SUGARCANE PATHOLOGY RESEARCH - 2023

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The Sugarcane Pathology Program is dedicated to enhancing sugarcane cultivation through a comprehensive approach. The program focuses on providing data on sugarcane variety susceptibility, efficacy of chemical treatments to improve billet planting performance, diagnosing sugarcane diseases for growers, and studying the impact of diseases on sugarcane yields. Projects conducted during the 2023 season included screening for smut resistance, testing chemical treatments for sugarcane billets, and providing support to the Healthy Seedcane Programs in Louisiana to manage systemic diseases such as Ratoon Stunting Disease (RSD) and other viral infections.

Susceptibility of sugarcane varieties to diseases

Resistance to smut was assessed during the 2023 season at the Sugar Research station in Saint Gabriel. Two varieties of the 20 HoCP series and 15 varieties of the 21 L series were evaluated for smut susceptibility, comparing them to five previously released varieties. 12 of the 15 assessed L21 varieties were resistant, two were moderately susceptible, and one was susceptible to sugarcane smut (Table 1). Variety HoCP20-568 was moderately susceptible to smut, whereas HoCP20-570 was resistant to the disease (Table 1).

Healthy seedcane program support

The sugarcane pathology lab maintained eight cultivars in the local quarantine greenhouse in Baton Rouge, LA. Prior to their planting in October 2022, meticulous testing was carried out on sugarcane stalks for mosaic, RSD, yellow leaf disease, and leaf scald. Upon confirmation of their disease-free status, these plants were transferred to the local quarantine greenhouse on LSU's main campus in Baton Rouge, LA. Subsequent retesting in May 2023 confirmed their disease-free status before they were provided to two tissue culture companies in Louisiana. These plants served as foundation stock plants for apical meristem tissue culture performed by the two tissue culture companies. 4582 samples were received for Sugarcane Yellow Leaf Virus (ScYLV) testing. This effort is part of the certification of seed cane, if fields are over 10% of infection, they cannot be commercialized as certified seed cane. Out of the 4582 samples, 108 were positive for ScYLV, not allowing certification for 10 of the 140 fields tested for ScYLV.

Evaluating chemical treatments for billets

A field experiment was conducted during the 2022-2023 sugarcane season at the Sugar Research Station located in Saint Gabriel, Louisiana, aiming to assess the efficacy of fungicides and some insecticides against red rot disease, caused primarily by *Colletotrichum falcatum* in Louisiana. Red rot affects plant cane germination, reduces juice quality in standing cane when affecting mature plants, and can greatly reduce yield. Given the increasing adoption of billet planting in Louisiana, efforts were directed towards understanding the impact of various treatments on red rot incidence. The description of treatments and associated abbreviations, used throughout this report, are described in Table 2.

The treatment with the highest weights of sugar per acre (Figure 1A) and sugarcane tons per acre (Figure 1B) was Quilt+Platinum. Non-treated whole stalks performed statistically similar to Quilt + Platinum, Xyway + Platinum, Xyway + Vantacour, Xyway + Platinum, Xyway + Zironar, Xyway, Vantacour, and Quilt. The treatments with the lowest performance were Zironar and non-treated billets, although their performance was comparable to Xyway + Zironar, Xyway, and Vantacour.

Table 1. Smut resistance ratings determined in an inoculated test for commercial check and experimental sugarcane varieties during 2023.

Variety	Rating (Percent smut)	Variety	Rating (Percent smut)
CP73-351	4(11)	L21-080	1(0)
LCP82-089	3(8)	L21-081	3(5)
HoCP89-846	1(0)	L21-086	6(28)
HoCP96-540	1(0)	L21-091	1(0)
L99-226	4(9)	L21-092	1(0)
HoCP20-568	4 (12)	L21-093	3(5)
HoCP20-570	3(8)	L21-095	4(9)
L21-074	9(53)	L21-096	1(0)
L21-077	2(1)	L21-097	3(8)
L21-078	1(0)	L21-099	1(0)
L21-079	1(0)	L21-102	1(0)

Resistance ratings assigned on a 1-9 scale in which 1-3 = resistant, 4-6 = moderately susceptible, and 7-9 = highly susceptible.

