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RESISTÊNCIA DE GENÓTIPOS DE CANA-DE-AÇÚCAR AO *Sugarcane mosaic virus* (SCMV)

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RESISTÊNCIA DE GENÓTIPOS DE CANA-DE-AÇÚCAR AO *Sugarcane mosaic virus* (SCMV)

RESUMO - A resistência a doenças constitui o principal fator de substituição de cultivares na cana-de-açúcar, sendo o mosaico uma das principais doenças da cultura, com registros em quase todos os países produtores. O presente estudo teve como objetivo avaliar a resistência de 79 genótipos de cana-de-açúcar, incluindo variedades e clones elite, inoculados artificialmente com o *Sugarcane mosaic virus* (SCMV) Rib-1 e estimar os parâmetros genéticos associados à resistência por meio de análise de variância. Avaliações de sintomas por escala de notas foram feitas em associação com o teste serológico *Plate Trapped Antibody*-ELISA em um experimento conduzido em estufa e levado em condições de campo. Os genótipos IACSP982053, IACSP972028, RB855156, IACSP993009, IACSP977543, IACSP972000, IACSP962100, IACSP986202, IAC912195, IACSP953028, IAC862480, IACSP972098, IACSP955000, SP701143, IACSP952078, IACSP972020, IACSP967569, IACSP985046, SP803280, IACSP993085, IACSP972055 e IACSP977065 apresentaram-se resistentes à estirpe em estudo. A herdabilidade no sentido amplo calculada foi de 19,37% ao nível de plantas individuais e de aproximadamente 62,18% ao nível de média de parcelas, indicando uma alta influência das condições ambientais na manifestação dos sintomas de mosaico. Acessos de cana-de-açúcar pertencentes à Coleção de Germoplasma do Centro de Cana do Instituto Agronômico de Campinas também foram avaliados em um segundo experimento, com o objetivo de identificar possíveis fontes de resistência ao SCMV para serem utilizadas nos programas de introgressão genética. Foi realizada uma avaliação de sintomas de mosaico por meio de escala de notas em associação com o teste serológico PTA-ELISA em 43 acessos, ao todo, incluindo as espécies *Saccharum officinarum*, *S. barberi*, *S.spontaneum* e *S.robustum*, mantidos em campo em condições de infecção natural. Os clones IS76-155, IJ76-418 red, NG57-50, Ceram red, Badila, Sac,off. 8276, Fiji19 IJ76-313, US 57-141-5, Krakatau, IN8458, IN84-88, IN84-82, Gandacheni e Chin foram apontados como possíveis fontes de resistência. Também foi observado um comportamento diferenciado entre as espécies do gênero *Saccharum*, com maior suscetibilidade em acessos de *S. officinarum* seguidos por acessos de *S. robustum* e resistência nos acessos de *S. barberi* e *S. spontaneum*.

Palavras chave: Fontes de resistência, mosaico, parâmetros genéticos

RESISTANCE OF SUGARCANE GENOTYPES TO *Sugarcane mosaic virus* (SCMV)

ABSTRACT – The resistance to diseases constitutes the main factor of cultivar replacement in sugarcane, being mosaic one of the main diseases of this crop, with records in almost all the major sugarcane growing countries. This study aimed to evaluate the resistance of 79 sugarcane genotypes, including varieties and elite clones, artificially inoculated with *Sugarcane mosaic virus* (SCMV) R1b-1 and estimate genetic parameters associated to mosaic resistance by variance analysis. Evaluations of symptoms by grade scale associated with serological test *Plate Trapped Antibody*-ELISA were performed in a greenhouse experiment that was later taken to field conditions. The genotypes IACSP982053, IACSP972028, RB855156, IACSP993009, IACSP977543, IACSP972000, IACSP962100, IACSP986202, IAC912195, IACSP953028, IAC862480, IACSP972098, IACSP955000, SP701143, IACSP952078, IACSP972020, IACSP967569, IACSP985046, SP803280, IACSP993085, IACSP972055 and IACSP977065 were resistant to the strain in study. The broad-sense heritability at individual level and means based was 19.37% and 62.18%, respectively, which shows a great influence of environmental conditions on the expression of mosaic symptoms. Wild sugarcane germplasm were also evaluated for SCMV resistance in a second experiment, in order to identify new sources of mosaic resistance for future introgression crosses. An evaluation of symptoms by grade scale associated with serological test *Plate Trapped Antibody*-ELISA were performed for 43 clones, including *Saccharum officinarum*, *S. barberi*, *S. spontaneum* and *S. robustum* species, maintained under natural infection conditions. The clones IS76-155, IJ76-418 red, NG57-50, Ceram red, Badila, Sac.off. 8276, Fiji19 IJ76-313, US 57-141-5, Krakatau, IN8458, IN84-88, IN84-82, Gandacheni and Chin possibly represents resistant sources. A differential behavior among *Saccharum* species were also observed, with higher susceptibility in *S. officinarum* clones, followed by *S. robustum* clones, and a resistant behavior of *S. barberi* and *S. spontaneum* clones.

Keywords: Sources of resistance , mosaic, genetic parameters

CAPÍTULO 1 - Considerações gerais

1. INTRODUÇÃO

Em 2012, 1,832 MT (milhões de toneladas métricas) de cana-de-açúcar foram produzidas pelo mundo em uma área de 26,1 milhões de hectares. O Brasil é o maior produtor, contribuindo com 39,4% da produção mundial (721 MT), seguido por Índia (347,8 MT), China (123,4 MT), Tailândia (96,5 MT), Paquistão (58,4 MT), Colômbia (38 MT), Filipinas (30 MT), Estados Unidos (27,9 MT), Indonésia (26,3 MT), Austrália (25,9 MT), Argentina (25 MT) e Guatemala (21,8 MT) (FAOSTAT, 2012).

Segundo a CONAB (2013), a cultura continua em expansão no Brasil, com maiores índices de aumento de área plantada na região Centro-Sul, sendo São Paulo, Minas Gerais, Goiás e Mato Grosso do Sul os estados com maior acréscimo de áreas com 95,9 mil hectares, 60,1 mil hectares, 92,5 mil hectares e 81,4 mil hectares, respectivamente. Devido ao alto potencial produtivo dos programas de melhoramento de cana-de-açúcar existentes no país, é grande o número de variedades em cultivo, o que permite adaptação às diversas condições agroclimáticas. Isso também possibilita uma restrição natural da área plantada com um único cultivar, reduzindo riscos econômicos de perdas com possíveis epidemias (LANDELL; BRESSIANI, 2008).

A resistência a doenças constitui o principal fator de substituição de cultivares na cana-de-açúcar (BRESSIANI, 2001), sendo o mosaico uma das principais doenças da cultura, com registros em quase todos os países produtores (GONÇALVES et al., 2012; VISWANATHAN; MOHANRAJ, 2001). Devido às expressivas perdas causadas pela doença, avaliações para resistência ao vírus constituem um importante passo em programas de melhoramento de cana-de-açúcar.

No Brasil a doença foi relativamente controlada com o desenvolvimento de programas de melhoramento e a adoção de práticas culturais como o roguing em

viveiros, o uso de mudas sadias e o constante monitoramento de campos comerciais (GONÇALVES et al., 2012). No entanto, condições epidemiológicas favoráveis à disseminação da doença em algumas regiões do país e a descrição de novos isolados do vírus, como o *Sugarcane mosaic virus* (SCMV) Rib-1 (GONÇALVES et al., 2007a) responsável por surtos de mosaico no Estado de São Paulo em cultivares e clones até então considerados resistentes demonstram a importância atual da doença no desenvolvimento de novos cultivares.

Existem poucas informações na literatura quanto aos parâmetros genéticos associados à resistência ao mosaico em cana-de-açúcar, principalmente em cultivares utilizados no Brasil. Em adição, as avaliações de seleção de resistência realizadas em experimentos de campo pela maioria dos programas de melhoramento estão sujeitas a um possível comportamento diferenciado de clones e cultivares às diferentes estirpes de SCMV que prevalecem nas diferentes áreas experimentais, além da manifestação de sintomas visuais não ser uma expressão definitiva da patologia viral (HUCKETT e BOTHA 1996).

Estudos utilizando a inoculação artificial de uma estirpe de SCMV conhecida, em associação com a confirmação da infecção por diagnósticos serológicos ou moleculares são de grande contribuição para a seleção de resistência, mesmo em genótipos que não apresentam sintomas característicos.

A diversidade do germoplasma é essencial para o melhoramento genético sustentável de uma cultura. Devido a constatações de uma base genética estreita em populações melhoradas de cana-de-açúcar, o cruzamento entre cultivares comerciais e espécies selvagens de coleções mundiais de germoplasma tem sido realizado em diversos programas de melhoramento pelo mundo. A avaliação de acessos selvagens permite identificar possíveis fontes de resistência ao SCMV e orientar futuros cruzamentos para introgressão de genes de interesse.

2. REVISÃO DE LITERATURA

2.1. A cultura e o melhoramento da cana-de-açúcar

A cana-de-açúcar pertence ao gênero *Saccharum* L., tribo *Andropogoneae*, família *Poaceae*. O gênero possui seis espécies, onde quatro (*Saccharum officinarum* L., *S. sinense* Roxb., *S. barberi* Jesw., e *S. edule* Hassk) tem sido cultivadas tradicionalmente enquanto *S. spontaneum* L. e *S. robustum* Brandes & Jesw. ex Grassl são consideradas espécies selvagens (D' HONT et al. 1998; GRIVET et al. 2004), constituindo o complexo 'Saccharum' juntamente com gêneros próximos como *Erianthus*, *Miscanthus*, *Narenga* e *Sclerostachya*, (DANIELS et al., 1975).

Os programas de melhoramento de cana-de-açúcar têm como objetivo identificar, selecionar e multiplicar os genótipos superiores de uma população de forma que se tornem cultivares que ampliem a produtividade de energia (açúcar, álcool e fibra). Novos cultivares de cana-de-açúcar são produzidos por hibridação direcionada e propagadas vegetativamente após a obtenção de sementes sexuadas. A seleção é praticada em todas as fases do melhoramento: na escolha dos genitores, na escolha dos cruzamentos, nos cruzamentos após o teste de progênie e na população de indivíduos oriundos dos cruzamentos realizados (BRESSIANI, 2001).

As populações utilizadas para a seleção geralmente são produzidas por cruzamentos de cultivares comerciais ou clones pré-comerciais (LANDELL; BRESSIANI, 2008), sendo a magnitude da variância genética liberada no cruzamento entre clones, uma função do número de alelos diferentes por loco em cada clone (SOUZA JUNIOR, 1995). O genótipo de cada planta pode ser transmitido integralmente através de gerações, sendo multiplicado via clonagem através dos colmos, proporcionando o aproveitamento de todos os efeitos gênicos. Essa maior simplicidade de reprodução dos genótipos permite avaliações com altos níveis de precisão através de parcelas maiores, várias repetições, por diferentes locais e anos, sendo a classificação, a seleção e a multiplicação dos indivíduos superiores realizados de forma bastante segura. Para ser liberado como cultivar, o genótipo superior deve se situar próximo da extremidade da distribuição para diversos caracteres simultaneamente (BRESSIANI, 2001).

A estimativa da variância genética, da covariância genética, da herdabilidade e do ganho de seleção para determinado caráter são fundamentais para um

programa de melhoramento vegetal por fornecer informações sobre a variabilidade disponível no germoplasma e se esta é suficiente para o melhoramento. Além disso, possibilita uma estimativa dos gastos necessários para se testar um grupo de genótipos e indica os métodos mais eficientes para produzir um ganho genético apreciável (DUDLEY e MOLL, 1969).

Aproximadamente 100 doenças em cana-de-açúcar causadas por vírus, fungos, bactérias e nematoides foram registradas em diversos países produtores (ROTT et al., 2000). Breaux (1985) ressaltou a dificuldade adicional para o melhoramento quanto à resistência a doenças devido ao surgimento de novas estirpes e raças de patógenos já estabelecidos e a introdução de novas doenças que frequentemente ocorrem no intervalo médio de 8 a 10 anos do melhoramento clássico da cana-de-açúcar. Ainda assim, ganhos com resistência ao mosaico (BREAUX, 1985), escaldadura (CHAO et al., 1989; CHAO et al., 1990) e ferrugem (BISHOFF e GRAVOIS, 2004) foram alcançados.

A seleção para resistência a viroses é um processo importante, sendo conduzido pela maioria dos programas de melhoramento em experimentos de campo sob condições ambientais favoráveis a incidência da doença em áreas de alta infestação, geralmente durante os primeiros estágios de seleção. Avaliações em campo, no entanto, dependem do desenvolvimento de sintomas visuais, os quais não são expressões definitivas da patologia viral (HUCKETT e BOTHA 1996), mas que também dependem da estirpe ou isolado viral que prevalece na área experimental. O mosaico causado pelo SCMV possui várias estirpes (GONÇALVES et al. 2007a, 2007b; PEREIRA et al. 2009; GONÇALVES et al. 2011), levando a respostas diferenciadas em cultivares e clones de cana-de-açúcar.

Estudos de resistência a vírus realizados em casa de vegetação (SCHENCK; LEHRER 2000; PINTO et al. 2013) empregando diferentes métodos de inoculação artificial para SCMV (SRISINK et al. 1994; GEMECHU et al. 2004; CHAVES-BEDOYA et al. 2011) em combinação com diagnósticos moleculares ou serológicos (HUCKETT e BOTHA 1996; BALAMURALIKRISHNAN et al. 2004; PINTO et al. 2013) permitem a seleção de resistência para uma estirpe conhecida mesmo em variedades que não apresentam sintomas, confirmando a presença do vírus e a identificação de estirpe.

2.2. Germoplasma

Segundo Ming et al.(2006), a história das hibridações em cana-de-açúcar pode ser agrupada em cinco períodos distintos: (1) cruzamentos entre canas nobres (*S. officinarum*); (2) nobilização da cana-de-açúcar a partir de cruzamentos entre *S. officinarum* e *S. spontaneum* ; (3) cruzamentos entre os cultivares nobilizados para produzir os cultivares híbridos; (4) cruzamentos entre os híbridos para a produção dos cultivares atuais; e (5) cruzamentos para aumentar a base genética.

