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RESEARCH ARTICLE

REACTION OF SUGARCANE GENOTYPES TO STRAINS OF THE *SUGARCANE MOSAIC VIRUS*

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ABSTRACT

Sugarcane crops are cultivated in nearly all tropical and sub-tropical regions worldwide andhave a prominent position in the agricultural scenario in Brazil. However, viral diseases can threaten the production of this important commodity leading to large production losses. In the present study, we evaluatedthe reaction of 20 sugarcane genotypes independently inoculated with two different strains of *Sugarcane mosaic virus* (SCMV) isolated from naturally infected sugarcane (SCMV-SGC) and maize (SCMV-MZ). The maize inbred line L19 was used as a control of susceptibility to the SCMV-MZ strain. Symptoms intensity was evaluated through a visual scale with three levels of severity: weak, intermediate, and intense. The viral infection was confirmed by PCR and DNA sequencing. We observed that, although both strains were able to infect sugarcane genotypes and the maize inbred line, SCMV-SGC was more aggressive, resulting in only four resistant genotypes: IN84-58 (*S. spontaneum*), RB855536, RB 928064, and SP71-6163. Thirteen genotypes were resistant to SCMV-MZ: IN84-58 (*S.spontaneum*), NA56-79, CB47-355,CB49-260, RB72454, RB855113, RB855536, RB867515, RB928064, SP70-1143, SP71-1406, SP71-6163, and SP81-3250. This is the first report showingSCMV strains capable of cross-infectingand causing mosaic in sugarcane and maize. Our data emphasize the importance of continuous monitoring and screening for virus resistantgenotypes to be used in breeding programs for the development of new resistant cultivars.

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INTRODUCTION

Sugarcane (*Saccharum* spp Hybrids) is considered an important clean, renewable, and sustainable source of energy for the cogeneration of electricity and cellulosic ethanol from bagasse (Hofsetz and Silva, 2012). Brazil is the world's leading sugarcaneproducer, with a planted area of 9.05 million hectares and a production of 657.18 million tons in the 2016/17 harvest (Conab, 2016).The country is also top sugar producer and exporter, and the second leading ethanol producer and exporter (Assunção *et al*., 2016). However, sugarcane production can be threatened by viral diseases, which can lead to epidemics and result in large losses.

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Seven species of potyvirus have been reported as causing mosaic in various cereals and grasses worldwide, including: *Sugarcane mosaic virus* (SCMV), *Sorghum mosaic virus* (SrMV), *Maize dwarf mosaic virus* (MDMV), *Johnsongrass mosaic virus* (JGMV) (Shukla *et al*., 1994), *Sugarcane streak mosaic virus* (SCSMV) (Hall *et al*., 1998), *Zea mosaic virus* (ZeMV) (Sheifers *et al*., 2000), and *Pennisetum mosaic virus* (PenMV) (Deng *et al*., 2008). Although belonging to the same subgroup as SCMV, the species MDMV, JGMV, ZeMV, and PenMV have never been isolated from sugarcane (Chatenet *et al*., 2005), thus indicating that only SCMV, SrMV, and SCSMV can infect the crop under natural conditions (Gonçalves *et al*., 2012). In Brazil, the two main viruses infecting sugarcane are the *Sugarcane mosaic virus* (SCMV) and the *Sugarcane yellow leaf virus* (SCYLV) (Gonçalves *et al*., 2012). SCMV stands out as one of the most economically relevant, (Silva, 2014), reducing yields in terms of tons of cane

