306 (40.68 ± 0.29 µm) and SL 92 5588 (38.25 ± 0.17 µm). It was low in variety SLC 2009 01 (11.88 ± 0.01 µm). Phloem area in leaf-large vascular bundle was high in variety Co 775 (2658.18 ± 19.44 µm) and it was lower in variety SL 8306 (1264.72 ± 2.62 µm).

Table 3. Variation of distance from epidermis to vascular parenchyma (µm), distance from epidermis to vascular bundle (µm), thickness of phloem fiber layer (µm), area of phloem (µm^2) and width of vascular bundle (µm)on TVD of test sugarcane variety/accession

Variety	Distance from epidermis to vascular parenchyma (µm)	Distance from epidermis to vascular bundle (µm)	Thickness of phloem fiber layer (µm)	Area of phloem (µm^2)	Width of vascular bundle (µm)
Leaf-Large vascular bundles					
Co 775	25.88 ± 0.19^{d}	060.65 ± 0.67^{e}	14.09 ± 0.02^{e}	2658.18 ± 19.44^{a}	227.74 ± 1.16^{c}
SL 83 06	30.67 ± 0.27^{c}	112.49 ± 0.29^{a}	40.68 ± 0.29^{a}	1264.72 ± 02.62^{f}	271.62 ± 0.31^{b}
SL 92 5588	40.98 ± 0.48^{a}	079.86 ± 0.44^{b}	38.25 ± 0.17^{a}	1976.42 ± 03.21^{c}	218.67 ± 5.10^{d}
SL 96 128	17.83 ± 0.02^{d}	057.63 ± 0.10^{f}	12.83 ± 0.04^{e}	2056.11 ± 02.86^{b}	350.09 ± 0.63^{a}
SL 96 328	38.11 ± 0.15^{ab}	067.46 ± 0.26^{c}	13.30 ± 0.25^{e}	2059.14 ± 27.92^{b}	236.22 ± 2.40^{c}
SL 97 1442	14.35 ± 0.09^{d}	062.91 ± 0.01^{d}	13.38 ± 0.01^{e}	1296.96 ± 01.98^{f}	184.26 ± 0.37^{f}
SLT 4921	31.60 ± 0.25^{c}	063.00 ± 0.06^{d}	26.51 ± 0.29^{c}	1403.47 ± 02.62^{d}	169.55 ± 0.04^{f}
SLC 2009 01	22.64 ± 0.23^{d}	057.05 ± 0.03^{f}	11.88 ± 0.01^{e}	2088.01 ± 00.05^{b}	181.47 ± 0.29^{g}
SLC 92 95	40.26 ± 0.17^{a}	083.58 ± 0.49^{b}	19.38 ± 0.23^{d}	1289.67 ± 00.42^{e}	170.89 ± 0.06^{g}
SLC 92 77	26.66 ± 0.18^{d}	066.91 ± 0.04^{c}	31.34 ± 0.08^{b}	1563.15 ± 00.58^{d}	206.00 ± 0.57^{e}
Leaf-Medium vascular bundles					
Co 775	21.71 ± 0.15^{e}	60.11 ± 0.06^{d}	12.37 ± 0.02^{e}	1095.40 ± 00.31^{d}	099.35 ± 0.28^{f}
SL 83 06	30.66 ± 0.17^{b}	90.23 ± 0.39^{a}	30.25 ± 0.15^{b}	1020.76 ± 00.63^{d}	142.47 ± 9.74^{d}
SL 92 5588	42.42 ± 0.27^{a}	66.15 ± 0.05^{b}	37.57 ± 0.05^{a}	1269.25 ± 76.24^{b}	170.53 ± 0.36^{b}
SL 96 128	25.54 ± 8.44^{d}	62.97 ± 1.60^{c}	20.56 ± 8.50^{d}	1421.74 ± 01.19^{a}	183.81 ± 6.65^{a}
SL 96 328	31.41 ± 0.22^{b}	66.73 ± 0.38^{b}	11.52 ± 0.01^{e}	1238.58 ± 02.26^{b}	180.72 ± 0.65^{a}
SL 97 1442	13.17 ± 0.03^{f}	31.36 ± 0.03^{f}	19.66 ± 0.04^{d}	0916.13 ± 00.03^{f}	162.41 ± 0.30^{b}
SLT 4921	29.54 ± 0.04^{c}	64.94 ± 0.03^{c}	27.54 ± 0.01^{c}	0977.41 ± 00.89^{e}	150.77 ± 0.67^{b}
SLC 2009 01	21.77 ± 0.09^{e}	56.07 ± 0.07^{e}	10.07 ± 0.03^{e}	1206.42 ± 00.30^{c}	157.80 ± 0.15^{c}
SLC 92 95	32.96 ± 0.14^{b}	68.63 ± 0.07^{b}	13.82 ± 0.18^{e}	0564.56 ± 01.77^{g}	144.70 ± 0.25^{d}
SLC 92 77	20.30 ± 0.01^{e}	67.80 ± 0.20^{b}	37.67 ± 0.09^{a}	0902.36 ± 00.33^{f}	131.92 ± 0.04^{e}