Foi constatado que boa parte dos cultivares são híbridos interespecíficos derivados de poucos clones de quatro espécies de *Saccharum: S. officinarum*, *S. spontaneum*, *S. robustum* e *S. sinensis* (ARCENEAUX,1967), e que a base genética de produção de cana-de-açúcar em populações melhoradas pode ser inferior à existente no início do melhoramento, devido à seleção direcional realizada por mais de cem anos (WALKER, 1987). Com isso tornou-se relevante o esforço para aumentar a diversidade do germoplasma disponível.

Diversas expedições foram realizadas nos centros de origem da espécie em busca de genótipos resistentes às doenças, de elevadas produtividades ou de alto conteúdo de sacarose (BERDING e ROACH, 1987) e depositados em coleções mundiais na Índia e nos Estados Unidos, servindo como fonte de variabilidade para o desenvolvimento de novos cultivares, mais produtivos e resistentes às pragas e doenças. Nos últimos cinquenta anos, programas de incorporação genética têm sido realizados em vários países por meio de cruzamentos entre canas selvagens e cultivares comerciais, destacando-se os programas de Barbados e da Louisiana (LANDELL; BRESSIANI, 2008). Mesmo com esses esforços, segundo D' Hont et al. (1998), as espécies *S. officinarum* e *S. spontaneum* tem contribuído para o desenvolvimento da maioria dos cultivares modernos.

No Brasil, no começo do século XX, surtos de mosaico foram responsáveis pela substituição de vários cultivares, levando à introdução de híbridos interespecíficos de *Saccharum* importados de Java para conter a rápida disseminação da doença em canas nobres (*S. officinarum* L.) cultivadas até então (KOIKE; GILLASPIE, 1989). O surgimento de novas estirpes do vírus levou ao

lançamento de novos cultivares e, em busca de novas fontes de resistência, a avaliação do germoplasma da coleção mundial, nos Estados Unidos foi realizada com o objetivo incorporar a resistência ao mosaico de espécies selvagens em novos cultivares (GRISHAM et al., 1992).

No Brasil, em 1989, um programa de ampliação da base genética foi realizado pela extinta Copersucar com o objetivo de produzir parentais com características específicas para uso em cruzamentos comerciais a fim de melhorar a performance das progênies. O programa envolveu três linhas de nobilização: (1) cruzamentos entre *S. officinarum* e *S. spontaenum* para introgredir vigor e capacidade de brotação das soqueiras; (2) cruzamentos entre *S. officinarum* ou híbridos comerciais e *S. robustum* para introgredir vigor e maior teor de sacarose; e (3) cruzamentos entre *S. officinarum* ou híbridos comerciais e *Erianthus arundinaceus* para introgredir fibra, vigor e brotação de soqueira (LANDELL; BRESSIANI, 2008). A avaliação de acessos selvagens pode apontar novas fontes de resistência ao mosaico e direcionar futuros cruzamentos de introgressão.

2.3. Sugarcane mosaic virus (SCMV)

O mosaico da cana-de-açúcar é uma das doenças mais disseminadas no mundo todo, afetando diferentes espécies de poáceas, sendo causada pelos vírus do subgrupo do *Sugarcane mosaic vírus* (SCMV) do gênero *Potyvirus*, família *Potyviridae* (GONÇALVES et al., 2012; VISWANATHAN; MOHANRAJ, 2001). Sete espécies distintas de *Potyvirus* são reconhecidas no grupo: *Sugarcane mosaic virus* (SCMV); *Sorghum mosaic virus* (SrMV), *Sugarcane streak mosaic virus* (SCSMV), *Johnsongrass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV), *Pennisetum mosaic virus* (PenMV) e *Zea mosaic virus* (ZeMV). Segundo Chatenet et al. (2005), as últimas quatro espécies nunca foram isoladas a partir da cana-de-açúcar, mesmo pertencendo ao subgrupo do SCMV, indicando que apenas as espécies SCMV, SrMV e SCSMV podem infectar a cultura em condições naturais. Infecções mistas de SCMV e SrMV foram descritas na Argentina (PERERA et al. 2009) e de SCMV com SCSMV em alguns países da Ásia (CHATENET et al. 2005). No Brasil apenas a

espécie SCMV é encontrada infectando naturalmente a cana-de-açúcar (GONÇALVES et al.,2004, 2007a, 2011).

Os vírions dos potyvirus variam de 700 a 900 nm de comprimento com 11 nm de largura, sendo que o SCMV possui partículas filamentosas flexíveis de 700 a 750 nm de comprimento (HARRISON et al., 1971; ADAMS et al., 2005). Como outros potyvirus, o SCMV é constituído de uma fita simples de RNA senso positiva de aproximadamente 10 Kb de comprimento caracterizada por uma região não codificadora 5', um quadro de leitura aberta (ORF1) e uma cauda de poli adenina (poli A) na região 3'. A região ORF1 compreende dez proteínas funcionais, a proteína 1 (P1), o componente auxiliar de proteinase (HCPro), a proteína 3 (P3), a 6 K1, a proteína de inclusão cilíndrica (CI), a 6 K2, a proteína viral covalentemente ligada ao genoma (VPg), a principal protease da proteína de inclusão a, NIa (NIa-Pro), a proteína de inclusão nuclear b (NIb), a proteína capsidial (CP) (SHUKLA et al., 1988; PADHI e RAMU, 2011) e mais recentemente foi descrita a ORF2, codificando a proteína PIPO (CHUNG et al., 2008; WEI et al., 2010).

A capacidade de um vírus em infectar plantas depende de vários fatores. Dentre os principais encontra-se a sua habilidade de interagir com o plasmodesma para o movimento célula a célula e, particularmente, com o plasmodesma associado ao tecido vascular para o movimento a longas distâncias (GILBERTSON e LUCAS, 1996; LUCAS, 2006). Essa interação é geralmente alcançada com a ação de proteínas de movimento, sendo uma de suas importantes funções a de dilatar os microcanais do plasmodesma, aumentando o tamanho do limite de exclusão de passagem molecular, para que formas infecciosas do vírus possam, então, mover-se para as células adjacentes (CILIA e JACKSON, 2004; LUCAS, 2006).

As proteínas especializadas de movimento ainda não foram descritas para os potyvirus e sabe-se ainda menos sobre a interação com proteínas da planta hospedeira, mas a proteína CP, a HCpro e a helicase do *Tobacco etch vírus* (TEV) parecem ser necessárias para o movimento célula a célula e a longas distâncias (KASSCHAU et al., 1997; CARRINGTON et al., 1998). Foi sugerido por Wei et al.(2010) que a CI e o complexo P3N-PIPO coordenam a formação de estruturas associadas que facilitam o movimento intercelular de potyvirus em plantas infectadas. Estudos realizados por Chavez-Bedoya et al. (2011) encontraram

evidências da presença de vírions em colmo de milho, sugerindo a capacidade do SCMV de se movimentar a longas distâncias em linhagens suscetíveis. Os autores também observaram que o isolado SCMV-Ver-1 é capaz de passar pelos estágios iniciais de replicação em linhagens resistentes de milho e em plantas não hospedeiras, cana-de-açúcar, podendo ser detectado em sua forma replicativa cinco dias após a inoculação, o que sugere a existência de RNA viral sem capsídeo nos complexos de replicação.

Diversos isolados do subgrupo do SCMV que infectam naturalmente a cana-de-açúcar, milho, sorgo e milheto têm sido registrados em diferentes partes da Índia, mas apenas uma pequena parte destes isolados foi purificada e analisada bioquimicamente (ROYCHAUDHURI, 1977; KONDAIAH e NAYUDU, 1984, 1985; GOPAL e REDDY, 1988; HEMA et al., 1997; RAO, 1998b; MISHRA et al., 1998; RAO et al., 1996 a, b, 1998a, c). Estes isolados foram diferenciados com base em propriedades físicas, espécies hospedeiras, morfologia de partículas e dados serológicos.

Segundo Gaur et al.(2003), as características do genoma devem ser o critério final para a classificação correta dos vírus em seus grupos taxonômicos. A natureza do genoma viral, sua sequência de nucleotídeos, o mecanismo de replicação e montagem da partícula viral devem permitir afirmações precisas acerca do grupo de vírus em plantas. Outro critério de grande importância refere-se às sequências parciais de nucleotídeos, particularmente as sequências responsáveis pela proteína capsidial, devido à composição característica de aminoácidos de cada grupo (DONIER et al., 1987). De acordo com Chen et al. (2002), Adams et al. (2005), Viswanathan et al. (2009) e Wang et al. (2010), os dados de sequência de nucleotídeos da proteína capsidial dos *Potyvirus* permitem uma classificação hierárquica dentro da família *Potyviridae*. Espécies distintas do gênero apresentam identidades de sequência que variam de 38 a 71% ou de 75 a 89%, enquanto estirpes de um mesmo vírus exibem de 90 a 99% de identidade (GONÇALVES et al. 2011). Além disso, segundo Shukla et al.(1994) e Perera et al. (2009), existe no genoma viral uma região altamente conservada entre os *Potyvirus*, a região C-terminal do gene Nib, o que confere a possibilidade de desenho de oligonucleotídeos degenerados universais para *Potyvirus* a partir de sequências conhecidas.

No Brasil, até a metade da década de 2000 não havia informações precisas quanto à ocorrência e diversidade de espécies do subgrupo do SCMV. Quase todos os resultados eram baseados exclusivamente em sintomas em hospedeiras e exames de microscopia eletrônica de transmissão (TEM), com registros das espécies SCMV e MDMV. Mais recentemente, em análises realizadas de 2003 a 2007 com amostras das regiões sul, sudeste e centro-oeste do Brasil, em culturas de cana-de-açúcar, milho e sorgo, foram encontrados diversos isolados do vírus por meio de testes biológicos em plantas indicadoras, ELISA, RT-PCR, e análise de sequências (GONÇALVES et al. 2004, 2007a, 2007b, 2010). Foi constatado que o único potyvirus causador de mosaico nestas culturas no país foi o SCMV, contrastando com os registros anteriores de MDMV infectando milho (GONÇALVES et al. 2011).

Os vírus do subgrugo do SCMV e suas diversas estirpes são transmitidos por várias espécies de afídeos, dentre elas estão o *Rhopalosiphum maidis* e *Schizaphis graminum* (TEAKLE et al., 1989), comumente encontrados em diversas culturas do Brasil (GONÇALVES et al., 2007a). A transmissão é do tipo não persistente e os períodos de acesso de aquisição e transmissão duram de poucos segundos a minutos; a retenção por sua vez pode durar até algumas horas, como observado para o SCMVMDB (SHUKLA et al.,1994). Devido ao fato do SCMV ser transmitido naturalmente por afídeos de maneira não persistente o controle químico dos vetores não é muito efetivo no controle da doença (GONÇALVES et al.,2012).

Os sintomas iniciais de infecção segundo Gonçalves et al. (2007a), são caracterizados por pontos cloróticos de distribuição linear no meio ou mais comumente na base das folhas, que evoluem para áreas alongadas formando um mosaico típico, podendo aumentar de severidade com a idade da folha. Pode haver acentuada redução no crescimento das plantas dependendo da espécie e estirpe do vírus, da variedade de cana-de-açúcar e da infecção ocorrer nos estágios iniciais de desenvolvimento da planta. Variedades altamente suscetíveis podem ocasionalmente apresentar riscas e estrias nos colmos e encurtamento dos entre-nós.

As células das áreas cloróticas são pouco desenvolvidas, havendo nestas regiões um crescimento reduzido com pouca ou nenhuma diferenciação de células e

tecidos estruturais. Os cloroplastos das áreas cloróticas são pequenos e em pouco número como resultado da ação inibitória da doença (AGNIHOTRI, 1983). Irvine (1971) observou redução na fotossíntese por unidade de área em plantas de cana-de-açúcar infectadas pelas estirpes B e D de SCMV e uma significante redução na taxa de fotossíntese em três das quatro variedades estudadas. Bhargava et al. (1971) observaram concentração de sacarose e coeficiente de pureza menores em caldo extraído de plantas de cana-de-açúcar infectadas por mosaico, e efeitos inibidores sobre conteúdo de clorofila, fixação de CO_2, enzimas carboxilases e metabolismo de sacarose. Plantas infectadas mostraram acúmulo de açúcares redutores e diminuição na síntese de açúcares não redutores. Efeitos adversos na taxa líquida de fotossíntese foram observados por Viswanatham e Karuppaiah (2005) em cana-de-açúcar sob infecção severa por SCMV, o que pode levar a reduções em vários parâmetros de crescimento das variedades testadas.

Perdas de rendimento causadas pela doença dependem de fatores como variedade de cana-de-açúcar, estirpe de vírus, idade da cultura e condições ambientais (AGNIHOTRI, 1996). Balamuralikrishnam et al. (2003) observaram diminuição porcentagem de infecção por SCMV e no progresso da doença, proporcionais a idade da cana-de-açúcar, sendo apontados como prováveis causas, modificações no hospedeiro como espessamento de parede celular, formação de lignina, entre outras. Perdas de até cinquenta por cento em produção foram observadas na América do Sul (COSTA e MULLER, 1982) e mais de 40 por cento na Austrália (SMITH et al., 1992). Agnihotri et al. (1996) relataram danos expressivos ocasionados pela doença em variedades suscetíveis e ressaltaram que mesmo perdas de dez a quinze por cento são altamente significativas, devido ao cultivo extensivo da cultura.

A busca por novas variedades foi responsável pela introdução da doença nos Estados Unidos, Cuba e outros países (AGNIHOTRI, 1983). Epidemias frequentes de mosaico ocorreram no início do século XX na Argentina, Brasil, Cuba, Porto Rico e Estados Unidos ocasionando o quase colapso da indústria do açúcar (ABBOTT 1961; YANG; MIRKOV 1997). Desde então sua ocorrência tem sido relatada em quase todos os países produtores de cana-de-açúcar e as expressivas perdas causadas pela doença estimularam o estudo de sua epidemiologia e controle.

