



# Screening Sugarcane Wild Accessions for Resistance to *Sugarcane Mosaic Virus* (SCMV)

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**Abstract** In order to identify new sources of resistance for future introgression crosses, wild sugarcane germplasm was screened for resistance to *Sugarcane mosaic virus* (SCMV) under high inoculum pressure. Evaluation of symptoms by a grade scale associated with serological diagnostic by plate-trapped antigen-enzyme-linked immunosorbent assay (PTA-ELISA) with a specific antiserum were performed in 43 accessions, encompassing *Saccharum officinarum*, *S. barberi*, *S. spontaneum* and *S. robustum* species and hybrids. Differential patterns of resistance and susceptibility were observed among the *Saccharum* species, with *S. officinarum* followed by *S. robustum* accessions showing higher susceptibility, whereas *S. barberi* and *S. spontaneum* had the most resistant accessions. The results indicate that accessions IS76-155, IJ76-418 red, NG57-50, Ceram red, Badilla, Sac.off 8276, Fiji19, IJ76-313, US57-141-5, Krakatau, IN84-58, IN84-88, IN84-82, Gandacheni, and Chin, possibly consist in good sources of resistance to mosaic.

**Keywords** Sources of resistance · *Saccharum* spp. · Sugarcane mosaic

## Introduction

Mosaic is one of the most disseminated viruses in sugarcane, maize, sorghum and other poaceus worldwide, being caused by viruses of *Sugarcane mosaic virus* (SCMV) subgroup of the family *Potyviridae* (Gonçalves et al. 2012; Viswanathan and Mohanraj 2001). Six distinct species of the genus *Potyvirus*, including SCMV, *Sorghum mosaic virus* (SrMV), *Johnson-grass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV), *Pennisetum mosaic virus* (PenMV), *Zea mosaic virus* (ZeMV), and one of the new genus *Poacevirus* (Li et al. 2011; Parameswari et al. 2013; Viswanathan et al. 2008; Xu et al. 2010), *Sugarcane streak mosaic virus* (SCSMV) are recognized in this group. Under natural conditions, there are reports only of SCMV, SrMV and SCSMV isolated from sugarcane, whereas the last four viruses have never been isolated from this crop (Chatenet et al. 2005). It was reported that the single potyviridae causing mosaic in sugarcane in Brazil is SCMV (Gonçalves et al. 2011, 2007, 2004), despite JGMV has been recently reported infecting *Pennisetum purpureum*, a tropical grass used for pasture in the country (Silva et al. 2013).

The disease is responsible for varying economic impacts on sugarcane, depending on the virus species and strain and on the sugarcane variety. In Brazil, the disease is relatively controlled due to action of breeding programs and practical field approaches, but screening for resistance to mosaic remains as an essential step in reason of factors like favorable epidemiological conditions to mosaic dissemination and recent descriptions of new isolates, which reinforce the current importance of the disease (Gonçalves et al. 2012, 2007, 2004).

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The emergence of new virus strains reinforces the need of searching for new sources of resistance in sugarcane germplasm collections in order to support the development of new sugarcane varieties resistant to SCMV. Such studies were performed for SCMV strain H (Grisham et al. 1992), for SrMV HH (Li et al. 2013) with artificial inoculation under greenhouse conditions and for *Sugarcane yellow leaf virus* (SCYLV), another important virus infecting sugarcane, under natural infection conditions (Comstock et al. 2001).

Sugarcane belongs to the genus *Saccharum*, tribe *Andropogoneae* of the family *Poaceae*. The genus includes six species named *Saccharum officinarum*, *S. sinense*, *S. barberi*, *S. edule*, *S. spontaneum* and *S. robustum* that jointly with the related genera *Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya*, constitutes the ‘Saccharum’ complex (Daniels and Daniels 1975) which represents sources of genetic variation for sugarcane breeding programs worldwide. Modern sugarcane varieties have an interspecific origin derived from introgressions with the wild species *S. spontaneum* and *S. robustum* into the cultivated species *S. officinarum*, *S. sinense* and *S. barberi* (D’Hont et al. 2008; Grivet et al. 2006; Irvine 1999; Grivet and Arruda 2002). The major limitation of sugarcane breeding is its narrow genetic base, as all the modern varieties descend from a few ancestors or foundation clones (Deren 1995; Berding and Roach 1987).