Leaf-Small vascular bundles					
Co 775	19.79 ± 0.01^{e}	25.99 ± 0.03^{e}	07.85 ± 0.04^{d}	976.74 ± 01.12^{a}	099.63 ± 0.02^{a}
SL 83 06	33.50 ± 0.09^{b}	52.49 ± 0.07^{b}	27.57 ± 0.16^{a}	840.00 ± 00.01^{b}	090.54 ± 0.03^{b}
SL 92 5588	40.11 ± 0.03^{a}	49.05 ± 0.01^{b}	22.63 ± 0.02^{b}	451.13 ± 00.07^{e}	089.78 ± 0.01^{b}
SL 96 128	24.67 ± 7.71^{d}	50.43 ± 0.70^{b}	15.63 ± 3.50^{c}	534.37 ± 41.62^{d}	083.89 ± 2.94^{c}
SL 96 328	28.54 ± 0.03^{c}	63.14 ± 0.09^{a}	04.94 ± 0.02^{e}	916.49 ± 00.01^{a}	097.45 ± 0.03^{a}
SL 97 1442	11.22 ± 0.01^{g}	31.37 ± 0.02^{d}	15.87 ± 0.09^{c}	055.37 ± 00.10^{g}	075.45 ± 0.04^{d}
SLT 4921	29.52 ± 0.02^{c}	54.46 ± 0.02^{b}	26.84 ± 0.01^{a}	810.98 ± 00.52^{b}	083.7 ± 0.006^{c}
SLC 2009 01	17.73 ± 0.01^{f}	49.35 ± 0.18^{b}	08.08 ± 0.01^{d}	469.11 ± 00.06^{e}	105.33 ± 0.03^{a}
SLC 92 95	32.80 ± 0.01^{b}	48.06 ± 0.03^{c}	08.99 ± 0.01^{d}	390.69 ± 00.15^{f}	057.09 ± 0.01^{e}
SLC 92 77	28.90 ± 0.01^{c}	61.49 ± 0.01^{a}	29.22 ± 0.00^{a}	666.35 ± 00.32^{c}	087.76 ± 0.00^{c}

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

Table 4. Variation of number of available vascular bundles, width of epidermis and thickness of leaf blade on TVD of test sugarcane variety/accession