No Brasil a doença causou perdas econômicas drásticas na década de 1920, com novos ciclos epidêmicos em meados de 1930. Com o desenvolvimento de programas de melhoramento e de práticas culturais como o uso de mudas sadias, a prática de roguing em viveiros e o constante monitoramento de campos comerciais, foi obtido um controle relativo da doença (GONÇALVES et al., 2012). No Brasil, a maioria dos cultivares de cana-de-açúcar é considerada tolerante ou de resistência intermediária ao mosaico, apesar de registros constantes de novos casos em campo (GONÇALVES et al., 2012). A proximidade das culturas de cana-de-açúcar e de milho em algumas regiões do país forma uma condição favorável à disseminação do SCMV, uma situação que vem se intensificando com o uso de cultivares de milho adaptados a condições mais frias e secas, caracterizando culturas de inverno que aumentam a fonte de inóculo de SCMV e a sobrevivência de vetores afídeos no campo (GONÇALVES et al., 2007a,2007b, 2012; DUDIENAS et al.,1997; FERNANDES e OLIVEIRA, 1997; WAQUIL et al., 1996). Além disso, Gonçalves et al. (2007a) descreveram um isolado de SCMV, denominado Rib-1, responsável por surtos de mosaico no estado de São Paulo, ocasionando sintomas severos em cana-de-açúcar e encontrado infectando variedades comerciais e clones até então considerados resistentes ao mosaico. Essas informações reforçam que o mosaico continua uma importante doença a ser considerada durante a seleção e desenvolvimento de novos cultivares de cana-de-açúcar.

3. OBJETIVOS GERAIS

Avaliar a resistência ao mosaico de variedades, clones elite e acessos de cana-de-açúcar para identificar fontes de resistência e estimar parâmetros genéticos associados à resistência ao mosaico.

4. REFERÊNCIAS

ABBOTT, E. V.; HUGHES, C.G; MARTIN, J.P., J.P.; Mosaic. In:_____. **Sugarcane diseases of the world**, Amsterdam: Elsevier Pub. Co., 1961. v. 1, p. 407-425.

ADAMS, M. J.; ANTONIW, J. F.; BEAUDOIN, F. Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. **Molecular Plant Pathology**, Chichester, v. 6, n. 4, p. 471-487, 2005.

AGNIHOTRI, V. P. Current sugarcane disease scenario and management strategies. **Indian Phytopathology**, New Delhi, v. 49, n. 2, p. 10-126,1996.

AGNIHOTRI, V. P. **Diseases of sugarcane**. New Delhi: Oxford & IBH Publishing Co., 1983. p. 383.

ARCENEAUX, G. Cultivated sugarcanes of the world and their botanical derivates. In: [ISSCT CONGRESS], 1967, [S.l.]. **Proceedings…** [Quatre-Bornes: ISSCT], 1967. v. 12, p. 844-854.

BALAMURALIKRISHNAN, M.; DORAISAMY, S.; GANAPATHY, T.; VISWANATHAN, R. Comparison of antibody- and genome-based diagnostic techniques for Sugarcane mosaic virus in sugarcane. **Phytoparasitica**, Dordrecht, v. 32, n. 1, p. 52-56, 2004.

BALAMURALIKRISHNAN, M.; DORAISAMY, S.; GANAPATHY, T.; VISWANATHAN, R. Sugarcane mosaic virus infection progress in relation to age of sugarcane. **Sugar Tech**, Gandharva Mahavidyalaya, v. 5, n. 1-2, p. 21-24, 2003.

BERDING, N.; ROACH, B. T. Germoplasm collection, maintenance and use. In: HEINZ, D. J. (Ed.). **Sugarcane improvement through breeding**. Amsterdam: Elsevier Press, 1987. p. 143-210.

BHARGAVA, K. S.; JOSHI, R. D.; LAL, K. M. Strain D of sugarcane mosaic virus in India. **Sugarcane Pathologist's Newsletter**, [Sydney], v. 8, p. 23, 1971.

BISHOFF, K. B.; GRAVOIS, K. A. The development of new sugarcane varieties at the LSU Ag center. **Journal American Society of Sugar Cane Technologists**, Baton Rouge, v. 24, p. 142-164, 2004.

BREAUX, R. D. Controlling diseases of sugar cane in Louisiana by breeding: Potentialities and realities. **Journal American Society of Sugar Cane Technologists**, Baton Rouge, v. 4, p. 58-61, 1985.

BRESSIANI, J. A. **Seleção seqüencial em cana-de-açúcar**. 2001. 133 f. Tese (Doutorado em Agronomia) - Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2001.

CARRINGTON, J. C.; JENSEN, P. E.; SCHAAD, M. C. Genetic evidence for an essential role for Potyvirus CI protein in cell-to-cell movement. **The Plant Journal**, Oxford, v. 14, n. 4, p. 393-400, 1998.

CHAO, C. P.; HOY, J. W.; MARTIN, F. A. Evaluation of traits associated with resistance to sugarcane smut caused by *Ustilago scitaminea*. **Journal of the American Society of Sugar Cane Technologists**, Belle Glade, v. 9, p. 6-18, 1989.

CHAO, C. P.; HOY, J. W.; SAXTON, A. M.; MARTIN, F. A. Heritability of resistance and repeatability of clone reactions to sugarcane smut in Louisiana. **Phytopathology**, St. Paul, v. 80, n. 7, p. 622-626, 1990.

CHATENET, M.; MAZARIN, C.; GIRARD, J. C.; FERNADEZ, E.; GARGANI, D.; RAO, G. P.; ROYER, M.; LOCKHART, B. E. L.; ROTT, P. Detection of Sugarcane streak mosaic virus in sugarcane from several Asian countries. In: [ISSCT CONGRESS, 25]., 2005, Jakarta. **Proceedings…** [Quatre-Bornes: ISSCT], 2005. v. 25, p. 656-662

CHAVES-BEDOYA, G.; ESPEJEL, F.; ALCALÁ-BRISEÑO, R. I.; HERNÁNDEZ-VELA, J.; SILVA-ROSALES, L. Short distance movement of genomic negative strands in a host and nonhost for Sugarcane mosaic virus (SCMV). **Virology Journal**, London, v. 8, p. 1-8, 2011.

CHEN, J.; CHEN, J.; ADAMS, M. J. Characterisation of potyviruses from sugarcane and maize in China. **Archives of Virology**, Vienna, v. 147, n. 6, p. 1237-1246, 2002.

CHUNG, B. Y.; MILLER, W. A.; ATKINS, J. F.; FIRTH, A. E. An overlapping essential gene in the Potyviridae. **Proceedings of National Academy of Sciences**, Washington, DC, v. 105, n. 15, p. 5897-5902, 2008.

CILIA, M. L.; JACKSON, D. Plasmodesmata form and function. **Current Opinion in Cell Biology**, Oxford, v. 16, n. 5, p. 500-506, 2004.

CONAB (COMPANHIA NACIONAL DE ABASTECIMENTO). **Acompanhamento da safra brasileira**: cana-de-açúcar, safra 2013/2014, segundo levantamento. Brasília, DF, 2013. Disponível em: <http://www.conab.gov.br/OlalaCMS/uploads/arquivos/13_08_08_09_39_29_boletim_cana_portugues_-_abril_2013_1o_lev.pdf >. Acesso em: 12 fev. 2014.

COSTA, A.S.; MÜLLER, G.W. General evaluation of the impacts of virus disease of economic crops on the development of Latin American countries. In: Conferência Internacional sobre o Impacto das Doenças Virais no Desenvolvimento dos Países Latino-Americanos e da Região do Caribe, 1., 1982, Rio de Janeiro. **Anais**... Rio de Janeiro: [s.n.], 1982. p.216-230.

DANIELS, J.; DANIELS, C. A. Geographical, historical and cultural aspects of the origin of the indian and chinese sugarcanes *S. barberi* and *S. sinense*. **Sugarcane Breeding Newsletters**, [S.l.], v. 36, p. 4–23, 1975.

D' HONTH, A.; ISON, D.; ALIX, K.; ROUX, C.; GLASZMANN, J. C. Determination of basic chromosome numbers in the genus Saccharum by physical mapping of ribosomal RNA genes. **Genome**, Ottawa, v. 41, n. 2, p. 221-225, 1998.

DOMIER, L. L.; SHAW, J. G.; RHOADS, R. E. Potyviral proteins share amino acid sequence homology with picorna, como and caulimoviral proteins. **Virology**, Maryland Heights, v. 158, n. 1, p. 20-27,1987.

DUDIENAS, C.; DUARTE, A.P.; PATERNIANI, M.E.G.Z; RIBEIRO, J.L.; BIANCHINI, M.T.; KANTACCH, R.A.D.; CASTRO, J.L.; SILVEIRA, L.C.P; DENUCCI, S.; JUNIOR, J.S.; BOLONHESI, D.; DE SORDI, G. Severidade de doenças no milho safrinha no estado de São Paulo em 1996. In: SEMINÁRIO SOBRE A CULTURA DO MILHO SAFRINHA, 1997, [S.l.]. **Anais…** [S.l.]: IAC/CDV, 1997. p. 107-115.

DUDLEY, J. W.; MOLL, R. H. Interpretation and use of estimates of heritability and genetic variances in plant breeding. **Crop Science**, Madison, v. 9, p. 257-262, 1969.

FAOSTAT. Rome: Food and Agriculture Organization of the United States, 2014. Área colhida, produção mundial e produção nos principais países produtores de cana-de-açúcar. Disponível em: <http://faostat.fao.org/>. Acesso em: 01 fev. 2014.

FERNANDES, F. T.; OLIVEIRA, E. **Principais doenças na cultura do milho**. Sete Lagoas: Embrapa Milho e Sorgo, 1997. (Circular Técnica, 26). Disponível em: <www.infoteca.cnptia.embrapa.br/bitstream/doc/478849/1/circ261.pdf>. Acesso em: 01 fev. 2014.

GAUR, R. K.; MANEESHA SINGH; RAO, G. P. Molecular characterization of sugarcane mosaic virus isolate from North Eastern Region of India. **Sugar Tech**, Gandharva Mahavidyalaya, v. 5, n. 3, p. 149-153, 2003.

GEMECHU, A. L.; CHIEMSOMBAT, P.; ATTATHOM, S.; LERSRUTAIYOTIN, R.; REANWARAKORN, K. The variations among isolates of sugarcane mosaic virus in Thailand as determined by virus-host interaction. **Kasetsart Journal**, Bankok, v. 38, n. 3, p. 369-379, 2004.

GILBERTSON, R. L.; LUCAS, W. J. How do viruses traffic on the 'vascular highway'? **Trends in Plant Science**, Kidlington, v. 1, n. 8, p. 260-268, 1996.

GONÇALVES, M.C. Atualidades na etiologia de viroses da cana-de-açúcar e reflexos no melhoramento genético de variedades. **Tropical Plant Pathology**, Brasília, DF, v. 35, n. , p. 64-67, 2010. Edição especial.

GONÇALVES MC; GALDEANO, D. M.; MAIA, I. G.; CHAGAS, C. M. Variabilidade genética de *Sugarcane mosaic virus*, causando mosaico em milho no Brasil. **Pesquisa Agropecuária Brasileira** 46, p. 362-369, 2011.

GONÇALVES, M.C.; MAIA, I. G; GALLETI, S. R.; FANTIN, G. M. Infecção mista pelo *Sugarcane mosaic virus* e *Maize rayado fino virus* provoca danos na cultura do milho no estado de São Paulo. **Summa Phytopathologica**, 33, p. 22-26, 2007b.

GONÇALVES, M.C.; MOREIRA, Y. J. C. B.; MAIA, I. G.; SANTOS, A. S.; FANTIN, G. M.; CHAVES, A. Identificação e caracterização de isolados pertencentes ao subgrupo do Sugarcane mosaic virus no estado de São Paulo. **Fitopatologia Brasileira**, 29, p. 129-129, 2004.

GONÇALVES, M.C. ; PINTO, L. R.; SOUZA, S. C; LANDELL, M. G. A. Virus Diseases of Sugarcane. A Constant Challenge to Sugarcane Breeding in Brazil. **Functional Plant Science & Biotechnology**, Middleex, v. 6, n. 2, p. 108-116, 2012. Special.

GONÇALVES, M.C; SANTOS, A. S.; MAIA, L. G.; CHAGAS, C. M.; HARAKAVA, R. Caracterização de um isolado do *Sugarcane mosaic virus* que quebra a resistência de variedades comerciais de cana-de-açúcar. **Fitopatologia Brasileira**, Brasília, DF, v. 32, n. 1, p. 32-39, 2007a.

GOPAL, K.; REDDY, K. S. Studies on mosaic disease of sugarcane (*Saccharum officinarum* L.). **Plant Pathology News**, [S.l.], v. 6, p. 1-2, 1988.

GRISHAM, M. P.; BURNER, D. M.; LEGENDRE, B. L. Resistance to the H Strain of Sugarcane mosaic virus among wild forms of sugarcane and relatives. **Plant Disease**, St. Paul, v. 76, n. 4, p. 360-362, 1992.

GRIVET, L.; DANIELS, C.; GLASZMANN, J. C.; D' HONT, A. A review of recent molecular genetics evidence for sugarcane evolution and domestication. **Ethnobotany Research and Application**, Honolulu, v. 2, p. 9–17, 2004.

HARRISON, B. D.; FINCH, J. T.; GIBBS, A. J.; HOLLINGS, M.; SHEPHERD, R. J.; VALENTA, V.; WETTER, C. Sixteen groups of plant viruses. **Virology**, Maryland Heights, v. 45, n. 2, p. 356-363, 1971.

HEMA, M.; SAVITHRI, H.S.; SREENIVASULU, P. Taxonomic postion of *Sugarcane streak mosaic virus* in the family Potyviridae. **Archives of Virology**, Vienna, v. 147, n. 10, p. 2002-2007, 1997.

HUCKETT, B.; BOTHA, F. C. Progress towards a definitive diagnostic test for sugarcane mosaic virus infection. **Proceedings of the South African Sugarcane Technologists Association**, Mount Edgecombe, v. 70, p. 11-13, 1996.

IRVINE, E. J. Phytosynthesis in sugarcane varieties infected with strains of sugarcane mosaic virus. **Physiologia Plantarum**, Malden, v. 24, n. 1, p. 51-54, 1971.

KASSCHAU, K. D.; CRONIN, S.; CARRINGTON, J. C. Genome amplification and longdistance movement functions associated with the central domain of tobacco etch potyvirus helper component-proteinase. **Virology**, Maryland Heights, v. 228, n. 2, p. 251-262, 1997.

KOIKE, H.; GILLASPIE, A. G. JUNIOR. Mosaic. In: RICAUD, C.; EGAN, B. T.; GILLASPIE JUNIOR, A. G.; HUGHES C. G. (Ed.). **Diseases of sugarcane**: major diseases. Amsterdam: Science Publishers, 1989. p. 301-322.