In Brazil, sugarcane breeding programs use mainly commercial varieties as parents (Barbosa 2001; Duarte Filho et al. 2010) in their crosses, which have a common genetic basis. Besides the need to broaden the genetic basis of sugarcane, the current focus on biomass for ethanol and energy production, has stimulated sugarcane breeders to start introgression programs involving crosses between commercial varieties and wild accessions of *S. spontaneum* and *S. robustum*. Therefore, screening sugarcane wild accessions for resistance to SCMV will help breeders in the choice of appropriate accessions to be used as parents in the introgression programs either for bioenergy or sugar production. The present study aimed to evaluate SCMV infection in some accessions of the germplasm collection maintained at the Campinas Agronomic Institute (IAC) Sugarcane Research Centre, Brazil, in order to identify possible sources of resistance and guide future introgression crosses.

## Materials and Methods

### Genotype Panel

The resistance to mosaic was evaluated in 43 accessions, which include 30 *S. officinarum*, 3 *S. barberi*, 6 *S.*

*spontaneum* and 4 *S. robustum* accessions maintained at the IAC Sugarcane Breeding Station, Brazil (Table 1).

### Experimental Design and Evaluation of Mosaic Incidence and Severity

The experiment was conducted in complete randomized block design with three replicates and 1 m plots spaced 1 m between plots and 1.5 m between rows, adopting IACSP95-5000 and IACSP93-046 varieties as checks. The field experiment was installed in October 2011 in an area previously known as of high mosaic and sugarcane aphid incidence in Ribeirão Preto, São Paulo Brazil. Sugarcane accessions were evaluated for mosaic incidence and severity under natural infection conditions in 6 months first ratoon cane, in May 2013. A slightly modified symptom grade scale based in the one described by Pinto et al. (2013) was used to evaluate the severity of mosaic symptoms, as following: symptom absence (0); mild mosaic in one or more leaves (1); intense mosaic in two or more leaves (2); generalized intense mosaic, along with reduction in plant growth (3).

### SCMV Detection by PTA-ELISA

In order to confirm the virus infection, the serological test plate-trapped antigen-enzyme-linked immunosorbent assay (PTA-ELISA) with an antiserum specific to SCMV was carried out in the second youngest sugarcane leaf collected from each accession. Leaf samples were grounded in carbonate buffer 0.05 M, pH 9.6, 1:10 ratio (weight: volume), to cover ELISA plates. The antiserum was diluted in phosphate buffered saline with 0.05 % Tween-20, 2 % polyvinylpyrrolidone and 0.2 % ovalbumin (PBS-TPO) 1:6000, with 1 % of skim milk powder and the alkaline phosphatase-conjugated “anti-rabbit” IgG was diluted 1:3000. The enzyme substrate *p*-nitrophenylphosphate (PNPP) was used in the concentration of 1 mg/ml of diethanolamine buffer pH 9.8. Four absorbance readings at 405 nm ( $A_{405nm}$ ) were done in an ELISA plate reader at every 20 min. Samples with  $A_{405nm}$  reading mean three times above the negative control’s reading means were considered as SCMV infected.

### Statistical Analysis

Variance analysis was performed on grade symptoms results, using the Statistical Analysis System (SAS) software (SAS 2008) general linear model procedure (Proc GLM), and means comparisons by Least significant difference test (LSD Test) at  $p < 0.05$ , according to the statistical model:  $Y_{ijk} = \mu + B_j + S_i + G_{k(i)} + e_{ijk}$ , where  $Y_{ijk}$  = observation of  $k$ th genotype (accession) nested to