ha-[1] and tons of sucrose ha-1 (Yao *et al*., 2017). Mosaic epidemics were first reported in sugarcane in the 1920's in Argentina, Brazil, Cuba, Puerto Rico, and theUSA, leading to a near collapse of the sugarcane industry (Abbott, 1961; Yang and Mirkov, 1997). In Brazil, mosaic epidemics were observed between the 1920's and 1930's as a consequence of the planting of the susceptible varieties (Gonçalves *et al*., 2012). The virus was subsequently controlled using resistant hybrids. However, the alleged eradication of the mosaic, susceptible varieties began to be planted again, thus causing the resurgence of new cycles of the disease (Gonçalves *et al*., 2005). Roguing (digging out and destroying diseased plants) is another virus control practice adopted in nurseries intended for the production of commercial seedlings. However, rouguing is not considered feasible when the infection level is higher than 5% (Rott *et al*., 2015). Thus, the most important strategy in disease controlcontinues to be the cultivation of resistant genotypes. Miller (2008) recommends the use of a large number of varieties, each occupying considerable areas, but never exceeding 10%-20%. This strategy minimizes the risk of loss of productivity in case of epidemics by viruses, with the rapid replacement of the susceptible variety by a resistant one (Barbosa *et al*., 2012). Although studies have shown that the mosaic in sugarcane in Brazil is caused only by strains of the SCMV species (Gonçalves *et al*., 2007, Gonçalves *et al*., 2012), these are different from the SCMV strains previously reported infecting maize and sorghum, which constitute a distinct monophyletic group (Souza *et al*., 2012). In addition, the ability of SCMV to infect diverse *Poaceae* weeds increases the likelihood of new strains or potyvirus species from the mosaic complex to spread in the field (Gonçalves *et al*., 2007). Moreover, the maize-sugarcane consortium in forage production (Botelho and Cabezas, 2007), the use of cornethanolas an alternative raw material in the sugarcane inter-harvest period (Ondei, 2016), and the extensive planting of maize in areas close to sugarcane fields have contributed to spread diseases common to these two Poaceae (Gonçalves *et al*., 2007). Indeed, it is very common to observe mosaic symptoms in sugarcane varieties that are not resistant to this disease when grown near sorghum or corn plantations (personal communication co-author Márcio Henrique Pereira Barbosa). The relevance of the common mosaic viruses and the threat that they represent for the production of sugarcane emphasize the need forcontinuous monitoring and screening for virus-resistant genotypes to be used in breeding programs aiming to develop new resistant cultivars. The present study evaluated the reaction of sugarcane genotypes to SCMV strainsinfecting the sugarcane genotype RB72454 (SCMV-SGC, NCBI N.MF682978) and corn cultures (SCMV-MZ, NCBI N.MF682979) under field conditions.

MATERIALS AND METHODS

Planting and experimental protocol

The study was conducted at Embrapa Maize and Sorghum, SeteLagoas (state of Minas Gerais, Brazil), from April to August / 2015, in 26-liter pots, in a greenhouse with exhaust fan cages protected with an anti-aphid screen. The experimental unit of sugarcane constituted of a pot containing approximately six tillers with 30 days of vegetative development after the regrowth of the cut plants. To establish the plants, each pot was planted with two stem segments with 2-3 buds each. For maize, three plants/pot were used, with two replicates per treatment. Preventive insecticide application was performed to avoid the occurrence of insects. The mean values recorded inside the greenhouse during the experimental period were: daytime temperature = 25.56^{o}C, maximum temperature = 30.90^{o}C, minimum temperature = 14.56^{o}C, and relative humidity = 58.66%. The pots were filled with dark red Latosol soil with pH corrected and fertilized according to soil analysisat a rate of400 kg/ha of the formula08-28-16 + 0.3% Zn and 40 kg/ha of FTE Br 12. Eight months after the planting of the sugarcane genotypes, the plants were cut and cover fertilized with nitrogen and potassium at a rate of 400 kg / ha of the formula 20-00-20.

Genotypes

Twenty genotypes of sugarcane were selected, including the varieties currently cultivated in Brazil (RB855536, RB867515, RB92579, RB928064, and RB937570) and varieties that have been widely used in genetic improvement breeding programs (NA56-79, CO413, CO740, CB45-3, CB47-355, CB49-260, RB72454, RB855113, RB925268, SP70-1143, SP71-406, SP71-6163, and SP81-3250). One genotype of *S. officinarum* (Cana Blanca) and one of *S. spontaneum* (IN84-58) were also included. As controls, we used the sugarcane genotype RB72454 (from which the SCMV-SCG strain was isolated), and the maize inbred line L19, susceptible to the SCMV-MZ strain.