KONDAIAH, E.; NAYNDU, M. V. Strain N-A new strain of *Sugarcane mosaic virus*. **Sugarcane**, [S.l.], v. 4, p. 11-14, 1985.

KONDAIAH, E.; NAYUDU, M. V. *Sugarcane mosaic virus*-H, a new record from India. **Current Science**, Delran, v. 53, n. 5, p. 273-275, 1984.

LANDELL, M. G. de A.; BRESSIANI, J. A. Melhoramento genético, caracterização e manejo varietal. In: LANDELL, M. G. de A. et al. **Cana-de-açúcar**. Campinas: IAC 2008. Cap. 5, p. 101-127, 149.

LUCAS, W. J. Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. **Virology**, Maryland Heights, v. 344, n. 1, p. 169-184, 2006.

MING, R. P. H.; MOORE, K. K.; WU, A. D.; HONT, J. C.; GLASZMANN, T. L.; TEW, T. E.; MIRKOV, J.; DA SILVA, J.; JIFON, M.; RAI, R. J.; SCHNELL, S. M.; BRUMBLEY, P.; LAKSHMANAN, J. C.; COMSTOCK, A. H. Sugarcane improvement through breeding and biotechnology. **Plant Breeding Reviews**, Hoboken, v. 27, p. 15-118, 2006.

MISHRA, M.; RAO, G. P.; UPADHYAYA, P. P. Identification and characterization of Maize dwarf mosaic potyvirus in India. **Indian Journal of Virology**, New Delhi, v. 14, n. 1, p. 75-79, 1998.

PADHI, A.; RAMU, K. Genomic evidence of intraspecific recombination in sugarcane mosaic virus. **Virus Genes**, New York, v. 42, n. 2, p. 282-285, 2011.

PERERA, M. F.; FILIPPONE, M. P.; RAMALLO, C. J.; CUENYA, M. I.; GARCÍA M. L.; PLOPER, L. D.; CASTAGNARO, A. P. Genetic diversity among viruses associated with sugarcane mosaic disease in Tucumán, Argentina. **Phytopathology**, St. Paul, v. 99, n. 1, p. 38-49, 2009.

PINTO, L. R.; GONÇALVES, M. C; GALDEANO, D. M.; PERECIN, D.; MEDEIROS, C. N. F.; LANDELL, M. G. Preliminary investigation of sugarcane mosaic virus resistance and marker association in a sugarcane family sample derived from a bi-parental cross. In: [CONGRESS OF THE INTERNATIONAL SOCIETY OF SUGARCANE TECHNOLOGISTS], 28., 2013, São Paulo. **Proceedings…** [Quatre-Bornes: ISSCT], 2013.

RAO, G. P. Identification of sugarcane mosaic potyvirus on sorghum and bajra in India. **Indian Journal of Virology**, New Delhi, v. 14, n. 1, p. 81-84, 1998.

RAO, G. P.; JAIN, R. K.; LIRA, H. S.; FORD, R. E. Identification of Sugarcane mosaic potyvirus infecting in India. **Review of Plant Pathology**, Oxfordshire, v. 460, n. 514A, 1996a. Abstract.

RAO, G. P.; JAIN, R. K.; VARMA, A. Occurrence of MDMV on maize and Sudan grass in India. **Plant Disease**, St. Paul, v. 80, p. 711, 1996b.

ROTT, P.; BAILEY, R. A.; COMSTOCK, J. C.; CRAFT, B. J.; SAUMTALLY, A. S. **A guide to sugarcane diseases**. Montepellier: CIRAD Publication services, 2000. p. 339.

ROYCHAUDHURI, S. P. **Manual of virus disease of tropical plants.** New Delhi: The Macmillan Company, 1977. 299 p.

SCHENCK, S.; LEHRER, A. T. Factors affecting the transmission and spread of sugarcane yellow leaf virus. **Plant Disease**, St. Paul, v. 84, n. 10, p. 1085-1088, 2000.

SHUKLA, D. D.; STRIKE, P. M.; TRACY, S. L.; GOUGH, K. H.; WARD, C. W. The N and C termini of the coat proteins of potyviruses are surface- located and the N terminus contains the major virus-specific epitopes. **Journal of General Virology**, Berks, v. 69, p. 1497-1508, 1988.

SHUKLA, D. D.; WARD, C. W.; BRUNT, A. A. **The potyviridae**. Cambridge: Cambridge University Press, 1994. 516 p.

SMITH, G. R.; FORD, F.; FRENKEL, M. J.; SHUKLA, D. D.; DALE, J. L. Transient expression of the coat protein of suagrcane mosaic virus in sugarcane protoplasts and expression in *Escherishia coli*. **Archives of Virology**, Vienna, v. 125, n. 1-4, p. 19-23, 1992.

SOUZA JUNIOR, C. L. **Melhoramento de espécies de reprodução vegetativa**. Piracicaba: ESALQ, Departamento de Genética, 1995. 41 p.

SRISINK, S.; TAYLOR, P. W. J.; STRINGER, J. K.; TEAKLE, D. S. An abrasive pad rubbing method for inoculating sugarcane with sugarcane mosaic virus. **Australian Journal of Agricultural Research**, Collingwood, v. 45, n. 3, p. 625-631, 1994.

TEAKLE, D.S.; SHUKLA, D.D.; FORD, R.E. Sugarcane mosaic virus. In:_____. **AAB descriptions of plant viruses**. UK, Wellesbourne: Warwick, 1989. n. 342.

VISWANATHAN, R.; KARUPPAIAH, R.; BALAMURALIKRISHNAN, M. Identification of new variants of SCMV causing sugarcane mosaic in India and assessing their genetic diversity in relation to SCMV type strains. **Virus Genes**, New York, v. 39, n. 3, p. 375-386, 2009.

VISWANATHAN , R.; KARUPPAIAH , R.; BALAMURALIKRISHNAN, M. Impact of Mosaic Infection on Growth and Yield of Sugarcane. **Sugar tech**, Gandharva Mahavidyalaya, v. 7, n. 1, p. 61-65, 2005.

VISWANATHAN, R.; MOHANRAJ, D. Detection of sugarcane viral diseases by serological, techniques. In: RAO, G. P.; FORD, R. E.; TOSIC, M. S.; TEAKLE, D. S. (Ed.). **Sugarcane pathology**. Enfield (NH): Virus and Phytoplasma Diseases, Science Publishers, 2001. v. 11, p.195-208.

WALKER, D. I. T. Manipulating the genetic base of sugarcane. In: COPERSUCAR INTERNATIONAL BREEDING WORKSHOP, [S.l.], 1987. **Proceedings…** Piracicaba: Coopersucar Technology Center, 1987. p. 321-334.

WANG, J. G.; ZHENG, H. Y.; CHEN, H. R.; ADAMS, M. J.; CHEN, J. P. Molecular diversities of Sugarcane mosaic vírus and Sorghum mosaic virus isolates from Yunnan province, China. **Journal of Phytopathology**, Berlin, v. 158, n. 6, p. 427-432, 2010.

WAQUIL, J. M.; OLIVEIRA, E.; PINTO, N. F. J.; FERNANDES, F. T.; CORRÊA, L. A. Efeito na produção e incidência de viroses em híbridos comerciais de milho. **Fitopatologia Brasileira**, Brasília, DF, v. 21, n. 4, p. 460-463, 1996.

WEI, T. Y.; ZHANG, C. W.; HONG, J.; XIONG, R. Y.; KASSCHAU, K. D.; ZHOU, X. P.; CARRINGTON, J. C.; WANG, A. M. Formation of complexes at plasmodesmata for potyvirus intercellular movement is mediated by the viral protein P3N-PIPO. **Plos Pathogens**, San Francisco, v. 24, n. 6, p. e1000962, 2010.

YANG, Z. N.; MIRKOV, T. E. Sequence and relationships of sugarcane mosaic and sorghum mosaic virus strains and development of RT-PCR-based RFLPs for strain discrimination. **Phytopathology**, St. Paul, v. 87, n. 9, p. 932-939, 1997

CAPÍTULO 2 - Evaluation of Brazilian sugarcane genotypes resistance to *Sugarcane mosaic virus* under greenhouse and field conditions

Abstract

This study aimed to evaluate the resistance of 79 sugarcane genotypes, including varieties and elite clones, artificially inoculated with a severe *Sugarcane mosaic virus* (SCMV) strain by estimating genetic parameters associated to SCMV resistance. Evaluations of symptoms by a grade scale and confirmation of infection by the serological test *Plate Trapped Antibody*-ELISA were performed in plants conducted in a greenhouse experiment, later implemented under field conditions. The mean incidence of mosaic was low under greenhouse conditions, with higher values in the field assay. Based on field results, variance analysis revealed significant genotype and genotype x environmental conditions. The interaction of sugarcane genotypes with days of evaluation revealed a differential behavior in mosaic symptom expression, including the recovery in some of them. The broad-sense heritability at individual level and means based were 19.37% and 62.18%, respectively. Twenty two genotypes showed to be resistant to SCMV.

Keywords: Mosaic, genetic parameters, artificial inoculation.

1. INTRODUCTION

Mosaic is one of the main viruses affecting sugarcane worldwide, with records in almost all the major sugarcane growing countries (Gonçalves et al., 2012; Viswanathan and Mohanraj, 2001). Recurrent epidemics occurred in the beginning of XXth century in Argentina, Brazil, Cuba, Porto Rico and United States, causing near outbreak of the sugar industry (Abbott 1961; Yang and Mirkov 1997) and the replacement of many sugarcane varieties due to yield losses up to fifth percent (Singh et al., 1997).

Mosaic disease affects several poaceous species, and may be caused by viruses of the

Sugarcane mosaic virus (SCMV) subgroup of the genus *Potyvirus*, family *Potyviridae*. Seven distinct potyviruses are recognized in this group: *Sugarcane mosaic virus* (SCMV); *Sorghum mosaic virus* (SrMV), *Sugarcane streak mosaic virus* (SCSMV), *Johnsongrass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV), *Pennisetum mosaic virus* (PenMV) and *Zea mosaic virus* (ZeMV). According to Chatenet et al. (2005), only SCMV, SrMV and SCSMV can naturally infect sugarcane since the last four viruses have never been isolated from this crop. In Brazil only SCMV is found naturally infecting sugarcane (Gonçalves et al., 2007a, 2012).

In Brazil the sugarcane mosaic disease was controlled with the implementation of breeding programs and field practical approaches such as the use of healthy seed cane, the roughing in nurseries and the constant monitory of commercial fields (Gonçalves et al., 2012). However, favorable epidemiological aspects in some regions of the country, such as the proximity between sugarcane, sorghum and maize crops, and the use of new maize cultivars adapted to the colder and dry season, increases the source of inoculum of SCMV and the survival of its main aphid vector, *Rhopalosiphum maidis* in these crops. (Gonçalves et al., 2007a, 2007b, 2012; Dudienas et al.,1997; Fernandes e Oliveira, 1997; Waquil et al., 1996). In addition, Gonçalves *et al.* (2007a) described the SCMV isolate so-called Rib-1 which caused mosaic outbreaks in sugarcane in the state of São Paulo, the main sugarcane and ethanol producing area in Brazil. This isolate causes severe symptoms and was found infecting sugarcane commercial varieties and clones previously considered to be resistant to mosaic.

The majority of sugarcane breeding programs in Brazil screen clones to virus resistance in field assays under favorable environmental conditions to the disease incidence (Gonçalves et al., 2012). Evaluations under these conditions are based on the development of visual symptoms, which are not definitive expressions of the viral pathology (Huckett and Botha 1996), depending also on the prevalent virus strain in the experimental area. The mosaic disease can be caused by different SCMV strains (Gonçalves et al. 2007a; Perera et al. 2009; Gonçalves et al. 2011), leading to distinguished responses in sugarcane varieties and clones.

Methods of artificial inoculation for SCMV were developed and evaluated in their efficiency (Srisink et al. 1994; Gemechu et al. 2004; Chaves-Bedoya et al. 2011) as studies of virus resistance have been conducted under greenhouse conditions (Schenck e Lehrer 2000;

Pinto et al. 2013). These studies allow evaluations of genotypes resistance against a known virus strain and the combination with molecular or serological diagnosis (Huckett and Botha 1996; Balamuralikrishnan et al. 2003; Pinto et al. 2013) allows the evaluation of resistance even in symptomless varieties, confirming the presence of the virus.

Although the resistance and susceptibility of some sugarcane varieties have been studied in breeding programs, there is little information about their genetic parameters and heritability. The objective of this study was to evaluate the resistance of sugarcane genotypes to a severe SCMV strain (SCMV Rib-1), as well as to estimate the broad-sense heritability associated to this resistance.

2. MATERIAL AND METHODS

2.1. Genotypes panel

The resistance to mosaic was evaluated in 79 genotypes, collected from different breeding centres from Brazil: 5 (SP and CTC varieties), 5 (RB varieties), and 17 IAC varieties, along with 50 IAC elites clones (Table 1). Stems were collected from shoots without mosaic symptoms, maintained at the IAC Breeding Station, Brazil for seedling production. Leaf samples of every individual shoot were tested by PTA-ELISA (*Plate Trapped Antibody*-ELISA) in order to verify the virus sanity.

2.2. Greenhouse experimental design

The experiment was conducted in a greenhouse with aphid proof screen (50 mesh). Seedlings were planted in small plastic pots in two randomized complete blocks, arranged on stands inside the greenhouse. Every plot was composed by six seedlings of each genotype plus one mock inoculated seedling in its center serving as healthy control during evaluations.

During experiment conduction, the seedlings were monthly treated with calcium nitrate and Map fertilizers Nutriplant, constantly monitored for the presence of aphids, and three

insecticide applications of imidacloprid.

2.3. SCMV detection by PTA-ELISA

The serological test PTA-ELISA with an antiserum specific to SCMV was performed in sugarcane leaves collected from individual shoots, in order to identify previous virus infections. Leaf samples were grounded in carbonate buffer 0.05M, pH 9.6, 1:10 ratio (weight:volume), to cover ELISA plates. The antiserum was diluted in PBS-TPO 1:6000, with 1% of skim milk powder. The alkaline phosphatase-conjugated "anti-rabbit" IgG was diluted 1:3000. The enzyme substrate p-nitrophenylphosphate (PNPP) was used in the concentration of 1mg/ml of diethanolamine buffer pH 9,8. Four absorbance readings at 405nm ($A405_{nm}$) were done in an ELISA plate reader at every twenty minutes. Samples with A405nm reading mean three times above the negative control's reading means were considered as SCMV infected.