Table 2. Description of treatments and associated abbreviations evaluated during the 2022-2023 sugarcane season in field trials conducted at the Sugar Research Station in Saint Gabriel, Louisiana.

Treatment and rate per acre, if applicable	Abbreviation
Non-treated Billets	B
Non-treated Whole Stalks	W
Xyway 15 oz	X
Xyway 15 ox + Zironan 12 oz	XZ
Xyway 15 oz + Vantacor 2 oz	XV
Xyway 15 oz + Platinum 5.7 oz	XP
Zironar 12 oz	Z
Vantacour 2 oz	V
Quilt Xcel 21 oz	Q
Quilt Xcel 21 oz + Platinum 5.7 oz	QP

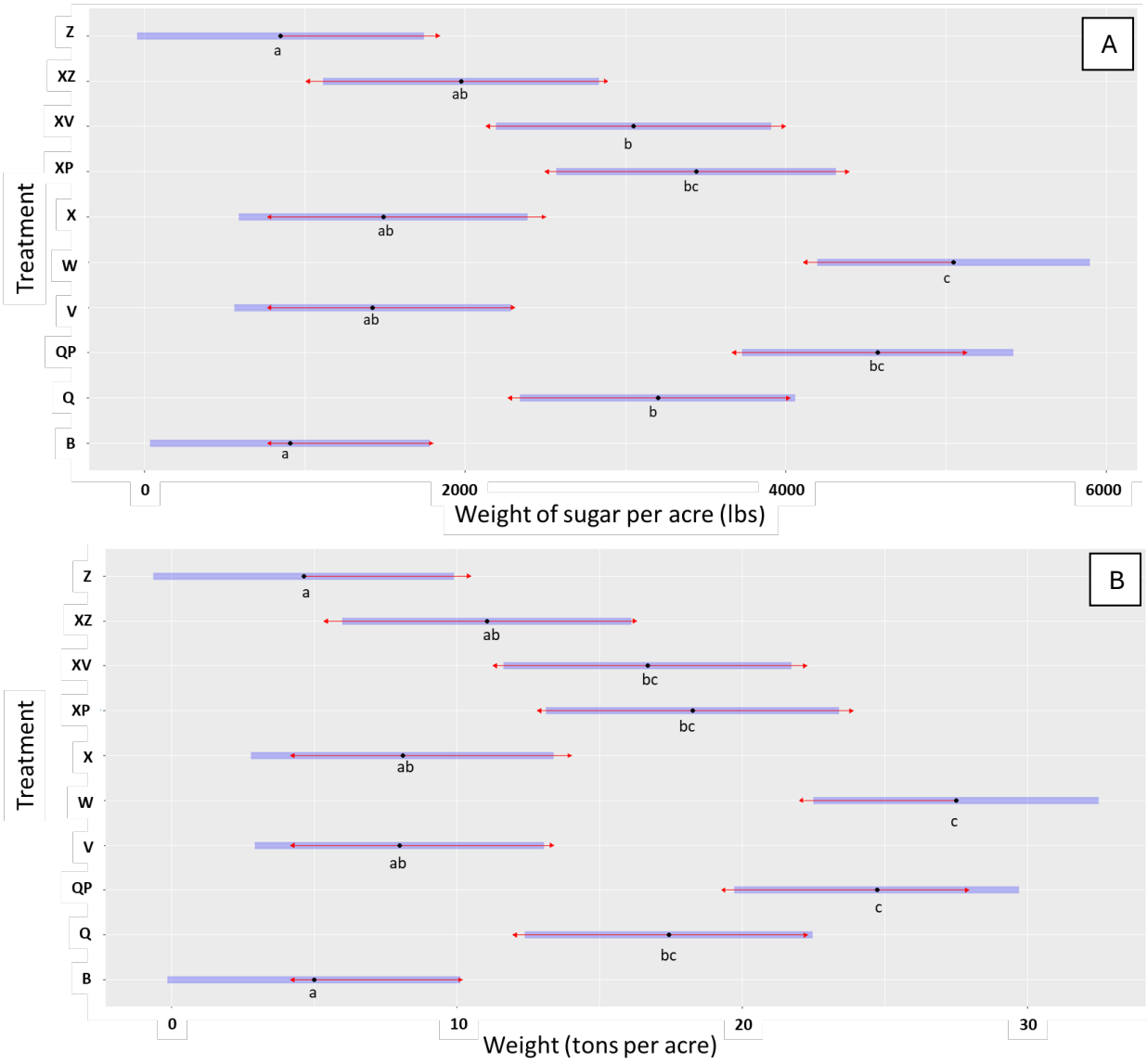

Figure 1. Estimated marginal means for weight of sugar per acre, in lbs, (A) and weight of cane in tons per acre, divided by treatments, obtained using generalized linear mixed models using a normal distribution (after normality was verified via Shapiro-Wilk test), reps as random factors, and treatments as fixed factors. Plots were harvested on 10-19-2023 and weights were recorded per plot. Blue bars represent the confidence interval for the estimated marginal means. Red arrows account for significant differences. Overlapping red arrows do not differ significantly at alpha=0.05. Letters denoting statistical differences were added to facilitate interpretation, means accompanied by the same letter do not differ significantly at alpha=0.05.

NEMATOLOGY RESEARCH

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Nematode research at LSU AgCenter addresses important management considerations for plant-parasitic nematodes affecting sugarcane in Louisiana. The goal of the program is to provide farmers with effective strategies to minimize nematode-associated yield loss. During the 2023 growing season we monitored an ongoing nematicide efficacy experiment located at the Sugar Research Station in St. Gabriel, Louisiana.

Nematicide Field Trial – St. Gabriel, Louisiana