Variety	Number of available vascular bundles (#)			Width of Epidermis (µm)	Thickness of Leaf blade (µm)	Distance from epidermis to vascular parenchyma (µm)	Distance from epidermis to vascular bundle (µm)	Thickness of phloem fiber layer (µm)	Area of phloem /A (µm^2)	Width of vascular bundle (µm)
	L	M	S							
Co 775	1 ± 0^{a}	2 ± 0^{c}	6 ± 0^{c}	7.62 ± 0.00^{e}	293.56 ± 0.2^{b}	35.64 ± 0.0^{e}	82.43 ± 0.0^{a}	14.05 ± 0.03^{e}	0162.50 ± 0.24^{e}	155.69 ± 0.10^{a}
SL 83 06	1 ± 0 ^{a}	3 ± 0^{b}	5 ± 0^{d}	11.68 ± 0.0^{a}	207.40 ± 5.9^{d}	50.25 ± 0.0^{a}	74.22 ± 0.1^{b}	28.95 ± 0.30^{a}	1033.16 ± 0.09^{b}	082.23 ± 0.07^{f}
SL 92 5588	1 ± 0 ^{a}	2 ± 0^{c}	4 ± 0^{e}	10.80 ± 0.0^{b}	223.16 ± 0.1^{c}	44.01 ± 0.0^{c}	75.93 ± 0.0^{b}	22.47 ± 0.01^{c}	0843.66 ± 0.23^{c}	089.62 ± 0.12^{e}
SL 96 128	1 ± 0 ^{a}	2 ± 0^{c}	7 ± 0^{b}	08.66 ± 0.3^{d}	328.63 ± 0.7^{a}	29.48 ± 7.3^{g}	66.03 ± 4.9^{c}	15.17 ± 3.65^{e}	1657.70 ± 1.28^{a}	139.18 ± 0.09^{b}
SL 96 328	1 ± 0 ^{a}	4 ± 0^{a}	4 ± 0^{e}	09.76 ± 0.0^{c}	331.00 ± 1.2^{a}	47.3 ± 0.03^{b}	84.56 ± 0.0^{a}	18.06 ± 0.06^{d}	1652.44 ± 0.27^{a}	140.2 ± 0.008^{b}
SL 97 1442	1 ± 0 ^{a}	4 ± 0^{a}	5 ± 2^{d}	08.38 ± 0.0^{d}	219.70 ± 1.2^{c}	20.76 ± 0.13^{i}	47.47 ± 0.2^{f}	20.59 ± 0.13^{c}	1633.09 ± 0.12^{a}	155.87 ± 0.01^{a}
SLT 4921	1 ± 0 ^{a}	2 ± 0^{c}	5 ± 0^{d}	07.49 ± 0.0^{e}	110.85 ± 0.1^{f}	26.82 ± 0.0^{h}	63.34 ± 0.0^{d}	25.04 ± 0.02^{b}	1007.97 ± 0.02^{b}	109.56 ± 0.30^{c}
SLC 2009 01	1 ± 0 ^{a}	3 ± 0^{b}	4 ± 0^{e}	06.94 ± 0.01^{f}	211.58 ± 4.2^{c}	35.22 ± 0.0^{e}	41.99 ± 0.0^{g}	10.27 ± 0.02^{f}	0610.16 ± 0.03^{d}	112.09 ± 0.10^{c}
SLC 92 95	1 ± 0 ^{a}	3 ± 0^{b}	8 ± 0^{a}	06.95 ± 0.08^{f}	179.93 ± 12.^{e}	33.12 ± 0.1^{f}	57.45 ± 0.2^{e}	10.33 ± 0.03^{f}	0829.37 ± 0.33^{c}	103.02 ± 0.04^{d}
SLC 92 77	1 ± 0 ^{a}	3 ± 0^{b}	3 ± 0^{f}	11.85 ± 0.3^{a}	173.60 ± 4.6^{e}	38.92 ± 0.0^{d}	68.79 ± 0.1^{c}	26.73 ± 0.02^{a}	0001.02 ± 0.59^{f}	106.19 ± 0.10^{d}

Note: In a column, means followed by common letters are not significantly different in 5% probability level.
L = Large; M = Medium; S = Small.

Table 5. Leaf N, P, K, Ca, Mg contents, leaf moisture per centage and chlorophyll (SPAD) and Leaf lamina epicuticle wax content in third leaves of four-month-old plants in test varieties