**Table 1** List of sugarcane accessions from the IAC germplasm collection and their respective originating species

Genotype	Species	Genotype	Species
Badilla java	F1 ( <i>S. officinarum</i> )	IS76-155	F1 ( <i>S. officinarum</i> )
IJ76-560	F1 ( <i>S. officinarum</i> × ?)	IJ76-418 red	F1 ( <i>S. officinarum</i> )
NG77-18	F1 ( <i>S. officinarum</i> )	NG57-50	Hybrid ( <i>S. officinarum</i> ) × ?
NG57-213	F1 ( <i>S. officinarum</i> )	Ceram red	<i>S. officinarum</i>
Caina fita	F1 ( <i>S. officinarum</i> )	Badilla	<i>S. officinarum</i> × NG96
Pitu	F1 ( <i>S. officinarum</i> )	Sac. off. 8276	Hybrid ( <i>S. officinarum</i> × ?)
Caiana	F1 ( <i>S. officinarum</i> )	Fiji19	F1 ( <i>S. officinarum</i> )
Formosa	F1 ( <i>S. officinarum</i> × ?)	IJ76-313	F1 ( <i>S. officinarum</i> )
IN84-126	F1 ( <i>S. officinarum</i> )	NG57-12	<i>S. robustum</i>
Manteiga	F2 [(F1) <i>S. officinarum</i> × ?]	IM76-229	<i>S. robustum</i>
Ajax	F2 ( <i>S. officinarum</i> )	IJ76-293	<i>S. robustum</i> × ?
IJ76-566	F1 ( <i>S. officinarum</i> × ?)	US57-141-5	<i>S. robustum</i>
IJ76-317	F1 ( <i>S. officinarum</i> × ?)	SES205A	<i>S. spontaneum</i>
NG21-21	F1 ( <i>S. officinarum</i> )	IN84-58	<i>S. spontaneum</i> × ?
IJ76-325	F1 ( <i>S. officinarum</i> × ?)	Krakatau	<i>S. spontaneum</i>
Sabura	Hybrid ( <i>S. officinarum</i> × ?)	US851008	<i>S. spontaneum</i> × US60-313
White transp.	<i>S. officinarum</i>	IN84-88	<i>S. spontaneum</i>
Cana alho	<i>S. officinarum</i>	IN84-82	<i>S. spontaneum</i> × ?
MZ151	F1 ( <i>S. officinarum</i> )	Gandacheni	<i>S. barberi</i> × ?
IN84-105	F1 ( <i>S. officinarum</i> × ?)	Chin	<i>S. barberi</i> × ?
Caiana risc.	F1 ( <i>S. officinarum</i> )	Chunnee	<i>S. barberi</i>
Zopilota	<i>S. officinarum</i> × ?		

ith specie in jth block,  $\mu$  = overall mean,  $B_j$  = effect of jth block,  $S_i$  = effect of ith specie,  $G_k$  = effect of kth genotype nested to ith specie, and  $e_{ij}$  = random error, estimating the experimental variance. Data were presented as grade mean and  $\ln(x + 5)$  transformed, in order to approach the normal distribution (Berry 1987).

The accessions with maximum grade from 2 to 3 were classified as susceptible. Accessions with maximum grade of 1 were classified as intermediary. Accessions without mosaic symptoms were classified as resistant; even those with positive results in PTA-ELISA, characterizing latent infections, but only accessions free from SCMV were pointed out as sources of resistance for future crosses.

## Results and Discussion

Variance analysis showed significant values ( $p < 0.01$ ) for specie and accession nested to species, whereas non-significant effect was observed for block, indicating variability among accessions and species. Unfolding the variance within species, significant value ( $p < 0.01$ ) was observed for *S. officinarum*, indicating variability in response to mosaic infection among accessions within this specie, while accessions within *S. barberi*, *S. robustum* and *S. spontaneum* did not vary significantly (Table 2). Accessions from *S. officinarum*, was

**Table 2** Variance analysis of mosaic symptoms grades in accessions from the IAC germplasm collection

F.V.	D.F.	Q.M.	F
Block	1	0.038	2.41 <sup>ns</sup>
Specie	3	0.102	6.45 <sup>**</sup>
Genotype nested in species (sp)	39	0.036	2.27 <sup>**</sup>
<i>S. barberi</i>	2	0.000	0.00 <sup>ns</sup>
<i>S. officinarum</i>	29	0.045	2.76 <sup>**</sup>
<i>S. robustum</i>	3	0.030	1.83 <sup>ns</sup>
<i>S. spontaneum</i>	5	0.000	0.00 <sup>ns</sup>
Residual	38	0.016	
CV (%)			7.29

ns =  $p > 0.05$

sp specie

\*\*  $p < 0.01$

the most susceptible according to LSD test ( $p < 0.05$ ), followed by *S. robustum*, while *S. spontaneum* and *S. barberi* were resistant (Table 3). The most susceptible accessions, according to the LSD test ( $p < 0.05$ ) were IJ76-560 and IN84-126, followed by Ajax, NG57-213, NG77-18 and Caiana fita, all belonging to *S. officinarum* (Table 4). According to Koike and Gillaspie (1989) within the genus *Saccharum* and depending on the SCMV strain involved, *S. officinarum* is the most susceptible to mosaic, while *S. barberi* and *S. robustum*