2.4. SCMV inoculation

The first mechanical artificial inoculation of seedlings was performed in April 2012, three weeks after planting the sugarcane stalk pieces in plastic pots. The virus strain SCMV Rib-1, originally isolated from commercial fields in the municipality of Guaíra, state of São Paulo, Brazil (Gonçalves et al., 2007a), was used in the present study. The virus inoculum was prepared from young plants of *Sorghum bicolor* (L.) 'Rio', previously inoculated with SCMV-Rib1, showing typical mosaic symptoms. The inoculum was obtained from grounding the young symptomatic sorghum leaves with 0.01M phosphate buffer, pH 7.2 at 4ºC, in the ratio 1:5 (mg:mL). The abrasive silicon carbide (600 mesh) was mixed with the inoculum and rubbed with the forefinger on the youngest expanded leaves. To avoid failures in seedlings' infection, virus inoculation was repeated two weeks later the first one.

2.5. Symptoms evaluation and statistical analysis

Biweekly evaluations of mosaic incidence and severity of symptoms started to be recorded two weeks after the second inoculation, during twelve weeks. A symptom grade scale (Pinto et al., 2013), was used to evaluate the severity of mosaic symptoms, as folowing:

0. Symptom absence;
1. Mild mosaic in one leaf;
2. Intense mosaic in two or more leafs;
3. Generalized intense mosaic, along with reduction in plant growth;

Variance analysis (F Test) was performed in the results, using the SAS (2008) *Proc GLM* procedure, and means comparisons by LSD Test ($p<0.05$), according to the statistical model: $Y_{ij} = \mu + B_j + G_i + (BG)_{ij} + e_{ij}$, where Y_{ij} = observation of i^{th} genotype in j^{th} block, μ = overall mean, B_j = effect of j^{th} block, G_i = effect of i^{th} genotype, $(BG)_{ij}$ = effect of interaction block x genotype, and e_{ij} = random error (seedlings inside genotype and block). Data were presented as grade mean and ln (x+5) transformed, in order to approach the normal distribution (Berry, 1987). SCMV infection was confirmed by PTA-ELISA in leaf samples collected ten weeks after the second inoculation.

2.6. Field evaluations

After the last symptom record in greenhouse conditions, i.e. 4 months after planting, the seedlings were pruned and, two months later, mechanically re-inoculated by the same procedure. One week after the third inoculation, the six inoculated seedlings of each plot were planted in a field assay, in the spacing of 1.5 meters between lines and 0.5 meters within lines, in a two complete randomized blocks, using the variety IACSP95-5000, resistant to SCMV, as side border. Planting in the field was carried out in October 2012, and six biweekly symptoms recording started one month after planting. Data were submitted to variance analysis, adopting the Split plot temporal arrangement, using SAS (2008) *Proc GLM* and *Proc MIXED* procedures according to the linear mixed model: $Y_{ijkw} = \mu + B_j + G_i + (BG)_{ij} + A_k + (AG)_{ik} + E_{ijk} + e_{ijkw}$, were Y_{ijkw} = observation of w^{th} plant of i^{th} genotype in j^{th} block in k^{th}

evaluation, μ = overall mean, B_j = effect of j^{th} block, G_i = effect of i^{th} genotype, $(BG)_{ij}$ = effect of interaction block x genotype, A_k = effect of k^{th} evaluation, $(AG)_{ik}$ = effect of interaction evaluation x genotype, E_{ijk} = mean experiment error inside each evaluation and e_{ijkw} = random error (seedlings inside genotype and block). Means were compared by LSD test ($p>0.05$) with data presented as grade means and ln (x+5) transformed. From the ANOVA, genetic variance (σ_G^2), variance between plots (σ_E^2) and variance between plants inside plot (σ_D^2) were determined. The broad sense heritability at individual level (h^2) was estimated using the components of variance according to the equation: $h^2 = \sigma_G^2/(\sigma_G^2+\sigma_E^2+\sigma_D^2)$, while the means based heritability ($h_{\bar{x}}^2$) was calculated with the equation: $h_{\bar{x}}^2 = \sigma_G^2/[\sigma_G^2+(\sigma_E^2/r)+(\sigma_D^2/nr)]$, with, r = blocks = 2, n = number of plants per plot = 6.

3. RESULTS AND DISCUSSION

3.1. Greenhouse and field trials

The variance analysis for mosaic symptom grades in greenhouse conditions showed significant F values ($p<0.01$) for block, genotype and interaction block x genotype (Table 2). The experiment showed a low mean incidence of mosaic, being observed in only 9 of 79 sugarcane genotypes, with no effects of evaluation and interaction genotype x evaluation. Even so, a significant difference among genotypes and between blocks was observed. The significant interaction between genotypes and blocks revealed that some genotypes had different behaviors between blocks. The genotypes IACSP97-2109 and IACSP96-1066, followed by IACSP96-3076, RB925211, IACSP97-7018 and IACSP93-2060, in a decreasing scale, were the most susceptible to mosaic by LSD test ($p<0.05$) (Table 3).

The variance analysis for mosaic symptom grades under field conditions showed significant F values ($p<0.01$) for block, genotype, interaction block x genotype, evaluation and interaction evaluation x genotype (Table 2). The significant F value for repetition inside block and genotype shows that seedlings of the same genotype had different behavior in the manifestation of mosaic symptoms, contributing to environmental variance. A higher mosaic

incidence and symptom grade mean were observed in the field assay, with 40 of 79 genotypes showing mosaic symptom in at least one evaluation. The higher mean allowed an observation of a more distinctive behavior among genotypes, which can be seen by significant effects of evaluation and their interaction with genotypes. The Split plot temporal arrangement allowed observing the behavior of genotypes during evaluations and the estimation of genetic variance free from interaction variance (Allard, 1971). The most susceptible genotypes by LSD test ($p<0.05$) were RB925211, followed by IACSP96-1066 (Table 4). The LSD test showed higher mosaic incidence values 75 and 105 days after planting, with major difference occurring among genotypes in these records, while symptoms records at 30 and 45 days after planting showed the minor differences among genotypes (Table 5).

Unfolding the interaction evaluation x genotypes, different symptoms expression behaviors were observed for the beginning of the records and the persistence of symptoms during the final records among genotypes. Among genotypes which has shown mosaic symptoms, it was observed a recovery behavior from mosaic symptoms during evaluations in the genotypes SP79-1011, IACSP96-2019, IACSP94-2101, IACSP96-3048, IACSP97-3313, IACSP97-3331, IACSP95-3018, IACSP97-3384, IACSP97-2084, IACSP99-4010 and SP86-42 (Table 6).

This phenomenon is recently related to RNA silencing or post transcriptional gene silencing, a sequence-specific RNA degradation described in plants and several eukaryotic organisms (Voinnet et al., 1999, Waterhouse et al. 2001). RNA silencing has been extensively studied for transgenes, but virus can also serve as initiators and targets of RNA silencing (Ratcliff et al., 1997; Voinnet et al., 1999). Recovery can be initiated naturally in some virus infections in a similar way of response to virus-induced recovery of transgenic plants, where the young leaves are symptom free, with reduced levels of virus and exhibit a strong and specific virus resistance (Covey et al., 1997; Ratcliff et al., 1997).

3.2. Variance components

The variance components (Table 7) estimated from symptoms grade records in field conditions indicated that the mosaic resistance inheritance tends to be qualitative, showing a

broad-sense heritability of 19.37% at individual level. The genetic variance (σ_G^2) magnitude was inferior to environmental variance (represented by the sum of σ_E^2 and σ_D^2), with 80.73% of variance due to environmental effects, indicating that individual selection to mosaic resistance is inefficient.

The means based broad-sense heritability was 62.17%, showing a higher efficiency of methods based on means, such as family selection, whose efficiency is based on tendency of the environmental deviations of individuals to cancel, approaching the average phenotypic value to the average genotypic value (Falconer and Mackay, 1996).

The individual analysis and means based heritability estimations, adapted from Skinner et al. (1987) by Landell & Bressiani (2008), showed low magnitude estimations for several sugarcane characters and highly superior values for family based estimations. According to Latter (1964) and Resende (2002), only characters with heritability at individual level over 50% would provide the same selection efficiency in individuals when compared to family selection.

The variance components analysis showed the mosaic symptom manifestation is under strong influence of the environment. The influence of environmental conditions in proportion of maize plants susceptible to SCMV and MDMV, was reported by Melchinger et al. (1998) and Tu & Ford (1969), respectively, and by Jensen et al. (1985) in terms of virus titre in MDMV infected sorghum. In all cases, temperature was the main environmental factor influencing both, viruses' incidence and titre. For maize, another poaceous crop which is taxonomically close to sugarcane, mosaic resistance has been attributed to two dominant genes, Scm1 and Scm2 (Melchinger *et al.* 1998). However, the authors observed that the presence of these genes is not sufficient for complete resistance to SCMV, indicating the action of other putative major or minor genes, whose expression is highly influenced by environment conditions. In addition, the genome of sugarcane is complex and most sugarcane traits are multi-genic, multi-allelic and quantitatively inherited (Hoarau et al, 2007) which reinforces the possibility of influence of environmental factors on the expression of resistance to SCMV. To better understand other genetic parameters of resistance to mosaic, future studies concerning prediction of favorable environmental conditions for the development of symptoms are required.

3.3. PTA-ELISA diagnosis

Twenty two out of the 79 sugarcane shoots from which cuttings were taken for the production of seedlings, corresponding to the genotypes IACSP99-4010, IACSP96-1005, IACSP95-5094, IACSP98-5010, IACSP02-2074, IACSP97-7077, RB925211, IACSP99-4007, IAC86-2210, IACSP99-4013, IACSP97-2053, IACSP99-1308, IACSP99-4011, IACSP96-3060, IACSP97-7018, IACSP96-2042, IACSP97-4039, IACSP97-6682, IACSP96-2019, IACSP98-2030, IACSP96-7506 and IACSP97-7074 showed to be previously infected by SCMV by PTA-ELISA analysis. Thirty one genotypes seedlings mechanically inoculated under greenhouse conditions confirmed infection by SCMV, after the second inoculation (Table 8).

The response of genotypes to SCMV infection in terms of symptom grades evaluations and PTA-ELISA results are showed in Table 8. The genotypes that showed maximum symptom grades varying from 2 to 3 during both evaluations under greenhouse and field conditions, along with the prevalence of mosaic symptoms in the end of assays, were classified as susceptible to SCMV. The genotypes with maximum grade equal 1, along with those that showed symptoms recovery in the end of evaluations in the field, were classified as intermediary. The genotypes without mosaic symptoms were classified as resistant, even those testing positive for SCMV infection by PTA-ELISA. The explanation for the virus presence in some of these isolates is the occurrence of latent infections.

The recovery from shooting latent infection was observed in the genotypes IACSP99-4007, IACSP98-5010, IACSP02-2074, IACSP97-7077, IACSP96-3060, IACSP97-6682, IACSP98-2030, IACSP97-7074, IACSP95-5094 and IACSP96-7506, ten weeks after inoculation in the greenhouse, along with absence of symptoms during the field evaluations, suggesting a cross protection effect. In fact, mixed infections may result in cross protection and usually occurs through the interaction of related strains of a same virus constituting a phenomenon whereby a pre-inoculated plant become resistant to subsequent inoculation. Krstic et al (1995) investigated cross protection among 53 strains of four viruses belonging to SCMV subgroup and observed unidirectional protection only between SCMV-MDB and SCMV-BC, indicating a quite restrict protective pattern for SCMV. Nevertheless as mentioned above, this phenomenon has been recently better explained as RNA gene silencing mediated by viruses, a very well-studied mechanism in plant response against viruses

(Mandadi & Scholthof, 2013). On the other hand, the establishment of resistance induced by virus infection may result in recovery, just as in the case of the response to SCMV observed in these genotypes.

In face of the strain SCMV-Rib1 used in our experiments prevails in Ribeirão Preto region (Gonçalves et al., 2007a) and there is little information regarding its presence in other Brazilian regions, the recovery behavior observed in the genotypes mentioned above requires more detailed studies to check if there was cross-protection by the previous presence of a mild SCMV strain in these plants. Nevertheless, the recovery was notorious in these cultivars and clones during the interaction of SCMV with sugarcane, indicating that this phenomenon may be a constant on disease development in the field.

Among genotypes with negative results for SCMV infection by PTA-ELISA even after the two inoculations in the greenhouse, symptomatic plants of IACSP99-4013, IACSP93-6006, IACSP93-3046, IAC87-3396, IACSP97-3406, IACSP96-3048, IACSP97-2084 IACSP99-4008, RB72454, IACSP95-5094, IACSP98-3020, IACSP94-4004, IACSP95-3018, IACSP94-2101, IACSP96-7506, IACSP97-3313, IACSP96-7586, SP86-42 and SP79-1011 were observed in field evaluations, revealing a higher virus titre that either has increased with plant development and new infection due to the third inoculation or with natural infection by aphids.

The genotypes IACSP98-2053, IACSP97-2028, RB855156, IACSP99-3009, IACSP97-7543, IACSP97-2000, IACSP96-2100, IACSP98-6202, IAC91-2195, IACSP95-3028, IAC86-2480, IACSP97-2098, IACSP95-5000, SP70-1143, IACSP95-2078, IACSP97-2020, IACSP96-7569, IACSP98-5046, SP80-3280, IACSP99-3085, IACSP97-2055 and IACSP97-7065 did not show symptomatic plants neither SCMV infection by PTA-ELISA, characterizing good sources of resistance to mosaic.

In the present study, it was observed resistance break down by SCMV-Rib1 in varieties RB925211, RB867515, IACSP93-6006, IAC91-2218, IAC86-2210, IACSP93-3046, IACSP95-5094, IAC87-3396 and RB72454, so far considered resistant to the disease (Landell & Bressiani, 2008), while IACSP94-2101 showed an intermediary behavior with mild symptoms. The varieties IACSP95-3028, IACSP95-5000, IACSP94-2094 and IACSP96-2042 had their resistance to mosaic confirmed. Nevertheless field infection records of varieties RB72454, IAC91-2218 and IACSP93-6006 had already been reported for this virus isolate by

Gonçalves et al. (2007a).