Variety	N	P	K	Ca	Mg	Moisture %	SPAD	Leaf lamina epicuticle wax
Co775	1.48 ± 0.003^{c}	0.20 ± 0.005^{c}	1.01 ± 0.005^{a}	0.34 ± 0.001^{c}	0.11 ± 0.0005^{a}	21.86 ± 0.03^{g}	41.668 ± 0.83^{d}	0.3320 ± 0.64 ^{d}
SL 83 06	1.60 ± 0.003^{b}	0.20 ± 0.200^{c}	0.95 ± 0.950^{b}	0.28 ± 0.210^{e}	0.09 ± 0.0900^{ab}	26.3 ± 0.003^{x}	37.004 ± 0.90^{fg}	2.4800 ± 0.07 ^{a}
SL 92 5588	1.11 ± 1.480^{g}	0.26 ± 0.260^{a}	1.01 ± 1.010^{a}	0.26 ± 0.260^{g}	0.10 ± 0.1000^{a}	27.03 ± 0.07^{e}	38.026 ± 0.47^{efg}	2.0794 ± 0.26^{a}
SL 96 128	0.13 ± 1.110^{h}	0.13 ± 0.130^{e}	0.75 ± 0.750^{f}	0.41 ± 0.410^{b}	0.09 ± 0.0900^{ab}	27.97 ± 0.26^{d}	50.278 ± 0.54^{b}	0.2300 ± 0.14^{d}
SL 96 328	1.17 ± 0.005^{f}	0.15 ± 0.000^{d}	0.94 ± 0.003^{b}	0.20 ± 0.001^{i}	0.09 ± 0.0003^{ab}	28.96 ± 0.03^{b}	41.411 ± 0.90^{de}	0.4663 ± 0.42^{d}
SL 97 1442	1.40 ± 1.400^{e}	0.20 ± 0.200^{c}	0.91 ± 0.910^{c}	0.19 ± 0.190^{j}	0.09 ± 0.0900^{ab}	27.99 ± 0.01^{d}	42.398 ± 0.48^{d}	0.8569 ± 0.67 ^{cd}
SLC 2009 01	1.44 ± 0.005^{d}	0.21 ± 0.003^{c}	0.86 ± 0.000^{d}	0.27 ± 0.005^{f}	0.07 ± 0.0003^{b}	28.4 ± 0.100^{c}	35.340 ± 0.21^{g}	0.7765 ± 0.96^{cd}
SLT 4921	1.58 ± 0.005^{b}	0.20 ± 0.005^{c}	0.86 ± 0.003^{d}	0.42 ± 0.005^{a}	0.13 ± 0.0005^{a}	30.3 ± 0.005^{a}	39.040 ± 0.81^{def}	1.3661 ± 0.45^{b}
SLC 92 95	1.95 ± 0.003^{a}	0.24 ± 0.005^{b}	0.82 ± 0.003^{e}	0.31 ± 0.003^{d}	0.10 ± 0.0050^{a}	29.07 ± 0.02^{b}	70.956 ± 0.38^{a}	0.2260 ± 0.10^{d}
SLC 92 77	1.48 ± 0.003^{c}	0.20 ± 0.200^{c}	0.91 ± 0.910^{c}	0.24 ± 0.240^{h}	0.09 ± 0.0900^{ab}	28.5 ± 0.003	46.396 ± 0.28^{c}	1.3803 ± 0.18^{bc}

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

f. leaf n, p, k, ca, and mg content

Significant difference was observed in percentage of leaf N ($F_{11, 18}$= 2267.52, $p<0.05$), P ($F_{11, 18}$= 160.28, $p<0.05$), K ($F_{11, 18}$= 104.30, $p<0.05$), Ca ($F_{11, 18}$= 160.51, $p<0.05$) and Mg ($F_{11, 18}$= 545.22, $p<0.05$) in the TVD of four-month-old plants in tested varieties. Higher percentage of leaf N was found on accession SL 92 95 (1.95 ± 0.003) and lower percentage (0.13 ± 1.11) of leaf N was found on variety SL 96128 (Table 5). The higher percentage of leaf P recorded on variety SL 92 5588 (0.26 ± 0.26) and lower percentage of leaf P was found on variety SL 96 128 (0.13 ± 0.13). Variety Co 775 (1.01 ± 0.005) recorded higher percentage of leaf K and it was lower in variety SL 96 128 (0.75 ± 0.75). Higher percentage of leaf Ca was found on variety SLT 4921 (0.42 ± 0.0005) and lower percentage of leaf Ca was found on variety SL 97 1442 (0.19 ± 0.19). Variety SLT 4921was associated with higher percentage of leaf Mg and it was lower in variety SLC 2009 01(0.07 ± 0.0003) (Table 5). Percentage of leaf N, P, K, Ca and Mg in third leaves of four-month-old plants in test varieties were in the range of leaf analysis critical levels in sugarcane i.e. 1.8, 0.19, 1.11, 0.2, and 0.08 correspondingly.

g. leaf moisture and leaf chlorophyll content

Leaf moisture percentage ($F_{11, 18}$= 66.31, $p<0.05$) and chlorophyll content ($F_{11, 18}$= 312.43, $p<0.05$) were also significantly varied among the sugarcane varieties/accessions. Chlorophyll content was highest in variety SLC 92 55 (70.956 ± 0.38) and it was lowest in SLC 2009 01 (35.340 ± 0.21). The higher moisture percentage was recorded on variety SLT 4921 (30.3 ± 0.005) and the lowest moisture percentage was recorded on variety Co 775 (21.86 ± 0.03)

III. PCA for physico-morphic plant characteristics of selected sugarcane varieties/ accessions with variable resistance to WLD

Two components of the plant morphological characteristics of selected sugarcane varieties/ accessions contributed 68.64 % of the total variance (Table 2). Dependent variable was associated with leaf length, number of leaves, and distance between leaves, spine density, and leaf angle. PC1 accounted for 38.44% of the total variation and PC2 accumulate 30.2% of total variation. The identified PC for dependent variable were illustrated below.