A small-plot field trial was planted at the Sugar Research Station in St. Gabriel, Louisiana in September 2022 with the sugarcane variety 'L01-299'. The field had a moderate infestation with lesion nematode (*Pratylenchus* sp.) and ring nematode (*Mesocriconema* sp.) at the time of planting. Nematicide treatments (Table 1) were initially applied in-furrow over the plant cane prior to closing the rows (September 2022) and were reapplied to select plots as a soil-directed spray (March 2023). Nematode soil population densities were monitored throughout the 2023 growing season. Early shoot emergence (March 2023), mid-season shoot height (July 2023) were measured, as well as cane tonnage and sucrose yield (November 2023).

Table 1. Nematicide treatments applied to the field.

Treatment	Rate	Timing	Method
Untreated	-	-	-
MOCAP 15G	3.6 lb/1,000 row ft	At plant	In-furrow
Nimitz 1 application	7 pints/A	At plant	In-furrow
Nimitz 2 applications	7 pints/A	At-plant and March 2023	In-furrow and soil-directed spray
Syngenta Experimental 1 application	100 mg a.i. /A	At plant	In-furrow
Syngenta Experimental 2 applications	100 mg a.i. /A	At-plant and March 2023	In-furrow and soil-directed spray

Lesion nematode soil population densities at harvest were significantly lower in plots treated with the Syngenta Experimental treatment relative to the untreated soil, regardless of application frequency (Figure 1). Ring nematode soil population densities showed the opposite trend (Figure 2), with higher numbers observed at harvest in plots treated with the Syngenta Experimental nematicide when applied twice. Overall, these data suggest that lesion nematode and ring nematode may compete for the sugarcane root system. Identification of additional nematicides with efficacy toward ring nematode may be required.