The mosaic susceptibleness was confirmed for IACSP93-2060, IAC91-1099 and IACSP94-4004. The variety CTC9, also considered susceptible (Landell & Bressiani, 2008), showed to be resitant to the strain used in the present study. The differences observed between the results presented here and the previous information of resistance and susceptibility of sugarcane varieties demonstrates that resistance to mosaic is dependent on the SCMV strain, as emphasized by Gonçalves et al. (2012). The artificial inoculation with a known strain showed to be essential for this kind of evaluation since different responses to the strain SCMV Rib-1 were confirmed here.

The combination of symptom evaluation by grade scale with the serological test ELISA for SCMV detection proved to be efficient for selection of sources of resistance to mosaic, detecting the virus in symptomless genotypes, and pointing out twenty two genotypes as resistant to the virus strain used in this study. According to our results, the resistance to mosaic tends to be a quantitative trait, having implications in the selection methods in order to obtain appreciable genetic gains.

Avaliação de genótipos de cana-de-açúcar para resistência ao *Sugarcane mosaic virus* sob condições de casa de vegetação e campo

Resumo

O presente estudo teve como objetivo avaliar a resistência de 79 genótipos de cana-de-açúcar, incluindo variedades e clones elite, inoculados artificialmente com uma estirpe severa de *Sugarcane mosaic virus* (SCMV) e estimar os parâmetros genéticos associados à resistência ao mosaico. Avaliações de sintomas por escala de notas e confirmação da infecção pelo teste serológico *Plate Trapped Antibody*-ELISA foi realizado em um experimento conduzido em estufa e posteriormente levado em condições de campo. A incidência média de mosaico foi baixa em condições de estufa, com maiores valores no ensaio em campo. Com base nos resultados de campo, a análise de variância mostrou valores significativos para

genótipo e interação entre genótipo e condições ambientais. A interação de genótipos dos genótipos com dias de avaliação revelou um comportamento diferenciado na expressão de sintomas de mosaico, incluindo a recuperação em alguns deles. A herdabilidade no sentido amplo calculada foi de 19,37% ao nível de plantas individuais e de 62,18% ao nível de média de parcelas. Vinte e dois genótipos mostraram-se resistentes à estirpe em estudo.

Palavras chave: Mosaico, parâmetros genéticos, inoculação artificial.

4. REFERENCES

Abbott EV, Hughes CG and Martin JP (1961) In: **Sugarcane Diseases of the world.** Publisher Elsevier, Amsterdam, The Netherlands. 407-425 p.

Allard, R W (1971) **Princípios do melhoramento genético de plantas.** Publisher E. Blücher, São Paulo, 381p.

Balamuralikrishnan M., Doraisamyd, SGT, Viswanathan R (2003) Sugarcane mosaic virus infection progress in relation to age of sugarcane. **Sugar Tech 5**: 21-2.

Berry, D A (1987) Logarithmic transformations in ANOVA. **Biometrics 43:** 439-456.

Chatenet M, Mazarin C, Girard JC, Fernadez E, Gargani D, Rao GP, Royer M, Lockhart BEL, Rott P (2005) Detection of Sugarcane streak mosaic virus in sugarcane from several Asian countries. In: **Proceedings of the International Society of Sugarcane Technologists 25.** Guatemala City, Guatemala, p 656-662.

Chaves-bedoya G, Espejel F, Alcalá-Briseño RI, Hernández-Vela J, Silva-Rosales L (2011) Short distance movement of genomic negative strands in a host and nonhost for *Sugarcane mosaic virus* (SCMV). **Virology Journal 8:** 1-8.

Covey S N, Al-Kaff N, La´ ngara, A and Turner D S (1997) Plants combat infection by gene silencing. **Nature 385**: 781–782.

Dudienas C, Duarte AP, Paterniani MEGZ, et al. (1997) Severidade de doenças no milho safrinha no estado de São Paulo em 1996. **Seminário sobre a cultura do milho safrinha.** IAC/CDV, p. 107-115.

Falconer DS and Mackay TFC (1996). **Introduction to quantitative genetics.** 4. Ed. Publisher Longman, London. 464 p.

Fernandes FT and Oliveira E (1997) Principais doenças na cultura do milho. **Empresa Brasileira de Pesquisa Agropecuária. Circular Técnica n. 26.**

Gemechu AL, Chiemsombat P, Attathom S, Lersrutaiyotin R, Reanwarakorn K (2004) The variations among isolates of Sugarcane mosaic virus in Thailand as determined by virus-host interaction. **Kasetsart Journal (Natural Science) 38:** 369-379.

Gonçalves MC, Pinto LR, Souza SC and Landell MGA (2012) Virus Diseases of Sugarcane. A Constant Challenge to Sugarcane Breeding in Brazil. **Functional Plant Science & Biotechnology 6:** 108-116.

Gonçalves MC, Galdeano DM, Maia IG, Chagas CM (2011) Genetic variability of *Sugarcane mosaic virus* genotypes causing maize mosaic in Brazil. **Pesquisa Agropecuária Brasileira 46**: 362-369.

Gonçalves MC, Santos AS, Maia LG, Chagas CM and Harakava R (2007a) Characterization of an isolate of Sugarcane mosaic virus breaking down resistance of commercial sugarcane varieties. **Fitopatologia Brasileira 32:** 32-39.

Gonçalves, MC, Maia, IG, Galleti, SR and Fantin, GM (2007b) Mixed infection by Sugarcane mosaic virus and Maize rayado fino virus causing breaking yields in maize in São Paulo state. **Summa Phytopathologica 33:** 22-26.

Hoarau JY, Souza G, D'Hont A et al. (2007) Sugarcane, a tropical crop with a highly complex genome. In: Morot-Gaudry JF, Lea P, Briat JF (eds.) **Functional Plant Genomics** Publisher INRA, France, 1-708 p.

Huckett B, Botha FC (1996) Progress towards a definitive diagnostic test for sugarcane mosaic virus infection. **Proceedings of the South African Sugarcane Technologists Association 70**. Durban, South Africa, p. 11-13

Jensen SG, Palomar MK, Ball EM, Samson R (1985) Factors influencing virus titer in maize dwarf mosaic virus infectted sorghum. **Phytopathology 75:** 1132-1136.

Krstic B, Ford RE, Shukla DD and Tosic M (1995) Cross-protection studies between strains of sugarcane mosaic, maize dwarf mosaic, Johnsongrass mosaic, and sorghum mosaic potyviruses. **Plant Disease 79:**135-138.

Landell MG de A, Bressiani JA (2008) **Melhoramento genético, caracterização e manejo varietal.** In: Dinardo-Miranda LL, Vasconcelos ACM, Landell MG de A et al. **Cana-de-açúcar.** Editora IAC, Campinas, p.101-127, 149.

Latter BDH (1964) Selection methods in the breeding of cross-fertilized pasture species. In: Barnard C (ed.) **Grasses and grasslands.** Publisher Macmillan, London, p.168-181.

Mandadi KK, Scholthof, KG (2013) Plant immune responses against viruses: how does a virus cause disease? **The Plant Cell 25**: 1489-1505.

Melchinger AE, Kuntze L, Gumber RK, Lübberstedt T, Fuchs E (1998). Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. **Theoretical and Applied Genetics 96**: 1151-1161

Pinto LR, Gonçalves MC, Galdeano DM, Perecin D, Medeiros CNF, Landell MG (2013) Preliminary investigation of sugarcane mosaic virus resistance and marker association in a sugarcane family sample derived from a bi-parental cross. **Proceedings Int. Soc. Sugarcane Technol. Brisbane: Int. Soc. Sugarcane Technol.** In: XXVIII ISSCT CONGRESS, São Paulo, v. 28.

Perera MF, Filippone MP, Ramallo CJ, Cuenya MI, García ML, Ploper LD, Castagnaro AP (2009) Genetic diversity among viruses associated with sugarcane mosaic disease in Tucumán, Argentina. **Phytopathology 99:** 38-49.

Ratcliff F, Harrison BD and Baulcombe DC (1997) A similarity between viral defense and gene silencing in plants. **Science 276**: 1558–1560.

Resende MDV (2002) **Genética, biométrica e estatística: no melhoramento de plantas perenes.** Embrapa Informação Tecnológica, Brasília, 975p.

SAS Institute. **SAS/STAT user's guide, version 9.2 SAS, Cary**. 2008.

Schenck S, Lehrer AT (2000) Factors affecting the transmission and spread of sugarcane yellow leaf virus. **Plant Disease 84:** 1085-1088

Singh SP, Rao GP, Singh J and Singh SB (1997) Effect of sugarcane mosaic potyvirus infection on metabolic activity, yield and juice quality. **Sugar Cane 5:** 19-23.

Srisink S, Taylor PWJ, Stringer JK, Teakle DS (1994) An abrasive pad rubbing method for inoculating sugarcane with sugarcane mosaic virus. **Australian Journal of Agricultural Research 45**: 625-631.

Tu JC, Ford RE (1969) Effect of temperature on maize dwarf mosaic virus infection, incubation, and multiplication in corn. **Phytopathology 59:** 699 - 702.

Viswanathan R, Mohanraj D (2001) Detection of sugarcane viral diseases by serological, techniques. In: Rao GP, Ford RE, Tosic MS, Teakle DS (eds). **Sugarcane Pathology. vol. 11. Virus and Phytoplasma Diseases.** Science Publishers, Enfield (NH), USA, p.195-208.

Voinnet O, Pinto Y M and Baulcombe DC (1999) Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. **Proceedings of the National Academy of Sciences USA 96:** 14147–14152.

Waquil JM, Oliveira E, Pinto NFJ, Fernandes FT and Corrêa LA (1996). Efeito na produção e incidência de viroses em híbridos comerciais de milho. **Fitopatologia Brasileira 21:** 460-463.

Waterhouse PM, Wang MB and Lough T (2001) Gene silencing as an adaptive defence against viruses. **Nature 411:** 834–842.

Yang ZN, Mirkov TE (1997) Sequence and relationships of sugarcane mosaic and sorghum mosaic virus strains and development of RT-PCR-based RFLPs for strain discrimination. **Phytopathology 87:** 932-939.

Table 1. List of sugarcane varieties (V) and elite clones (C) from the IAC germplasm collection used for screening resistance to mosaic.

SP70-1143(V)	IACSP99-4013(C)	IACSP96-2100 (C)	RB867515(V)
SP86-42(V)	RB925211(V)	IACSP97-7077(C)	IACSP96-3060(V)
RB72454(V)	IACSP02-2074(C)	IACSP98-2053(C)	IAC86-2210(V)
RB835486(V)	IACSP96-1066(C)	IACSP99-4010(C)	IAC91-1099(V)
SP79-1011(V)	IACSP94-2101(V)	SP80-3280(V)	IACSP93-3046(V)
IACSP98-5046(C)	IACSP96-7586(C)	IACSP96-1005(C)	IACSP94-2094(V)
IAC86-2480(V)	IACSP97-2055(C)	IAC91-2218(V)	IACSP94-4004(V)
IACSP96-3056(C)	RB855156(V)	IACSP97-2053(C)	IACSP95-3028(V)
IACSP95-2078(C)	IACSP98-6202(C)	IACSP97-3331(C)	IACSP95-5000(V)
IACSP98-3020(C)	IACSP99-4007(C)	IACSP97-4039(V)	IACSP96-2019(C)
IAC52-150(V)	IACSP93-6006(V)	IACSP98-2030(C)	IACSP96-3048(C)
IACSP97-2023(C)	IACSP97-7074(C)	IACSP99-3009(C)	IACSP96-7569(V)
IACSP99-1305(C)	IACSP97-3406(C)	IACSP95-5094(V)	IACSP97-2000(C)
IACSP97-2020(C)	IACSP98-3099(C)	IACSP96-2042(V)	IACSP97-2084(C)
IACSP95-1218(V)	IACSP99-4008(C)	IACSP96-7506(C)	IACSP97-2098(C)
CTC9(V)	IACSP93-2060(V)	IACSP97-2028(C)	IACSP97-2109(C)
IAC87-3396(V)	IACSP97-7065(C)	IACSP97-3384(C)	IACSP97-3313(C)
IACSP97-6682(C)	IACSP97-7543(C)	IACSP97-7018(C)	IACSP98-5010(C)
IACSP96-3076(C)	IACSP983021(C)	IACSP99-3085(C)	IACSP99-1308(C)
IAC91-2195(V)	IACSP95-3018(V)	IACSP99-4011(C)	

Table 2. Variance analysis for records of symptom grades after SCMV mechanical inoculation in sugarcane seedlings under greenhouse and field conditions.

Experiment	Source	D.F.	Mean Square
Greenhouse	**Block**	1	0,067**
	Genotype	78	0,021**
	Genotype* Block	78	0,013**
	Residual	962	0,003
	CV(%)		3,26
Field	**Block**	1	0,419**
	Genotype	78	0,150**
	Res(a)= Gen*Block	78	0,039
	Rep(block*genot.)	786	0,019**
	Evaluation	5	0,070**
	Evaluation*Genot.	390	0,005**
	Res(b)	4269	0,003
	CV(%)		3,30

Table 3. Symptom grade means comparisons by LSD test (p<0,05) for genotype evaluation under greenhouse conditions.