PC1= (Number of leaves X 0.8) + (Leaf angle X -0.82) + (Distance between leaves X 0.89)

PC2 = (Leaf length X 0.85) + (Spine density X 0.83) + (Internodal length X 0.54)

Table 6. Component loadings from the PCA of physico-morphic plant characteristics of selected sugarcane varieties/ accessions with variable resistance to WLD

Physico-morphic plant characteristics	Components	
	PC1	PC 2
Leaf length	0.01	0.85
Number of leaves	0.80	-0.24
Leaf angle	-0.82	-0.13
Distance between leaves	0.89	0.11
Spine density	0.25	0.83
Internodal length	-0.34	0.54

This PCA related to plant physico-morphic characteristics (Table 6) can use to select varieties by considering the appearance / plant architecture. Since leaf length, number of leaves, leaf angle, distance between leaves, and spine density on leaf sheath are the factors which are easily measurable; this can be used in selecting resistant varieties from large pool of varieties. Therefore, this is an efficient selection procedure for early selection stages of sugarcane varieties through a large pool of varieties.

IV. PCA for leaf colour, epicuticular wax, arrangement of trichomes, stomata, leaf nutrition, moisture and chlorophyll content of selected sugarcane varieties/ accessions with variable resistance to WLD

Principal component analysis revealed that the first five components accounted for 77.81% of the total variation among the selected sugarcane

varieties/accessions (Table 7). The first component explained 29.71% of the total variance, followed by the second (19.74%), third (10.50%), fourth (9.43%), and fifth (8.43%) components. Together, these five components captured the majority of the variability present in the dataset.

Table 7. Eigenvalues, variance explained, and factor loadings of principal components of sugarcane traits

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)
PC1	13.67	29.71	29.71
PC2	9.08	19.74	49.46
PC3	4.83	10.5	59.95
PC4	4.34	9.43	69.39
PC5	3.88	8.43	77.81

Principal component analysis identified ten components with eigenvalues greater than 1.0, collectively explaining 98.17% of the total variation among the sugarcane varieties/accessions. The first five principal components accounted for 77.81% of the total variability and were therefore considered sufficient for interpretation. PC1 explained 29.71% of the variation and was primarily associated with leaf colour traits, wax content, and epidermal anatomical characteristics (Table 8). PC2 explained 19.74% of the variation and was related mainly to stomatal and leaf morphological traits. PC3 accounted for 10.50% of the variation and was associated with vascular bundle and tissue development characteristics. PC4 (9.43%) was dominated by nutrient-related traits, while PC5 (8.43%) was largely influenced by leaf colour parameters.

The identified PC for dependent variable is illustrated below.

PC1= (Leaf lamina epicuticle wax) -0.745 + (Leaf chlorophyll content) 0.762 + (Fiber layer; large VB) 0.702 + (Fiber layer; medium VB) 0.894 + (Fiber layer; small VB) 0.842 + (Total number of vascular bundle) -0.769

PC2= (Leaf moisture) 0.501 + (Epidermis to vascular bundle) 0.775 + (Epidermis to vascular bundle) 0.94 + (Phloem area; small VB) 0.606

PC3= (Phloem Area) 0.946 + (Phloem Area) 0.75 + (Leaf width) 0.72

PC4 = (Red) 0.9 + (Blue) 0.81 + (Epidermis to vascular bundle) 0.557

PC5 = (Average length of trichomes) 0.66 + (Total number of trichomes per unit area)-0.86 + (Width of Stomata) 0.863

First 3 components (PC1, PC2, and PC3) accumulate 63 % of the total variation. PC1, PC2 and PC3 mainly related to leaf wax composition, SPAD (Chlorophyll content), epidermis to vascular bundle (Sucking distance) large, fiber layer large, area of phloem in large vascular bundle, epidermis to vascular bundle (Sucking distance) medium, fiber layer medium, area of phloem in medium vascular bundle, fiber layer, small and total number of vascular bundles from all the considered characteristics.