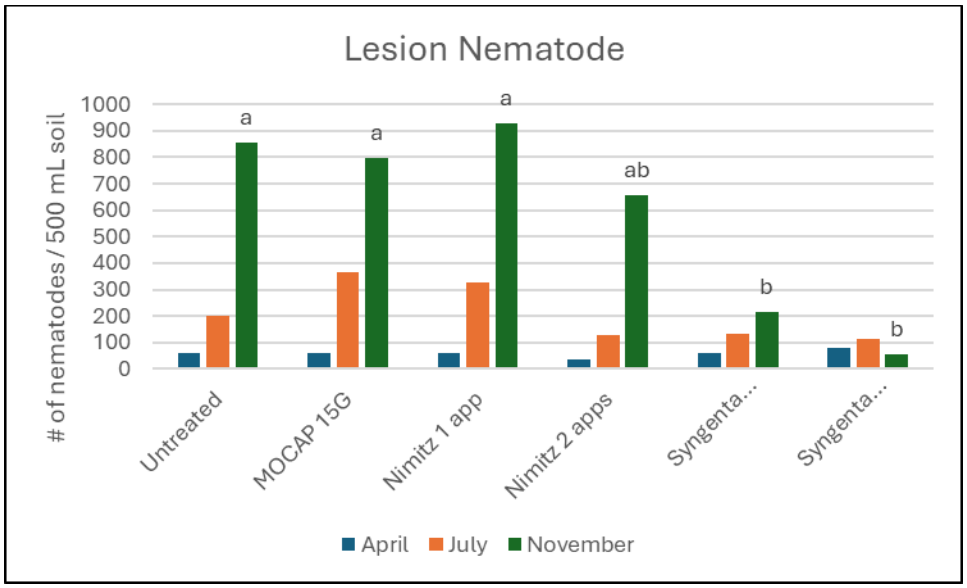

Figure 1. Influence of soil treatment on lesion nematode soil population densities.

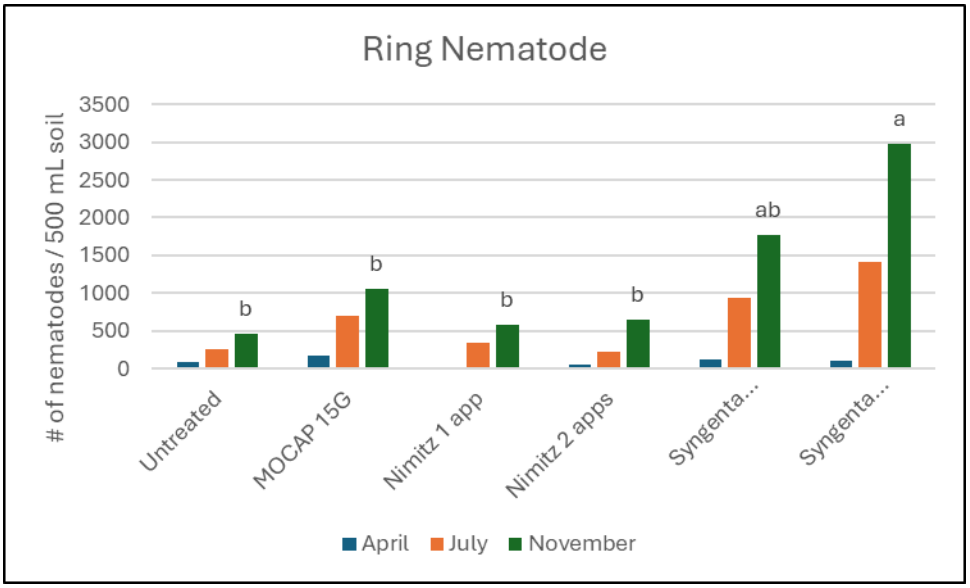

Figure 2. Influence of soil treatment on ring nematode soil population densities.

Treating soil with nematicides did not have an impact on any plant growth parameter measured during the growing season (Table 2). Cane tonnage and sugar yield were similarly unaffected by nematicide treatment; however, there was a trend toward greater yields in nematicide treated plots. Overall, the yield benefit of nematicide application on sugarcane remains to be determined. The impact of repeated nematicide applications on the first ratoon crop will be evaluated in 2024.

Table 2. Influence of soil treatment on plant growth parameters and yield.