Genotype	*Mean	**Mean		Genotype	*Mean	**Mean	
IACSP97-2109	0,991	(1,790)	a	IACSP02-2074	0,000	(1,609)	c
IACSP96-1066	0,770	(1,753)	a	IACSP99-4007	0,000	(1,609)	c
IACSP96-3076	0,719	(1,744)	ab	IACSP98-2053	0,000	(1,609)	c
RB925211	0,712	(1,743)	ab	IACSP97-2028	0,000	(1,609)	c
IACSP97-7018	0,633	(1,729)	ab	CTC9	0,000	(1,609)	c
IACSP93-2060	0,622	(1,727)	ab	RB835486	0,000	(1,609)	c
IACSP97-4039	0,496	(1,704)	bc	RB855156	0,000	(1,609)	c
IACSP97-2053	0,436	(1,693)	bc	IACSP97-2023	0,000	(1,609)	c
IAC91-1099	0,171	(1,643)	c	IACSP99-1308	0,000	(1,609)	c
IACSP93-6006	0,000	(1,609)	c	IACSP96-2042	0,000	(1,609)	c
RB867515	0,000	(1,609)	c	IACSP99-1305	0,000	(1,609)	c
IACSP98-3099	0,000	(1,609)	c	IACSP94-2094	0,000	(1,609)	c
IACSP98-3020	0,000	(1,609)	c	IACSP99-4011	0,000	(1,609)	c
IAC52-150	0,000	(1,609)	c	IACSP98-3021	0,000	(1,609)	c
IACSP99-4008	0,000	(1,609)	c	IACSP99-3009	0,000	(1,609)	c
IACSP93-3046	0,000	(1,609)	c	IACSP97-7543	0,000	(1,609)	c
IAC91-2218	0,000	(1,609)	c	IACSP97-2000	0,000	(1,609)	c
IACSP96-1005	0,000	(1,609)	c	IACSP96-2100	0,000	(1,609)	c
RB72454	0,000	(1,609)	c	IACSP98-5010	0,000	(1,609)	c
IACSP95-3018	0,000	(1,609)	c	IACSP98-6202	0,000	(1,609)	c
IAC87-3396	0,000	(1,609)	c	IAC91-2195	0,000	(1,609)	c
IACSP99-4013	0,000	(1,609)	c	IACSP95-3028	0,000	(1,609)	c
IACSP96-7586	0,000	(1,609)	c	IAC86-2480	0,000	(1,609)	c
IACSP96-3056	0,000	(1,609)	c	IACSP97-2098	0,000	(1,609)	c
IACSP97-2084	0,000	(1,609)	c	IACSP95-5000	0,000	(1,609)	c
IACSP97-3406	0,000	(1,609)	c	IACSP98-2030	0,000	(1,609)	c
IACSP94-4004	0,000	(1,609)	c	SP70-1143	0,000	(1,609)	c
IAC86-2210	0,000	(1,609)	c	IACSP95-2078	0,000	(1,609)	c
IACSP96-7506	0,000	(1,609)	c	IACSP97-2020	0,000	(1,609)	c
IACSP99-4010	0,000	(1,609)	c	IACSP96-7569	0,000	(1,609)	c
IACSP97-3313	0,000	(1,609)	c	IACSP98-5046	0,000	(1,609)	c
SP79-1011	0,000	(1,609)	c	IACSP97-6682	0,000	(1,609)	c
IACSP96-3048	0,000	(1,609)	c	SP80-3280	0,000	(1,609)	c
IACSP97-3384	0,000	(1,609)	c	IACSP97-7077	0,000	(1,609)	c
IACSP97-3331	0,000	(1,609)	c	IACSP99-3085	0,000	(1,609)	c
IACSP96-2019	0,000	(1,609)	c	IACSP96-3060	0,000	(1,609)	c
IACSP94-2101	0,000	(1,609)	c	IACSP97-2055	0,000	(1,609)	c
IACSP95-1218	0,000	(1,609)	c	IACSP97-7074	0,000	(1,609)	c
SP86-42	0,000	(1,609)	c	IACSP97-7065	0,000	(1,609)	c
IACSP95-5094	0,000	(1,609)	C				

*Mean: non transformed data. ** Mean: data transformed in ln (x+5).

Table 4. Symptom grade means comparisons by LSD test (p<0,05) for genotype evaluation under field conditions.

Genotype	***Mean**	****Mean**		**Genotype**	****Mean**	***Mean**	
RB925211	1,322	(1,844)	a	IACSP02-2074	0,000	(1,609)	p
IACSP96-1066	1,130	(1,813)	b	IACSP99-4007	0,000	(1,609)	p
IACSP93-2060	0,822	(1,762)	c	IACSP98-2053	0,000	(1,609)	p
IACSP97-4039	0,730	(1,746)	c	IACSP97-2028	0,000	(1,609)	p
IACSP93-6006	0,618	(1,726)	d	CTC9	0,000	(1,609)	p
RB867515	0,543	(1,713)	de	RB835486	0,000	(1,609)	p
IACSP97-2053	0,497	(1,704)	e	RB855156	0,000	(1,609)	p
IACSP98-3099	0,385	(1,684)	fg	IACSP97-2023	0,000	(1,609)	p
IACSP96-3076	0,318	(1,671)	gh	IACSP99-1308	0,000	(1,609)	p
IACSP98-3020	0,292	(1,666)	ghi	IACSP96-2042	0,000	(1,609)	p
IAC91-1099	0,276	(1,663)	hij	IACSP99-1305	0,000	(1,609)	p
IACSP97-7018	0,267	(1,661)	hij	IACSP94-2094	0,000	(1,609)	p
IAC52-150	0,265	(1,661)	hij	IACSP99-4011	0,000	(1,609)	p
IACSP99-4008	0,203	(1,649)	ijk	IACSP98-3021	0,000	(1,609)	p
IACSP97-2109	0,185	(1,646)	jk	IACSP99-3009	0,000	(1,609)	p
IACSP93-3046	0,131	(1,635)	kl	IACSP97-7543	0,000	(1,609)	p
IAC91-2218	0,123	(1,634)	kl	IACSP97-2000	0,000	(1,609)	p
IACSP96-1005	0,120	(1,633)	klm	IACSP96-2100	0,000	(1,609)	p
RB72454	0,113	(1,632)	klmn	IACSP98-5010	0,000	(1,609)	p
IACSP95-3018	0,089	(1,627)	lmno	IACSP98-6202	0,000	(1,609)	p
IAC87-3396	0,083	(1,626)	lmnop	IAC91-2195	0,000	(1,609)	p
IACSP99-4013	0,083	(1,626)	lmnop	IACSP95-3028	0,000	(1,609)	p
IACSP96-7586	0,071	(1,623)	lmnop	IAC86-2480	0,000	(1,609)	p
IACSP96-3056	0,071	(1,623)	lmnop	IACSP97-2098	0,000	(1,609)	p
IACSP97-2084	0,049	(1,619)	lmnop	IACSP95-5000	0,000	(1,609)	p
IACSP97-3406	0,049	(1,619)	lmnop	IACSP98-2030	0,000	(1,609)	p
IACSP94-4004	0,047	(1,619)	lmnop	SP70-1143	0,000	(1,609)	p
IAC86-2210	0,047	(1,619)	lmnop	IACSP95-2078	0,000	(1,609)	p
IACSP96-7506	0,033	(1,616)	lmnop	IACSP97-2020	0,000	(1,609)	p
IACSP99-4010	0,033	(1,616)	mnop	IACSP96-7569	0,000	(1,609)	p
IACSP97-3313	0,025	(1,615)	nop	IACSP98-5046	0,000	(1,609)	p
SP79-1011	0,025	(1,615)	nop	IACSP97-6682	0,000	(1,609)	p
IACSP96-3048	0,023	(1,614)	op	SP80-3280	0,000	(1,609)	p
IACSP97-3384	0,023	(1,614)	op	IACSP97-7077	0,000	(1,609)	p
IACSP97-3331	0,023	(1,614)	op	IACSP99-3085	0,000	(1,609)	p
IACSP96-2019	0,013	(1,612)	op	IACSP96-3060	0,000	(1,609)	p
IACSP94-2101	0,013	(1,612)	op	IACSP97-2055	0,000	(1,609)	p
IACSP95-1218	0,013	(1,612)	op	IACSP97-7074	0,000	(1,609)	p
SP86-42	0,013	(1,612)	op	IACSP97-7065	0,000	(1,609)	p
IACSP95-5094	0,013	(1,612)	op				

*Mean: non transformed data. ** Mean: data transformed in ln (x+5).

Table 5. Symptom grade means comparisons of evaluations (days after planting) by LSD test ($p<0,05$).

Days after planting	*Mean	**Mean	
75	0,264599	(1,661005)	a
105	0,258653	(1,659875)	a
90	0,215083	(1,651555)	b
60	0,170462	(1,642962)	c
45	0,098075	(1,628863)	d
30	0,096938	(1,62864)	d

*Mean: non transformed data. ** Mean: data transformed in ln (x+5).

Table 6. Genotype's symptom grade means at different length periods after planting.

Days after planting	30	45	60	75	90	105
RB925211	1,272	1,272	1,435	1,534	0,593	0,593
IACSP96-1066	0,669	0,669	1,039	1,531	1,432	1,432
IACSP93-6006	0,439	0,561	0,623	0,848	0,623	0,623
IACSP93-2060	0,439	0,288	1,082	1,221	1,327	1,327
IACSP98-3099	0,404	0,404	0,348	0,407	0,407	0,407
IACSP97-4039	0,348	0,348	0,783	1,325	0,848	0,848
RB867515	0,288	0,288	0,500	0,709	0,522	0,522
IAC91-1099	0,221	0,221	0,221	0,429	0,142	0,142
IACSP96-3076	0,200	0,200	0,348	0,407	0,348	0,348
IACSP97-2053	0,154	0,154	0,221	0,719	0,916	0,916
IACSP97-7018	0,142	0,142	0,348	0,200	0,348	0,348
IAC52-150	0,142	0,077	0,077	0,407	0,279	0,279
IACSP98-3020	0,142	0,142	0,348	0,407	0,380	0,380
IACSP93-3046	0,142	0,077	0,142	0,142	0,142	0,142
IACSP97-2109	0,000	0,000	0,215	0,303	0,303	0,303
SP79-1011	0,000	0,000	0,077	0,077	0,000	0,000
IACSP96-2019	0,000	0,077	0,000	0,000	0,000	0,000
IACSP94-2101	0,000	0,000	0,000	0,077	0,000	0,000
IAC86-2210	0,000	0,000	0,000	0,000	0,142	0,142
IACSP96-3048	0,000	0,000	0,000	0,142	0,000	0,000
IACSP96-7506	0,000	0,000	0,000	0,000	0,000	0,200
IACSP96-7586	0,000	0,000	0,000	0,142	0,142	0,142
IACSP94-4004	0,000	0,000	0,000	0,000	0,142	0,142
IACSP95-1218	0,000	0,000	0,000	0,000	0,077	0,077
IACSP97-3313	0,000	0,000	0,000	0,154	0,000	0,000
IACSP97-3331	0,000	0,000	0,000	0,142	0,000	0,000
IACSP96-3056	0,000	0,000	0,142	0,142	0,142	0,142
IACSP97-3406	0,000	0,000	0,000	0,000	0,000	0,301
IACSP95-3018	0,000	0,000	0,142	0,407	0,000	0,000
IACSP95-5094	0,000	0,000	0,000	0,000	0,142	0,142
IACSP97-3384	0,000	0,000	0,000	0,142	0,000	0,000
IACSP97-2084	0,000	0,000	0,077	0,221	0,000	0,000
IACSP99-4008	0,000	0,000	0,142	0,200	0,200	0,200
IACSP99-4010	0,000	0,000	0,000	0,200	0,000	0,000
IACSP99-4013	0,000	0,000	0,142	0,142	0,221	0,221
IAC87-3396	0,000	0,000	0,142	0,077	0,142	0,142
RB72454	0,000	0,000	0,200	0,200	0,142	0,142
IAC91-2218	0,000	0,000	0,142	0,200	0,200	0,200
IACSP96-1005	0,000	0,000	0,000	0,000	0,301	0,301
SP86-42	0,000	0,000	0,000	0,077	0,000	0,000

Non transformed data.

Table 7. Variance components estimate of mosaic symptom grades under field conditions.

Variance components	***Estimate**	****Estimate**
Phenotypic variance	0,007997	0,002491
Genetic variance	0,001549	0,001549
Variance between experimental plot	0,000972	0,000486
Variance among plants within plot	0,005476	0,000456
Heritability	0,1937	0,6218

*Estimate at individual level **Plot means based estimate.

Table 8. Symptom grades of mosaic and PTA-ELISA results for SCMV infection before and after mechanical inoculation of sugarcane genotypes.

Genótipo	ELISA shooting	Max. Grade Greenhouse	ELISA Greenhouse	Max. Grade Field	Symptoms recovery in the field	Class of resistance
RB925211	+	2	+	3	N	S
IACSP96-1066	-	3	+	3	N	S
IACSP93-2060	-	3	+	3	N	S
IACSP97-4039	+	3	+	3	N	S
IACSP93-6006	-	0	-	3	N	S
RB867515	-	0	+	3	N	S
IACSP97-2053	+	3	+	3	N	S
IACSP98-3099	-	0	+	3	N	S
IACSP96-3076	-	3	+	3	N	S
IACSP98-3020	-	0	-	3	N	S
IAC91-1099	-	3	+	3	N	S
IACSP97-7018	+	3	+	3	N	S
IAC52-150	-	0	+	3	N	S
IACSP99-4008	-	0	-	3	N	S
IACSP97-2109	-	3	+	3	N	S
IACSP93-3046	-	0	-	2	N	S
IAC91-2218	-	0	+	3	N	S
IACSP96-1005	+	0	+	2	N	S
RB72454	-	0	-	3	N	S
IACSP95-3018	-	0	-	3	S	I
IAC87-3396	-	0	-	2	N	S
IACSP99-4013	+	0	-	2	N	S
IACSP96-7586	-	0	-	2	N	S
IACSP96-3056	-	0	+	2	N	S
IACSP97-2084	-	0	-	2	S	I
IACSP97-3406	-	0	-	2	N	S
IACSP94-4004	-	0	-	2	N	S
IAC86-2210	+	0	+	2	N	S
IACSP96-7506	+	0	-	3	N	S
IACSP99-4010	+	0	+	3	S	I
IACSP97-3313	-	0	-	1	S	I
SP79-1011	-	0	-	1	S	I
IACSP96-3048	-	0	-	2	S	I
IACSP97-3384	-	0	+	2	S	I
IACSP97-3331	-	0	+	2	S	I
IACSP96-2019	+	0	+	1	S	I
IACSP94-2101	-	0	-	1	S	I
IACSP95-1218	-	0	-	1	N	I
SP86-42	-	0	-	1	S	I
IACSP95-5094	+	0	-	1	N	I
IACSP02-2074	+	0	-	0	N	R
IACSP99-4007	+	0	-	0	N	R
IACSP98-2053	-	0	-	0	N	R
IACSP97-2028	-	0	-	0	N	R
CTC9	-	0	+	0	N	R
RB835486	-	0	+	0	N	R
RB855156	-	0	-	0	N	R
IACSP97-2023	-	0	+	0	N	R
IACSP99-1308	+	0	-	0	N	R
IACSP96-2042	+	0	+	0	N	R
IACSP99-1305	-	0	+	0	N	R
IACSP94-2094	-	0	+	0	N	R
IACSP99-4011	+	0	+	0	N	R
IACSP98-3021	-	0	+	0	N	R
IACSP99-3009	-	0	-	0	N	R
IACSP97-7543	-	0	-	0	N	R
IACSP97-2000	-	0	-	0	N	R
IACSP96-2100	-	0	-	0	N	R

IACSP98-5010	+	0	-	0	N	R
IACSP98-6202	-	0	-	0	N	R
IAC91-2195	-	0	-	0	N	R
IACSP95-3028	-	0	-	0	N	R
IAC86-2480	-	0	-	0	N	R
IACSP97-2098	-	0	-	0	N	R
IACSP95-5000	-	0	-	0	N	R
IACSP98-2030	+	0	-	0	N	R
SP70-1143	-	0	-	0	N	R
IACSP95-2078	-	0	-	0	N	R
IACSP97-2020	-	0	-	0	N	R
IACSP96-7569	-	0	-	0	N	R
IACSP98-5046	-	0	-	0	N	R
IACSP97-6682	+	0	-	0	N	R
SP80-3280	-	0	-	0	N	R
IACSP97-7077	+	0	-	0	N	R
IACSP99-3085	-	0	-	0	N	R
IACSP96-3060	+	0	-	0	N	R
IACSP97-2055	-	0	-	0	N	R
IACSP97-7074	+	0	-	0	N	R
IACSP97-7065	-	0	-	0	N	R

PTA-ELISA results: (-) negative and (+) positive. Max. grade: higher grade for mosaic symptoms observed by genotype. Recovery in the field: development of visual symptoms and posterior recovery (S), symptom permanence (N) at the end of evaluations. Class of resistance: Susceptible (S), Intermediary (I) and Resistant (R).