**Table 3** Mean comparisons of *Saccharum* species of mosaic symptoms grade by LSD test ( $p < 0.05$ )

Species	Mean <sup>a</sup>	Mean <sup>b</sup>	
<i>S. officinarum</i>	0.953	(1.765)	a
<i>S. robustum</i>	0.500	(1.697)	ab
<i>S. spontaneum</i>	0.000	(1.609)	b
<i>S. barberi</i>	0.000	(1.609)	b

a, b means with the same letter are not significantly different

<sup>a</sup> Non transformed data

<sup>b</sup>  $\ln(x + 5)$  transformed data

are moderately susceptible, *S. sinense* and *S. spontaneum* are resistant. On the other hand, in early reports, accessions of *S. spontaneum* were immune to SCMV with few exceptions, attesting the resistance of this species (Summers et al. 1948; Brandes et al. 1939; Brandes and Sartoris 1936; Jeswiet 1930). In subsequent studies, several accessions of *S. spontaneum*

were found to be susceptible to SCMV, possibly due to the development of new strains of the virus (Koike 1980; Abbott and Todd 1963). Differences in the percentage means of infection by SCMV strain H were observed in sugarcane accessions by Grisham et al. (1992), being accessions of *Erianthus*, *S. spontaneum*, *S. barberi* and *S. sinense* the most resistant and accessions of *S. robustum* the most susceptible, while interspecific hybrids and accessions of *S. officinarum* were intermediate. The authors also observed differences in accessions response within each evaluated taxon, with a considerable range of mosaic incidence among accessions of *S. spontaneum*. Li et al. (2013) observed that 17 out of the 37 tested *S. spontaneum* accessions were highly to moderately resistant to SrMV, being pointed, along with *Erianthus arundinaceus*, as valuable germplasm in terms of resistance to SrMV HH.

To date, the response to SCMV of the accessions investigated in the present study was unreported. In

**Table 4** Mean comparisons of the accessions for mosaic symptoms grade by LSD test ( $p < 0.05$ )

Accessions	Species <sup>a</sup>	Mean <sup>b</sup>	Mean <sup>c</sup>		Genotype	Species <sup>a</sup>	Mean <sup>b</sup>	Mean <sup>c</sup>	
IJ76-560	S. off.	3.000	(2.079)	A	Sac.off. 8276	S. off.	0.000	(1.609)	d
IN84-126	S. off.	3.000	(2.079)	A	NG21-21	S. off.	0.000	(1.609)	d
Ajax	S. off.	2.464	(2.010)	Ab	Cana alho	S. off.	0.000	(1.609)	d
NG57-213	S. off.	2.464	(2.010)	Ab	IN84-105	S. off.	0.000	(1.609)	d
NG77-18	S. off.	2.464	(2.010)	Ab	Zopilota	S. off.	0.000	(1.609)	d
Caina fita	S. off.	2.464	(2.010)	Ab	IS76-155	S. off.	0.000	(1.609)	d
IJ76-325	S. off.	2.000	(1.946)	Abc	IJ76-418 red	S. off.	0.000	(1.609)	d
IJ76-293	S. rob.	1.450	(1.864)	Abc	NG57-50	S. off.	0.000	(1.609)	d
Caiana	S. off.	1.450	(1.864)	Abc	Badilla	S. off.	0.000	(1.609)	d
Pitu	S. off.	1.000	(1.792)	Abcd	Fiji19	S. off.	0.000	(1.609)	d
Manteiga	S. off.	1.000	(1.792)	Abcd	IJ76-313	S. off.	0.000	(1.609)	d
Badilla Java	S. off.	1.000	(1.792)	Abcd	IM76-229	S. rob.	0.000	(1.609)	d
Formosa	S. off.	1.000	(1.792)	Abcd	US85-1008	S. spo.	0.000	(1.609)	d
Caiana risc.	S. off.	0.732	(1.746)	Bcd	SES205A	S. spo.	0.000	(1.609)	d
White transp.	S. off.	0.732	(1.746)	Bcd	Krakatau	S. spo.	0.000	(1.609)	d
IJ76-317	S. off.	0.732	(1.746)	Bcd	IN84-58	S. spo.	0.000	(1.609)	d
IJ76-566	S. off.	0.732	(1.746)	Bcd	IN84-88	S. spo.	0.000	(1.609)	d
Sabura	S. off.	0.732	(1.746)	Bcd	IN84-82	S. spo.	0.000	(1.609)	d
MZ151	S. off.	0.732	(1.746)	Bcd	Chunnee	S. bar.	0.000	(1.609)	d
NG57-12	S. rob.	0.414	(1.689)	Cd	Gandacheni	S. bar.	0.000	(1.609)	d
US57-141-5	S. rob.	0.000	(1.609)	D	Chin	S. bar.	0.000	(1.609)	d
Ceram red	S. off.	0.000	(1.609)	D					

a, b, c, d means with the same letter are not significantly different

ns =  $p > 0.05$

\*\*  $p < 0.01$

<sup>a</sup> Species: (S. off.) *S. officinarum*, (S. rob.) *S. robustum*, (S. spo.) *S. spontaneum*, (S. bar.) *S. barberi*