Treatment	Shoot emergence (#/10 ft)	Shoot height (cm)	Cane tonnage (ton/A)	Sugar yield (lb/A)
Untreated	64	165	52.0	10,473
MOCAP 15G	67	161	53.6	10,847
Nimitz 1 app	70	165	54.0	11,000
Nimitz 2 apps	74	168	54.4	11,017
Syngenta Experimental 1 apps	67	167	50.6	10,572
Syngenta Experimental 2 apps	63	160	53.8	10,678

GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY MOLECULAR MARKERS ASSOCIATIATED WITH SORGHUM MOSAIC VIRUS RESISTANCE IN LOUISIANA SUGARCANE

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INTRODUCTION

Mosaic is a historically important disease affecting sugarcane in Louisiana and worldwide (Grisham, 2000). In the 1920s, mosaic nearly wiped-out Louisiana's sugarcane industry, which was saved by the importation of the first interspecific hybrid cultivars developed by crossing to the wild species *Saccharum spontaneum* as a source of resistance. Mosaic in Louisiana is now caused by the Sorghum mosaic virus (SrMV). Mosaic is managed primarily through the development and cultivation of resistant cultivars. But periodic outbreaks of mosaic caused by the emergence of new strains led to increased focus on basic breeding to introgress additional genes for mosaic resistance and other traits. The cultivation of resistant cultivars resulted in very low incidence of mosaic in the Louisiana industry. Therefore, observational detection of susceptibility through natural infection during the 12-year cultivar selection program became unreliable due to a lack of inoculum pressure. In 2016, mosaic was detected in breeding program on-farm yield trials and cultivar increase plots in multiple clones near commercial release. Subsequent extensive screening of the commercial recurrent selection parent population and basic introgression parent population by mechanical inoculation of clones in the greenhouse determined that susceptibility to SrMV infection had unknowingly begun to infiltrate the parent population.

The history and recent re-emergence of mosaic in Louisiana indicate that the disease continues to be a threat. Determining resistance reactions for clones by mechanical inoculation is laborious, time-consuming, and not always repeatable. Therefore, a study was conducted to identify markers significantly associated with resistance to SrMV using a mosaic diversity panel that can be used in marker-assisted selection of parents and progeny early in the variety development program.

MATERIALS AND METHODS

Materials

Mosaic resistance reactions were determined by extensive mechanical inoculations screening of the commercial recurrent selection and gene introgression parent populations of the cooperative sugarcane breeding program in Louisiana. A panel of 213 sugarcane clones with resistance responses ranging from highly resistant to highly susceptible was chosen from the screened parent populations for the marker-trait association study. Within the panel, 136 clones were commercial parents (*Saccharum* interspecific hybrids), 54 were introgression program hybrid clones intended for possible inclusion as parents in the recurrent selection program, and 23 accessions were included of which 19 were *S. spontaneum* and two each of *S. officinarum* and *S. barberi*.

Phenotyping and heritability for SrMV resistance

Mosaic resistance responses based on disease incidence data from mechanical inoculations served as the phenotype for each clone. A typical mosaic symptom is shown in Figure 2.1. Disease incidence was determined for each clone as the total number of symptomatic plants out of four to six inoculated plants converted to percentage of infected plants per clone. Infection percentage intervals were then used to assign resistance ratings of highly resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible (Table 1).

Table 1. Disease scoring and ratings assigned for inoculated clones.

Score (total no. of infected/total no. of plants inoculated)	Resistance rating
0/6 (0%)	Highly resistant
1/6 (17%)	Moderately resistant
2/6 (33%)	Moderately susceptible
3-4/6 (60-67%)	Susceptible
5-6/6 (83-100%)	Highly susceptible

Frequency distributions were determined for the different resistance ratings and for the three categories of clones: commercial hybrids, introgression hybrids, and accessions. Analysis of variance (ANOVA) of the phenotypic data was performed using JMP Pro version 16.0.0 (SAS Institute, Cary, NC). Residual analysis was also carried out using the studentized t-test with Bonferroni correction at alpha 0.05.

The clones were representative of the diversity in Louisiana breeding program germplasm, and broad-sense heritability of mosaic resistance was calculated as percentage of genotypic variance over the total phenotypic variance that included the genotype by year variance and the error variance components. Narrow-sense heritabilty was calculated using the marker information. Incidence ranges for the different resistance ratings were tested for significance by a Tukey-Kramer test. Actual and predicted incidence data were also compared using a simple linear regression model in JMP Pro 16.0.0.