SCMV strains

During the survey for common mosaicviral disease in maize and sugarcane crops carried out in the state of Minas Gerais (MG, Brazil) in the 2014/2015 crop season, plants presenting typical mosaic symptoms (i.e., small chlorotic areas interspersed with green areas) had their foliar tissue collected and the isolates identified through molecular analyzes. The sugarcane strain (SCMV-SGC) was collected from the variety RB72454 in the municipality of Pompéu (state of Minas Gerais, Brazil), while the maize strain (SCMV-MZ) was collected in the municipality of SeteLagoas (state of Minas Gerais, Brazil).The sequences of the gene and the translated coat protein (CP) of these strains were deposited inGenBank under the accession numbers MF682978andMF682979, respectively. The maize strain was maintained in the inbredline L19, which wasour control of susceptibility to SCMV-MZ. To maintain the sugarcane strain SCMV-SGC, parts of the stem of the naturally infected variety RB72454 were collected in the field and planted in the greenhouse.

Treatments, inoculations, and phenotypic evaluations

The treatments tested were: (i) inoculum of the SCMV strain from sugarcane, (ii) inoculum of the SCMV strain from maize, and (iii) a negative control for inoculation (mock treatment). The two SCMV strains (SCMV-SGC and SCMV-MZ) were confirmed molecularly and the inoculums were individually prepared using leaves of the plants symptomatic for the common mosaic macerated in cooled phosphate buffer (10 mM, pH 7.0), at a ratio of 1:3 (weight/volume) (Souza *et al*., 2008). Carborundum 600 mesh (Sigma-Aldrich) was added to the inoculum solution, which was kept on ice throughout the inoculation process. The first inoculation was performed one month after regrowth, in the middle part of the leaves, using mechanical friction with the aid of a sponge with anabrasive surface containing the inoculum. The same mechanical friction procedure was adopted for the mock treatmentusing phosphate buffer (10 mM, pH 7.0) containing 600 mesh carborundum.

Three inoculations were performed at weekly intervals. The phenotypic evaluations were initiated 15 days after the first inoculation and were performed weekly for three months so that late manifestations of mosaic symptom could also be evaluated. Phenotypes were evaluated based on symptoms intensity and classified using a visual scale with three levels of severity: weak, intermediate, and intense.

Molecular analysis and DNA sequencing

Plant leaf tissue of all genotypes, from all the three treatments, were collected and immediately frozen in liquid nitrogen. RNA was extracted using the RNeasy®Plant Mini Kit (Qiagen) and cDNA synthesized from 1.0 µg total RNA usingoligo $(dT)_{18}$ andthe SuperScript®III First-Strand Synthesis System (Invitrogen). Molecular confirmation of the strains was performed by a two-step PCR. In the first step, we used primers that amplify the conserved region of the CP of potyvirus (Table 1). Amplification at this first roundindicated the presence of the virus. Then, the positive samples werePCR amplified with primers specific for the CP and a partial sequence of the nuclear inclusion protein (NIb) (Table 1). These primer sets amplify the N-terminal portion of the CP, which encodes the virus-specific determinants, thus allowing the identification of the SCMV strain by sequencing (Shukla *et al*., 1989). All PCR reactions were performed according to Souza and Barros, 2016. Amplicons were purified using ExoSAP-IT For PCR Product Cleanup (USB) and sequenced. The obtained sequences were analyzed using the Sequencher 1.4.1 software, and tested by sequence similarity searches against the NCBI sequence database (GenBank) to confirm their identities.

Pairwise sequence comparison and phylogenetic analysis

In our analysis, we used a data set with 23potyviruses CP nucleotide sequences (including part of the NIb) containing SCMV maize and sorghum strains and five other species as outgroups:*Maize dwarf mosaic virus* A (MDMV-A_U07216.1), *Zea mosaic virus* (ZeMV_AF228693.1), *Pennisetum mosaic virus* (PenMV_JX070151.1), *Johnsongrass mosaic virus* (JGMV_U07217.1), and *Sorghum mosaic virus* (SrMV_KM025045.1). Sequences comprised the complete ORF of the protein or were at least 700 base pairs (bp) long. Sequences obtained in the present study were downloaded from the NCBI database, apart from the SCMV-MZ and SCMV-SGCsequences. Alignments were generated and visually verified using ClustalOmega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and MUSCLE (executed in MEGA7 - Kumar, Stecher, and Tamura, 2016). Pairwise comparisons were performed using SDTv. 1.2 (Muhire *et al*., 2014).