Except for a few factors, several plant characteristics as leaf colour, leaf composition, trichome and stomata arrangement and configuration of fibro vascular bundle contribute to the behaviour of vector and level of WLD infection directly.

PCA of leaf colour, epicuticle wax, arrangement of trichomes, stomata, leaf nutrition, moisture and chlorophyll content can be implemented to confirm the level of resistance and mode of resistance in sugarcane varieties/ accessions.

Table 8. Component loadings from the PCA of leaf colour, epicuticular wax, arrangement of trichomes, stomata, leaf nutrition, moisture and chlorophyll content of selected sugarcane varieties/ accessions with variable resistance to WLD

Plant character	Components				
	PC 1	PC 2	PC3	PC 4	PC 5
Leaf colour					
Red	-0.19	-0.05	0.036	0.90	-0.037

Blue	0.11	0.055	0.04	0.81	-0.104
Trichome					
Average length of trichomes	0.072	-0.169	0.174	-0.511	0.66
Total number of trichomes / unit area	0.270	0.041	-0.000	-0.148	-0.86
Stomata					
Width of Stomata	-0.041	-0.259	0.058	-0.260	0.863
Leaf composition					
Leaf lamina epicuticle wax	-0.745	0.281	-0.135	0.424	-0.028
Leaf moisture	0.220	0.501	0.433	0.373	-0.101
Leaf chlorophyll content/ SPAD	0.762	-0.11	-0.008	0.128	-0.556
Leaf fibro-vascular system					
Large					
Epidermis to vascular bundle	0.107	0.775	-0.390	-0.453	0.048
Fiber layer	0.701	0.627	-0.252	-0.153	-0.008
Phloem Area	-0.175	-0.124	0.946	0.032	0.006
Medium					
Epidermis to vascular bundle	0.089	0.964	0.027	0.026	-0.180
Fiber layer	0.894	0.272	-0.253	-0.055	-0.103
Phloem Area	0.278	0.091	0.750	0.103	0.490
Small					
Epidermis to vascular bundle	0.172	0.515	-0.089	0.557	-0.112
Fiber layer	0.842	0.274	-0.372	0.039	-0.108
Phloem Area	-0.090	0.606	0.472	0.262	-0.302
Total number of vascular bundle	-0.769	-0.102	-0.383	-0.364	-0.052
leaf width	-0.424	-0.055	0.723	-0.305	0.053

The principal component analysis highlighted that specific leaf traits, fibro vascular bundle configurations, and physiological properties significantly affected the plant's interaction with the insect vector *D. menoni.*

According to the results, PCA identified leaf lamina epicuticle wax, chlorophyll content, and the distance between the epidermis and vascular bundles as critical contributing factors in resistance to the vector. High levels of epicuticular wax, reduce the feeding efficiency of *D. menoni* and subsequent disease transmission. In the same way, specific leaf coloration i.e. red and green hues, deter the vector's feeding and longevity, highlighting their importance in screening varieties for resistance against the vector. Characteristics of fibrovascular bundle also appeared as significant indicators of resistance. Varieties with smaller vascular bundle sizes, thicker phloem fiber layers, and greater distances from the epidermis to vascular parenchyma demonstrated reduced vector feeding and oviposition which result lower disease transmission rate. These structural characteristics may cause limited access to vascular tissues, disrupting the insects feeding process and reducing its ability to act as a vector. Conversely,

characteristics such as increased trichome length and wider stomata were associated with higher feeding and survival rates of *D. menoni*, which can increase disease transmission.

The PCA outcomes give a clear, multivariate perspective of leaf characteristics and internal vascular features most strongly distinguish sugarcane varieties in terms of potential resistance *to Deltocephalus menoni.* Rather than considering each character separately, the PCA allows to see which combinations (principal components) capture the bulk of variation that likely underlies differential vector behaviour. In the current study, leaf characteristics as epicuticular wax, chlorophyll content, and epidermis-to-vascular bundle distance emerge as dominant axes of variation; meanwhile, characteristics like trichome length and stomatal

width contribute less to the major components that likely relate to resistance.