Genotyping

Genomic DNA was extracted from young leaves of sugarcane plants following the CTAB method (cetyltrimethylammonium bromide). The quality and quantity of the DNA were evaluated using 1% agarose gel as well as Nanodrop spectrophotometer (ND-1000). Five µg of DNA was used for library preparation followed by genotyping-by-sequencing. Sequence reads were filtered using appropriate in-house bioinformatics tools and clean reads were mapped to the sugarcane genome to call 64,187 variants including single nucleotide polymorphisms (SNPs) and insertions-deletions (Indels). Biallelic SNPs with a minimum allele frequency of 0.5 and a minimum sequencing depth of 50 reads per sample were selected for use. A total of 20,329 SNPs were used for the association analysis.

Marker-trait association (MTA) analysis

The association between markers and the trait of interest, SrMV infection resistance, was performed using GAPIT taking into account the population structure (Q) and kinship (K)

matrices derived from JMP Genomics program. Phenotypic data (BLUEs) were used as dependent variables. Bonferroni and false discovery rate (FDR) were also run as correction factors at alpha 0.01. Significant SNPs for MTAs were set at -log(P) > 3 and $R^2 > 0.1$.

RESULTS

Phenotypic Characterization

Out of the 213 association panel clones, 180 (85%) exhibited consistent disease responses in multiple mechanical inoculations ranging from highly resistant to highly susceptible, and 33 (15%) clones exhibited inconsistent (both resistant and susceptible) responses (Figure1). Resistant responses (highly and moderately resistant) were detected for 148 (69%) and 32 (15%) had susceptible (moderately susceptible, susceptible, and highly susceptible) responses (Table 2). The frequency of resistance was highest in the introgression hybrids followed by the accessions then the commercial hybrids (Table 2). Within the accessions, the frequencies of resistance were 16 of 19 (84%) for *S. spontaneum*, 0 of 2 (0%) for *S. barberi*, and 0 of 2 (0%) for *S. officinarum*.

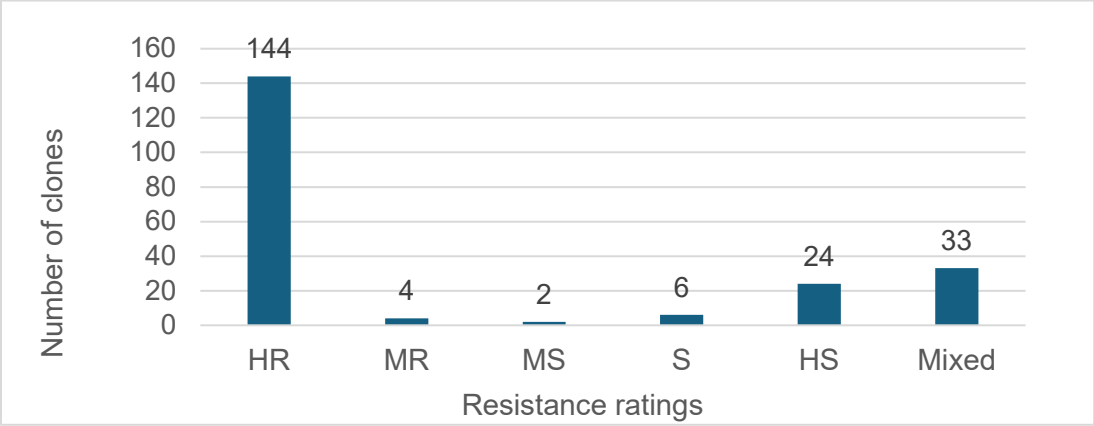

Figure 1. Frequency distribution of mosaic resistance ratings for sugarcane clones in the association panel. HR = highly resistant, MR = moderately resistant, MS = moderately resistant, S = susceptible, and HS = highly susceptible, Mixed = reaction varied between resistant and susceptible.

Table 2. Distribution of mosaic resistance and susceptibility in commercial hybrid, introgression hybrid, and accession clones included in association panel.