The phylogenetic reconstruction was carried out with MEGA7 (Kumar, Stecher, and Tamura, 2016) based on the Neighbor-Joining method (Saitou and Nei, 1987), and the evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). The tree was visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and the percentage of replicate trees in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985).

RESULTS

Expression of mosaic symptoms

After the inoculations with the SCMV-SGC and SCMV-MZ strains, the sugarcane and maize susceptible genotypes presented the typical mosaic, with variable patterns of symptoms. The initial symptoms of the disease were characterized by the appearance of chlorotic points with linear distribution in the central portion of the leaf, most commonly at the base (Gonçalves *et al*., 2007) that evolved into typical symptoms such assmall chlorotic areas interspaced by green or into bands following the direction of the leaf blade length (Figure 1). The phenotypic evaluation based onsymptoms intensity is shown in Table 2, and the intensity levels can be seen in the genotypes CO740 (intense) and CO413 (intermediate) inoculated with SCMV-SGC, andin the genotype CO740 (weak) inoculated with SCMV-MZ (Figure 1).

Molecular confirmation of the strains present in the inoculums and in the symptomatic samples of maize and sugarcane: The primers PZEOF and PZEOR, developed for the general detection of potyvirus, annealed in the DNA region corresponding to the conserved motifs of the potyvirus CP MVWCIENG and QMKAA, respectively, and produced amplicons of approximately 330 bp in the SCMV-infected symptomatic samples of sugarcane and maize (Table1). The samples inoculated with the sugarcane strain thatwas positive in the first step of the PCR(i.e., produced amplicons with the PZEOF/PZEOR primer set) produced amplicons of approximately 900 bp when the set of primers SCMV_F4/SCMV_R3 were tested. The primer SCMV_F4 is specific for the sequence corresponding to the amino acids VHFQAGTV. In this sequence, the first four amino acids correspond to the cleavage site between NIb and CP and the lastfour correspond to the initial portion of CP (Seifers *et al*., 2000). The use of this primer allows for the specific amplification of the SCMV-SGC CP, which begins with AGTV, while the CP originated from maize begins with SGTV. On the other hand, the samples inoculated with the maize strain that was positive in the first amplification produced amplicons of 1200 bp and 1072 bpwhen the sets of primers NI2/SCMVR3 and MDMV2/MDMV3 were tested,

Table 1. Nucleotide sequences and expected amplicon sizes of the primer pairs used for PCR amplification and detection ofpotyvirus and *Sugarcane mosaic virus* (SCMV)

Potyvirus	Primer	Sequence5'-3'	Amplicon (bp)	Reference
Potyviruses in general	PZEO_F	GTATGGTGCATCGAAAATGGT	330	SEIFERS *et al.*, 2000
	PZEO_R	TGCTGCTGCTTTCATCTG		
SCMV	MDMV2	GTATTCCATCAGTCGGGAACTG	1072	RESENDE *et al.*, 2004
	MDMV3	ACGAGGTAAAACCTCAC		
SCMV	[a]SCMV_F4	GTTTTYCACCAAGCTGGAACAGTC	900	ALEGRIA *et al.*, 2003; YANG & MIRKOV, 1997
	SCMV_R3	AGCTGTGTGTCTCTCTGTATTCTC		
SCMV	[a]NI2	GARGCATGGGGATA	1200	GONÇALVES *et al.*, 2011, YANG & MIRKOV, 1997
	SCMV_R3	AGCTGTGTGTCTCTCTGTATTCTC		

[a]Y= (C ou T); R = (A ou G).

respectively. The MDMV2/MDMV3 is specific for the detection of the Brazilian maize strain of SCMV (Resende*et al*., 2004). In agreement with these observations, sequencing of the strains confirmed that the SCMV-SGC was present in the sugarcane inoculumwhile SCMV-MZ was present in the maize inoculum. Among the 20 sugarcane genotypes evaluated for the SCMV-SGC strain, only four were resistant, asymptomatic, and showed negative results in the molecular analysis: IN84-58 (*S.spontaneum*) and the varietiesRB855536, RB928064, and SP71-6163. The maize line L19 was also susceptible to this strain.

we performed molecular analyses to confirm the absence of potyviruses in the genotypes that did not present mosaic symptoms. The same analysis was carried out on thenegative controls that were inoculated withphosphate buffer containing carborundum only. In these two situations, the analyzesproduced negative results, thus confirming the absence of viruses in these samples.