Epicuticular wax is strongly negatively loaded on PC1 (−0.745). This suggests that varieties with higher wax levels tend to have extreme (negative) PC1 scores, which may correlate with less attractiveness or lower feeding success by *D. menoni*. This finding fits well with previous studies e.g. epicuticular waxes (in forms such as wax crystals or wax "blooms") are known to interfere with insect attachment, making leaf surfaces slippery or reducing tarsal adhesion (Eigenbrode *et al.,* 2004). Waxes can change the micro-scale configuration and chemical environment of the cuticle surface such that insect legs, antennae, or stylets cannot maintain stable contact (Brennan, 2001). In cotton–whitefly systems, variation in wax chemistry and composition has been linked to lower insect population densities and reduced virus transmission (Ali *et al.,* 2021). Moreover, in rice mutants lacking normal wax deposition, resistance to herbivores like armyworms or weevils declines (Bernaola *et al.,* 2021). Therefore, wax accumulation is a well-supported, functional trait that can act as an antixenosis barrier to sap-feeding insects and vectors.

Similarly, chlorophyll content (SPAD) is positively loaded on PC1 (0.762) suggesting that greener, more photosynthetically active varieties fall on the same axis as lower-wax types. In literature on plant–insect interactions, higher chlorophyll content often correlate with higher nitrogen content and general nutritional value, rendering those leaves more attractive or suitable for insect feeding (Sousa-Souto *et al.,* 2018). Though chlorophyll content is not a defensive trait, it may serve as an indicator trait: breeders may wish to avoid inadvertently selecting low-vigour varieties. Thus, chlorophyll wax balance might act as a screening trade-off: high wax for deterrence, but optimum chlorophyll content to maintain yield and vigour.

Internal anatomical characteristics tied to vascular bundle configuration were prominent in PC2 and PC3. These loadings imply that varieties with greater epidermis-to-vascular bundle distance, thicker fiber sheath layers, and smaller phloem areas are tend to occur at different positions in the multivariate space that correspond to resistance traits. Structurally, a greater "buffer layer" the distance between the surface and the vascular tissue increases the mechanical and spatial challenge for the insect's stylet to reach the phloem. A robust fiber layer can act as a physical barrier. The more compact or narrower the vascular bundles and lower their number, the lower "open targets" are available for the insect (Chanchala *et al.,* 2022). They also reported negative correlations between epidermis-to-bundle distance, fibre layer thickness, and disease incidence or feeding behaviour of *D. menoni.*

Therefore, the combination of anatomical and surface traits give a composite view: vectors must initially land, adhere, probe through surface barriers (waxes), crossing internal tissues (epidermis, mesophyll, fiber sheath), and then reach vascular (phloem) tissue. Resistance can occur at any or multiple of these stages. Leybourne *et al,* (2022) in a meta-analysis of sap-feeding insect resistance found that resistant plants generally slow the time to first probe and reduce total feeding duration.

From a practical perspective, these findings have significant implications for variety screening and breeding programs. Researchers can measure the leaf wax content and examine leaf anatomy to assess each sugarcane variety. Based on these characteristics and the PCA results, varieties can be ranked for their potential resistance. Plants that have more wax on the leaf surface, a greater distance between the epidermis and vascular tissues, thicker fiber layers, and smaller or more compact vascular bundles are likely to be more resistant. By integrating the identified plant characteristics into selection criteria, resistant varieties can be more efficiently identified during the early stages of screening. These selected varieties can then be tested further to observe insect behavior (such as landing, probing, and feeding) and to evaluate how well they resist disease under field conditions. Breeders could use these insights to design sugarcane varieties that not only deter vector feeding but also minimize disease progression under natural conditions.

CONCLUSIONS

Sugarcane varieties with droopy canopy, wider leaf angles, and closer leaf spacing showed higher WLD incidence, indicating increased susceptibility. In contrast, higher red and green colour intensity, greater epicuticular wax, and shorter trichomes reduced attractiveness, feeding, and survival of *D. menoni,* contributing to resistance. Vector performance increased with longer trichomes and wider stomata, enhancing feeding, survival, and disease transmission. Resistance was associated

with greater distance from epidermis to vascular tissues, thicker phloem fiber layers, and smaller vascular bundles.Principal Component Analysis (PCA) supported early selection based on morphological traits, while behavioral traits confirmed resistance. These findings provide a practical framework for breeding sugarcane varieties with improved resistance to *D. menoni* and sustainable WLD management.

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