Population	Resistant	Susceptible	Mixed	Total clones
Commercial	90 (66%)	24 (18%)	22 (16%)	136
Introgression	42 (78%)	1 (2%)	11 (20%)	54
Accessions	16 (70%)	7 (30%)	0 (0%)	23
Total clones	148 (69%)	32 (15%)	33 (15%)	213

Analysis of variance (ANOVA) showed that the effect of genotype on phenotype was highly significant (Table 3). The inclusion of the clones with inconsistent reactions resulted in a significant year effect in the analysis. Broad-sense heritability for resistance was estimated to be 0.86, whereas narrow sense heritability was found to be 0.80. Pairwise comparisons using the Tukey-Kramer HSD indicated that the five mosaic resistance phenotypes were significantly different from each other (Table 4).

Table 3. Analysis of variance, test effect, and broad-sense heritability of resistance to SrMV infection in the association panel.

Source	DF	Sum of squares	Mean square	F ratio	Prob > F	Broad-sense heritability (H^2)
Genotype	212	433328.61	2044.00	6.5474	<.0001*	0.86
Residual	186	58066.73	312.19			
Effect Tests						
Genotype	212	431367.96		6.5503	<.0001*	
Year	1	4124.59		13.2779	0.0003*	

Table 4. Comparison of five different mosaic resistance phenotypes used to characterize disease responses for clones in the association panel.

Rating	Mean incidence (%) ± standard error[1]
Highly susceptible	93.5 ± 0.55 A
Susceptible	53.8 ± 0.94 B
Moderately susceptible	32.7 ± 1.09 C
Moderately resistant	18.2 ± 1.23 D
Highly resistant	0.0 E

[1]Means followed by different letters were significantly different (P=0.05) as determined by Tukey-Kramer HSD.

A linear regression model of actual and predicted incidence data generated a root mean square of 17.699 and R^2 of 0.88 at P = 0.0001. The results further demonstrated that the resistance ratings of phenotypes were highly significant with 88% of the variation explained by the model with no spurious data observed.

Population structure and kinship analysis

Population structure used identity by descent (IBD) matrix for principal component analysis (PCA). PCA calculations used the single-value decomposition with default parameter setting.

PCA1 and PCA2 were considered significant with eigenvalues greater than 10 or 10/100. The eigenvalues were the coefficients of the eigenvectors that measure the data's covariance. Since the eigenvalues dropped very little after PCA2, inclusion of additional PCs to the analysis was not considered informative. PCA revealed the population structure within the panel. The *S. spontaneum* clones were well distant from the commercial hybrids; the *S. barberi* clones separated from all the other clones; the recent introgression hybrids were separated but remained closer to the commercial hybrids; and the *S. officinarum* clones exhibited higher similarity to the commercial hybrids. The hierarchical cluster analysis showed that the *S. spontaneum* were grouped into one clade, while *S. officinarum* and *S. barberi* also converged into one clade. The hybrids (introgression and commercial) were interspersed in the dendrogram.

Marker-trait association analysis

The genome-wide association analysis identified 11 SNPs with significant associations with SrMV resistance at the criteria of FDR <0.05. Forty-four stable SNPs (P<0.001) occurring across multiple datasets and models were found.
Chromosome SH07 and SH05 showed the highest number of significant SNP markers (10) followed by SH03 (8). The lowest number of significant SNP (1) was found in chromosome SH10. Both 2017 and average dataset had 33 significant SNPs, whereas 2018 dataset had 27 significant MTAs.

Marker annotation

Genes closest to the 55 significant, non-redundant SNPs associated with SrMV resistance were identified. Genes linked to eight SNPs were related to plant defense, immunity and disease resistance, whereas other SNPs were related to other biotic stress resistance, abiotic stress tolerance, cellular functions and metabolic processes. Nine SNPs were linked to genes that coded for proteins with unknown function.

Because of high linkage disequilibrium (LD) due to short breeding history and the narrow genetic base of sugarcane, association mapping has been suggested to be preferred method to identify trait-associated markers. High heritability of mosaic resistance suggested that much of the resistance phenotype can be attributed to genetic variation, and that mosaic resistance could be amenable to marker-assisted breeding. Identification of multiple SNPs significantly associated with SrMV resistance suggested that SrMV resistance is a quantitative trait controlled by multiple genes. These SNPs have been validated using biparental populations for their use in marker-assisted selection.

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