Pairwise sequence comparison of the strains SCMV-SGC and SCMV-MZ: The hypervariable N-terminal portion of the CPs has different repeat sequences between the strains, and the

Table 2. **Observation records following artificial inoculationof sugarcane genotypes withthe strains SCMV-SGC and SCMV-MZ. The primers sets used and the symptoms intensity are indicated in the columns.The signals (+) and (-) represent the presence and absence of the amplicons, respectively**

Genotype	SCMV-SGC Strain					SCMV-MZ Strain				
	Primers Set				Symptoms Intensity	PrimersSet				Symptoms Intensity
	PZEO1 x PZEO2	MDMV2 x MDMV3	NI2 x SCMVR3	SCMVF4x SCMVR3		PZEO1 x PZEO2	MDMV2x MDMV3	NI2 x SCMVR3	SCMVF4 x SCMVR3	
Cana Blanca (*S.officinarum)*	+	-	-	+	Intense	+	+	+	-	Intense
IN84-58 (*S. spontaneum*)	-	-	-	-	-	-	-	-	-	-
NA56-79	+	-	-	+	Intense	-	-	-	-	-
CO413	+	-	-	+	Intermediate	+	+	+	-	Intemediate
CO740	+	-	-	+	Intense	+	+	+	-	Weak
CB45-3	+	-	-	+	Intense	+	+	+	-	Intense
CB47-355	+	-	-	+	Intense	-	-	-	-	-
CB49-260	+	-	-	+	Intense	-	-	-	-	-
RB72454	+	-	-	+	Intense	-	-	-	-	-
RB855113	+	-	-	+	Intense	-	-	-	-	-
RB855536	-	-	-	-	-	-	-	-	-	-
RB867515	+	-	-	+	Intense	-	-	-	-	-
RB925268	+	-	-	+	Intermediate	+	+	+	-	Intense
RB92579	+	-	-	+	Intense	+	+	+	-	Weak
RB928064	-	-	-	-	-	-	-	-	-	-
RB937570	+	-	-	+	Intense	+	+	+	-	Intense
SP70-1143	+	-	-	+	Weak	-	-	-	-	
SP71-1406	+	-	-	+	Intermediate	-	-	-	-	-
SP71-6163	-	-	-	-	-	-	-	-	-	-
SP81-3250	+	-	-	+	Intermediate	-	-	-	-	-
Maize L19	+	-	-	+	Weak	+	+	+	-	Intense