<sup>b</sup> Non transformed data

<sup>c</sup>  $\ln(x + 5)$  transformed data

**Table 5** Mosaic symptoms grades and PTA-ELISA specific for SCMV of sugarcane accessions after 6 months of exposition in the experimental field

Genotype	ELISA <sup>a</sup>	Maximum grade <sup>b</sup>	Mosaic resp. <sup>c</sup>	Accessions	ELISA <sup>a</sup>	Maximum grade <sup>b</sup>	Mosaic resp. <sup>c</sup>
<i>S. officinarum</i> accessions							
Badilla Java	+	1	I	Sabura	+	2	S
IJ76-560	+	3	S	White transp.	+	2	S
NG77-18	+	3	S	Cana alho	+	0	R
NG57-213	+	3	S	MZ151	+	2	S
Caina fita	+	3	S	IN84-105	+	0	R
Pitu	+	1	I	Caiana risc.	+	2	S
Caiana	+	2	S	Zopilota	+	0	R
Formosa	+	3	S	IS76-155	–	0	R
IN84-126	+	3	S	IJ76-418 red	–	0	R
Manteiga	+	3	S	NG57-50	–	0	R
Ajax	+	3	S	Ceram red	–	0	R
IJ76-566	+	2	S	Badilla	–	0	R
IJ76-317	+	2	S	Sac.off. 8276	–	0	R
NG21-21	+	0	R	Fiji19	–	0	R
IJ76-325	+	2	S	IJ76-313	–	0	R
<i>S. robustum</i> accessions							
IJ76-293	+	2	S	IM76-229	+	0	R
NG57-12	+	1	I	US57-141-5	–	0	R
<i>S. spontaneum</i> accessions							
US85-1008	+	0	R	Krakatau	–	0	R
SES205A	+	0	R	IN84-88	–	0	R
IN84-58	–	0	R	IN84-82	–	0	R
<i>S. barberi</i> accessions							
Chunnee	–	0	R	Chin	–	0	R
Gandacheni	–	0	R				

<sup>a</sup> PTA-ELISA results (–) negative and (+) positive

<sup>b</sup> Higher grade observed for mosaic symptoms

<sup>c</sup> *S* Susceptible, *I* Intermediary and *R* Resistant

addition, the strain SCMV-Rib1 that prevails in the experimental area where the trials were conducted was responsible for mosaic outbreaks in the state of São Paulo in cultivars previously thought to be resistant to mosaic (Gonçalves et al. 2007). The response to natural infection by mosaic observed among *Saccharum* species in our study was similar to that reported by Koike and Gillaspie (1989) for *S. officinarum* and *S. robustum* and to the results presented by Grisham et al. (1992) for *S. barberi* accessions.

The combined results from grade scale symptoms and PTA-ELISA indicate that the accessions IS76-155, IJ76-418 red, NG57-50, Ceram red, Badilla, Sac. off. 8276, Fiji19, IJ76-313 (*S. officinarum*); accession US 57-141-5 (*S. robustum*); accessions Krakatau, IN84-58, IN84-88 and IN84-82 (*S. spontaneum*); accessions Chunnee, Gandacheni and Chin (*S. barberi*) were SCMV free, characterizing promising sources for selecting resistance to mosaic. Although being the most susceptible specie, 8 out of the 30 tested *S.*

*officinarum* clones were pointed out as source of resistance. The higher number of *S. officinarum* accessions and the polycross origin of NG57-50 and Sac. off. 8296, which may have involved species resistant to mosaic, had contributed to the range of responses to mosaic here observed. Besides the expected variation within species, according to the literature, future studies involving a higher number of accessions by specie are required to a better understanding of specie response to mosaic. Despite symptomless, the accessions NG21-21, Cana alho, IN84-105, Zopilota, US85-1008 and SES205A had the presence of SCMV diagnosed by PTA-ELISA (Table 5), what partly prevents their recommendation for future crosses focusing on resistance to mosaic.

As the mosaic disease impairs photosynthetic efficiency, reduction in growth and yield parameters directly affecting biomass and consequently ethanol production are usually observed in infected crops (Viswanathan and Balamurali-krishnan 2005), therefore selection of wild *S. spontaneum*

and *S. robustum* accessions resistant to mosaic to be used as parents would be highly useful in the introgression programs focused in increase of biomass.

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