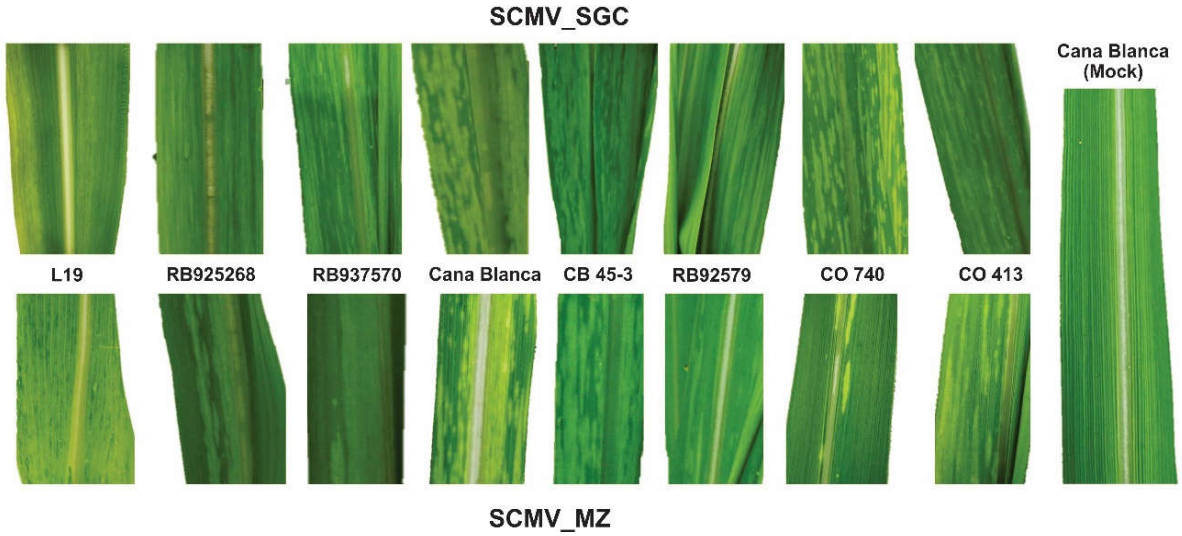

Figure 1. Mosaic symptoms following artificial inoculation of SCMV-SGC or SCMV-MZ in maize (L19) and sugarcane (RB925268, RB937570, Cana Blanca, CB 45-3, RB92579, CO 740, and CO 413) genotypes under greenhouse conditions. At the right side, asymptomatic sugarcane from the mock-treat control

The following sugarcane genotypes were susceptible to the SCMV-MZ strain and showed the typical symptoms of mosaic (Figure 1): Cana Blanca (*S.officinarum*), CB45-3, CO413, RB92579, RB937570, RB925268, and CO740. Similar symptoms were observed in the maize line L19, used as a control of susceptibility to this strain (Table 2). Additionally,

SCMV-SGC presents15 aminoacids less than SCMV-MZ. The level of identity between these strains for the partial Nib plus CPnucleotide and amino acid deduced sequences was estimated at 82.08% and 84.85%, respectively. Based onthe CP sequences, molecular data, phylogenetic analysis, and pairwise identity matrix the strains clustered distinctly (Figures 2 and

3A). The cluster formed by the SCMV strains that have sugarcane as host showed less variability than the strains from the maize cluster (Figure 3B).

These strains were isolated in monophyletic groups corresponding to their hosts (Figure 3A), in agreement with the phylogenetic tree presented by Souza *et al*. (2012) using Brazilian strains of SCMV isolated from sugarcane and maize.

```
SCMV_SGC        --------------------IEKYFKQFAKDLPGYLEDYNEEVFHQAGTVDAGAQGGGGN

SCMV_MZ         YIAETALRNLYLGTGIKEEEIEKYFKQFIKDLPGYIEDYNEDVFHQSGTVDAGAQGGTGN
                                    ******** ******:*****:****:********** **

SCMV_SGC        AGTQPPATGAAAQGGAQPPATGAATQP--PST---QGTQPPTG----------GATGGDG

SCMV_MZ         QGTTPPATGGTTGSAAPRTGSGGGTGTGTGATGGQAGTENGAGTGTGATGGQAGTGGGTG
                 ** *****.:: ..*   .:*..*     :*    **:  :*          *: ** *

SCMV_SGC        AQTGAGATGTVTGGQKDKDVD

SCMV_MZ         QTNTGSAGTGATGGQKDKDVD
                  . ..*   .**********
```

Figure 2. Alignment of part of the nuclear inclusion protein (NIb) and the N-terminal amino acid sequences of the coat protein (CP) of the strains SCMV-SGC and SCMV-MZisolated from sugarcane (SGC) and maize (MZ), respectively. The repeated and partially repeated sequences are in gray. The four underlined aminoacids represent the CP initiation

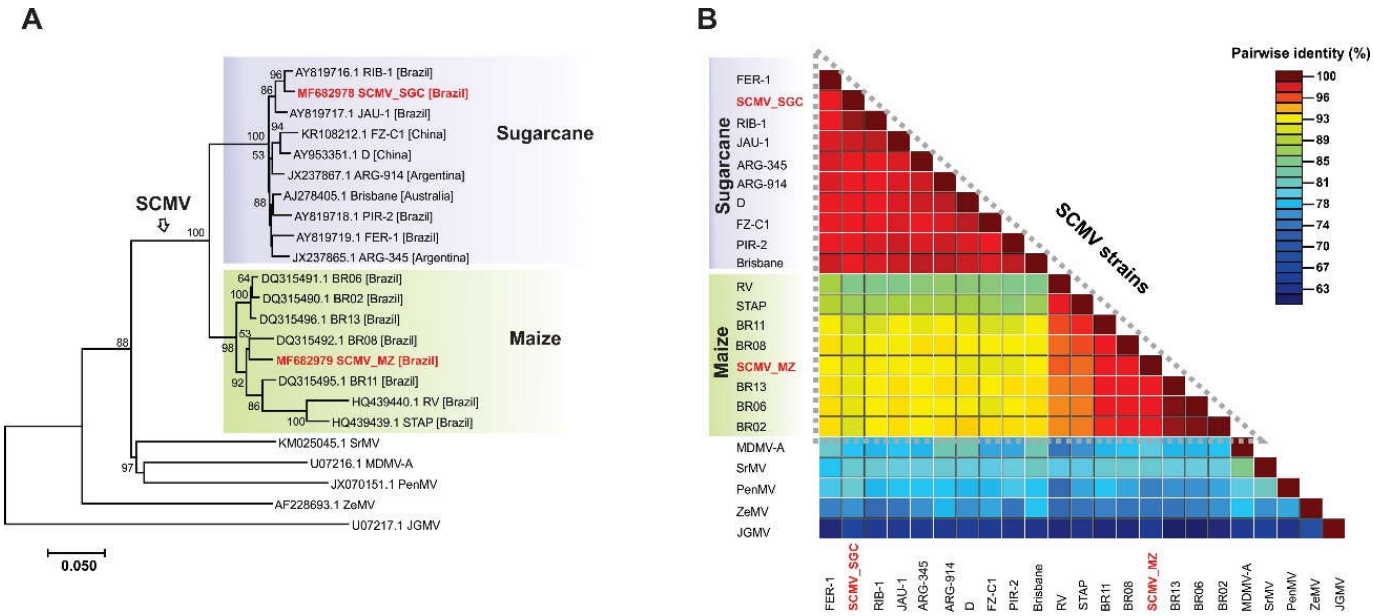

Figure 3. Neighbor-Joining phylogenetic tree inferred from 23 potyvirusescoat protein (CP) nucleotide sequences (including part of the nuclear inclusion protein, NIb) downloaded from GenBankand the SCMV-SGC and SCMV-MZ strains isolated from sugarcane and maize, respectively (A). Color-coded pairwise identity matrix generated from 23 potyviruses sequences. CP nucleotide sequences identity scores are represented by the colored squares (B)

DISCUSSION

The distinct variationsbetween the SCMV-SGC and SCMV-MZ strains were demonstrated by DNA sequencing and alignment of the CP sequences, showing identity percentages of 82.08% and 84.85% at the nucleotide and correspondentaminoacid sequences, respectively (Figures 2 and 3). The potyvirus species demarcation criteria of 76% and 82% identity at the nucleotide and aminoacid sequences, respectively (Wylie *et al*, 2017), allowed the classification of both strainsas belonging to the SCMV species. These percentages do not fit the criteria established by Shukla and Ward (1988), in which strains of individual viruses exhibited sequence homologies of 90 to 99% (average 99%) (Figure 3). The low sequence identity of the two CPs is due to the diversity in the N-terminal region, with a different repeat and partial repeat sequences, and SCMV-SGC is 15 aminoacids smaller in this region (Figure 2).

A higher number of sugarcane genotypes showed susceptibility to the SCMV-SGC strain than to SCMV-MZ. However, the latter was able to infect and produce mosaic symptoms of high intensity in four of the seven genotypes susceptible to this strain (Table 2). These results corroborate the observations in the field, where mosaic symptoms are very common in RB937570 and RB925268, especially when grown near sorghum and maize crops. Indeed, the varieties RB855536, RB928064, and SP71-6163 that were resistant to both strains are considered resistant under field cultivation conditions (personal communication co-author Márcio Henrique Pereira Barbosa). The varieties RB867515 and RB72454 were susceptible to the SCMV-SGC strain. Silva *et al*. (2014) showed that they are also susceptible to the strain SCMV-Rib1(GenBankAY819716;Gonçalves *et al*., 2011),whichwas found responsible for mosaic outbreaks in sugarcane crops in the state of São Paulo, Brazil (Gonçalves *et al*. 2007). On the other hand, the variety SP70-1143, that isresistant to SCMV-Rib1 (Silva *et al*., 2014), exhibitedmosaic symptoms of weak

intensity when inoculated with the SCMV-SGC strain. Altogether, the present data and other previously published results highlight the importance of the interaction between the genotype and the mosaic strain for the manifestation of the disease. Although mosaic symptoms may vary in intensity depending on the cane variety, growing conditions, and the strain of the virus involved (Comstock and Gilbert, 2009), in the present study, the pattern of the mosaic symptoms seemedmore associated withthe genotype. *S. spontaneum*isa widely adapted wild species that presentsgenes for disease and stress resistance (Cheavegatti-Gianotto, 2011). Cultivated sugarcane plants are hybrids derived mainly from crossings between *S. officinarum* and *S. spontaneum* (Dillon *et al*. 2007). Thisemphasizes the importance of the genotype IN84-58 (*S. spontaneum*), which was resistant to both strains tested here (Table 2) and is also resistant to SCMV-Rib1 (Silva *et al*., 2014). SCMV-Rib1 prevails in the municipality of Ribeirão Preto (state of São Paulo, Brazil) (Gonçalves *et al*., 2007), whereas SCMV-SGC was isolated from Pompéu (state of Minas Gerais, Brazil). Nevertheless, they form an isolated group and present 99% and 98% identities when comparing the nucleotide and amino acid sequences of the CP gene, respectively (Figure 3).

The identification of cultivars presenting resistance to only one of the two strains tested (Table 2) suggests the existence of different genes conditioning resistance to the mosaic disease caused by SCMV. Although RB855536, RB855113, and RB937570 are sister genotypes and present common ancestors, they showed different responses to the strains of SCMV, thus preventing the establishment of any kind of inference about the origin of resistance genes,especially considering that interspecific sugarcane hybrids present high genetic complexity (Manners *et al*., 2004). Nevertheless, evidence suggests a diploid-like mode of inheritance (Hogarth, 1987) and, according to Silva *et al*. (2014), resistance to mosaic disease tends to be a quantitative trait, which has implications for the selection methods toward genetic gains. Herein we identified sources of resistance in the germplasms of sugarcane currently cultivated, which helps breeding programs todefine better methods to be used for the incorporation of genes of resistance to SCMV in the development of cultivars. The ability of the SCMV-SGC and SCMV-MZ strains to infect both crops is an important aspect to be considered in extensive and nearby plantations and in maize crops during the sugarcane inter-harvesting. In these two conditions, it is necessary to adopt measures to minimize the possibility of losses in production and consequent economic damage. Barboza *et al*. (2008) highlighted the importance of quarantine measures to allow the movement and exchange of sugarcane germplasm, therebyavoiding the introduction of new SCMV strains in expansion areas of sugarcane, maize, and sorghum crops.

Among the measures of control of viral diseases, the most efficient is the use of resistant cultivars (Rott *et al*., 2015), which reinforces the strategic importance of genetic improvement programs in the development of disease-resistant cultivars. In this sense, the varieties RB855536, RB928064, and SP71-6163 stand out as being resistant to both SCMV strains tested. The genetic diversity identified in the sugarcane genotypes regarding the resistance to the mosaic caused by the SCMV strains tested herein provides important information for the programs that aim at the introgression of resistance genes.Our results demonstrate the importance and the need for continuous surveys and identification studies to allow the early detection of strains and potyvirus species of the mosaic complex not yet reported andcapable of infecting and being transmitted by vectorsamong sugarcane, maize, sorghum, and weeds. More comprehensive studies are also needed to understand the predominance of SCMV-SGC and SCMV-MZ infecting sugarcane and maize crops, respectively, in different producing regions. Additionally, as mentioned by Liu *et al*. (2002) and Dietzgen *et al*. (2016), the interactions between viruses and their insectvectors should be investigated in the process of host infection. Also, further studies on the characterization of alternative hosts not yet reported are needed for both strains.

Conclusion

In conclusion, here we evaluated thereaction of sugarcane genotypes to strains of the SCMV naturally infecting sugarcane and maize crops and described, for the first time, SCMV strains capable of cross-infecting and causing mosaic in sugarcane and maize. We identified sources of resistance in the germplasms of sugarcane currently cultivated thatcan be used in breeding programs developing new resistant cultivars to curb mosaic epidemics.

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