CAPÍTULO 3 - Screening sugarcane wild accessions for resistance to *Sugarcane mosaic virus* (SCMV)

ABSTRACT – Wild sugarcane germplasm were evaluated for SCMV resistance, in order to identify new sources of mosaic resistance for future introgression crosses. An evaluation of symptoms by grade scale associated with serological test *Plate Trapped Antibody*-ELISA were performed for 43 clones, including *Saccharum officinarum, S. barberi, S. spontaneum* and *S. robustum* species, maintained under natural infection conditions. The clones IS76-155, IJ76-418 red, NG57-50, Ceram red, Badila, Sac.off. 8276, Fiji19 IJ76-313, US 57-141-5, Krakatau, IN8458, IN84-88, IN84-82, Gandacheni and Chin possibly represents resistant sources. A differential behavior among *Saccharum* species were also observed, with higher susceptibility in *S. officinarum* clones, followed by *S. robustum* clones, and a resistant behavior of *S. barberi* and *S. spontaneum* clones.

Keywords: Sources of resistance, genus *Saccharum*, mosaic

1. Introduction

Mosaic is one of the most disseminated viruses in sugarcane, maize, sorghum and other poaceus across the world, being caused by virus of *Sugarcane mosaic virus* (SCMV) subgroup of the genus *Potyvirus*, family *Potyviridae* (Gonçalves et al. 2012; Viswanathan and Mohanraj 2001). Seven distinct potyviruses are recognized in this group: *Sugarcane mosaic virus* (SCMV); *Sorghum mosaic virus* (SrMV), *Sugarcane streak mosaic virus* (SCSMV), *Johnsongrass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV), *Pennisetum mosaic virus* (PenMV) and *Zea mosaic virus* (ZeMV). In natural conditions there are reports 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 only potyvirus causing mosaic in Brazil is the SCMV (Gonçalves et al. 2011, 2007, 2004).

The disease is responsible for varying economic impacts on sugarcane, depending on the species and strain and on the sugarcane variety. Recurrent mosaic epidemics in sugarcane in Argentina, Brazil, Cuba, Porto Rico and USA caused the near outbreak of the sugar industry (Abbott 1961; Yang and Mirkov 1997), leading to the introduction of *Saccharum* interspecific hybrids imported from Java in order to control the fast spreading of the disease in noble canes (*Saccharum officinarum*), cultivated in these countries until the beginning of XXth

century (Koike and Gillaspie 1989). In Brazil, the disease is relatively controlled due to breeding programs and practical field approaches, but screening for resistance to mosaic remains as an essential step because of factors like favorable epidemiological aspects to mosaic dissemination and a recent description of new isolates, which reinforce the current importance of the disease (Gonçalves et al. 2012, 2007, 2004). The development of new virus strains requires the release of new cultivars, making relevant the search for new sources of resistance in germplasm collections to increase the genetic diversity available for breeding. Such studies were performed for *Sugarcane yellow leaf virus* (SCYLV) under natural infection conditions (Comstock et al. 2001), for SCMV strain H (Grisham et al. 1992) and for SrMV HH (Li et al. 2013) under greenhouse conditions with artificial inoculation.

Sugarcane belongs to the genus *Saccharum* L., tribe *Andropogoneae* of the family *Poaceae*. The genus includes six species (*Saccharum officinarum* L., *S. sinense* Roxb., *S. barberi* Jesw., *S. edule* Hassk, *S. spontaneum* L. e *S. robustum* Brandes & Jesw. ex Grassl) that, jointly with related genus *Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya*, constitutes the 'Saccharum' complex (Daniels et al. 1975), which represents sources of genetic variation for sugarcane breeding programs. Modern cultivars corresponds to a complex of aneuploidy and polyploidy species (Grivet and Arruda 2002) from introgressions with 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). The major limitation of sugarcane breeding is its reduced genetic base, being modern cultivars descendants of a few ancestors or foundation clones (Deren 1995; Berding and Roach 1987). In Brazil, sugarcane breeding programs uses mainly national varieties as parental (Barbosa 2001), which are based in a common genetic basis obtained in the beginning of the century from successive crosses between *S. officinarum* and *S. spontaneum* (Duarte Filho et al. 2010).

Beyond necessity of increasing the genetic diversity in the available germplasm, the current focus on biomass production, for ethanol and energy production, has stimulated the implementation of a parallel introgression program between commercial varieties and wild accessions, involving mainly *S. spontaneum* and *S. robustum*, justifying the importance of the study of these accessions response to SCMV. In this regard, the present study aimed to evaluate SCMV infection in part of germplasm collection of Centro de Cana-de-açúcar IAC, in order to identify possible sources of resistance and guide future introgression crosses.

2. Material and Methods

2.1. Genotypes panel

The resistance to mosaic was evaluated in 43 accessions, which include 30 of *S. officinarum*, 3 of *S. barberi*, 6 of *S. spontaneum* and 4 of *S. robustum*, maintained at the IAC Breeding Station, Brazil (Table 1).

2.2. Experimental design and evaluation of mosaic incidence and severity

The experiment was carried out in a previous trial, planted in October 2011, in a three complete randomized block design consisting of plots of 1 meter spaced at 1 m between plots and 1.5 m between rows, which adopted the cultivars IACSP95-5000 and IACSP93-046 as control.

Sugarcane accessions were evaluated for mosaic incidence and severity in the sugarcane ratoon, under natural infection conditions, in May 2013. A symptom grade scale (Pinto et al. 2013), was used to evaluate the severity of mosaic symptoms, as following:

0. Symptom absence;
1. Mild mosaic in one leaf;
2. Intense mosaic in two or more leafs;
3. Generalized intense mosaic, along with reduction in plant growth;

2.3. SCMV detection by PTA-ELISA

The serological test PTA-ELISA with an antiserum specific to SCMV was performed in collected young sugarcane leaves of each accession, in order to confirm the virus infection. Leaf samples were grounded in carbonate buffer 0.05M, pH 9.6, 1:10 ratio (weight: volume), to cover ELISA plates. The antiserum was diluted in PBS-TPO 1:6000, with 1% of skim milk powder. The alkaline phosphatase-conjugated “anti-rabbit” IgG was diluted 1:3000. The enzyme substrate p-nitrophenylphosphate (PNPP) was used in the concentration of 1mg/ml of diethanolamine buffer pH 9,8. Four absorbance readings at 405nm ($A405_{nm}$) were done in an ELISA plate reader at every twenty minutes. Samples with A405nm reading mean three times above the negative control’s

reading means were considered as SCMV infected.

2.4. Statistical analysis

Variance analysis (F Test) was performed with grade results, using the SAS (2008) *Proc GLM* procedure, and means comparisons by LSD Test ($p<0.05$), 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 nested to i^{th} specie in j^{th} block, μ = overall mean, B_j = effect of j^{th} block, S_i = effect of i^{th} specie, G_k = effect of k^{th} genotype nested to i^{th} 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 clones (accessions) with maximum grade from 2 to 3 were classified as susceptible. Clones with maximum grade of 1 were classified as intermediary. Clones 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 as possible source of resistance.

3. Results and discussion

Variance analysis showed significant F values ($p<0.01$) for specie, genotype nested to specie and non-significant effect for block, indicating a variability among accessions and species. Unfolding the variance within species, significant F value ($p<0.01$) was observed for *S. officinarum*, indicating the variability among accessions within this specie for mosaic response, while clones within *S. barberi*, *S. robustum* and *S. spontaneum* did not vary significantly (Table 2). The specie *S. officinarum*, was the most susceptible according to LSD test ($p<0.01$), followed by *S. robustum*, while *S. spontaneum* and *S. barberi* were resistant (Table 3). The most susceptible clones by LSD test ($p<0.05$) were IJ76-560 and IN84-126, followed by Ajax, NG57-213, NG7718 and Caiana fita, all belonging to *S. officinarum* (Table 4). According to Koike and Gillaspie (1989), within genus *Saccharum*, *S. officinarum* is the most susceptible, while *S. barberi* and *S. robustum* are moderately susceptible, and *S. sinense* and *S. spontaneum* are resistant, depending on the SCMV strain involved. In early reports, clones of *S. spontaneum* were immune to SCMV with few exceptions, attesting the resistance of the specie (Summers et al. 1948; Brandes and Sartoris 1939; Brandes and Sartoris 1936; Jeswiet 1930). In subsequent studies, several

clones of *S. spontaneum* were found to be susceptible to SCMV, possibly due to the development of new strains of the virus (Koike 1980; Abbott 1963). Differences in taxon means percent infection by SCMV strain H was observed in clones of sugarcane relatives by Grisham et al. (1992), being clones of *Erianthus*, *S. spontaneum*, *S. barberi* and *S. sinense* the most resistant and *S. robustum* clones the most susceptible, while interspecific hybrid and *S. offcinarum* clones were intermediate. The authors also observed clone differences within each taxon evaluated, with a considerable range of responses to mosaic among clones of *S. spontaneum*. Li et al. (2013) observed that 17 of the 37 tested *S. spontaneum* clones were highly to moderately resistant to SrMV, being pointed as valuable germplasm resistant to SrMV HH, jointly with *E. arundinaceus*. The response to SCMV of clones investigated in the present study was unreported and, in addition, the strain that prevails in the experimental area, SCMV-Rib1, is responsible for mosaic outbreaks in São Paulo state in varieties previously thought to be resistant to mosaic (Gonçalves et al. 2007). Our results show a behavior among species similar to observed by Koike and Gillaspie (1989) for *S. officinarum* and *S. robustum* and similar to results presented by Grisham et al. (1992) for *S. barberi* clones. The higher number of *S. officinarum* accessions and the origin through polycross of accessions NG57-50 and Sac.off8296, which may have involved species resistant to mosaic, had contributed to the range of responses to mosaic here observed, besides the variation within specie be expected, according to literature.

The results by grade scale and PTA-ELISA indicate that accessions IS76-155, IJ76-418 red, NG57-50, Ceram red, Badila, Sac,off. 8276, Fiji19, IJ76-313 of *S. officinarum*; US 57-141-5 of *S. robustum*; Krakatau, IN8458, IN84-88 e IN84-82 of *S. spontaneum*; Chunee, Gandacheni and Chin of *S. barberi* were free from SCMV, characterizing possible sources of mosaic resistance. The symptomless accessions NG21-21, Cana alho, IN84-105, Zopilota, US851008 and SES205A had the presence of SCMV confirmed by serological test (Table 5).

In order to explore the production of ethanol and energy, a new concept of genotype emerged recently for breeding programs, aiming a higher biomass production. There are prospects for gains in traits of importance for biomass production, such as vigor, in *S. spontaneum* accessions and related genera as *Miscanthus* and *Erianthus* (Scortecci et al. 2012), being of great importance the association of resistance to mosaic during these introgressions in sugarcane. Preliminary results indicate the resistance of *S.spontaneum* clones, where four of the six accessions were found free of mosaic and latent infections by SCMV.

4. References

Abbott, E. V., E. H. Todd. 1963. Mosaic in clones of *Saccharum spontaneum* and in Kassoer. *Proc. Int. Soc. Sugarcane Technol.* 11: 753-755.

Abbott, E.V., C.G. Hughes and J.P. Martin .1961. Mosaic In *Sugarcane Diseases of the world*, ed. Martin, J.P. 407-425. Amsterdam, The Netherlands: Elsevier Pub. Co.

Barbosa, M.H.P. 2001. Study of genetic divergence in sugarcane varieties grown in Brazil using the parentage coeficiente. *International Sugar Journal*, London, v. 103, n. 1231: 294-295.

Berding, N.; B. T. Roach. 1987. Germoplasm collection, maintenance and use. In: *Sugarcane improvement through breeding*, ed. Heinz, D. J., 143-210. Amsterdam: Elsevier Press.

Berry, D.A. 1987. Logarithmic transformations in ANOVA. *Biometrics* 43: 439-456.

Brandes, E. W.; G. B. Sartoris,; C. O. Grassl. 1939. Assembling and evaluating wild forms of sugar cane and closely related plants. *Proc. Int. Soc. Sugar Cane Technol.* 6:128-154.

Brandes, E. W.; Sartoris, G. B. 1936. Sugarcane: Its origin and improvement. In: *Yearbook of Agriculture*, ed. Washington, D.C, 561-623. U.S: Government Printing Office.

Chatenet, M., C. Mazarin, J.C. Girard, E. Fernadez, D. Gargani, G.P. Rao, M. Royer, B.E.L. Lockhart, and P. Rott. 2005. Detection of Sugarcane streak mosaic virus in sugarcane from several Asian countries. In: *Proceedings of the International Society of Sugarcane Technologists* 25: 656-662.

Comstock, J. C., J. D. Miller, and R. J. Schnell. 2001. Incidence of Sugarcane yellow leaf virus in clones maintained in the world collection of sugarcane and related grasses at the United States national repository in Miami, Florida. *Sugar Tech* 3(4):128-133.

Daniels, J., and C.A. Daniels. 1975. Geographical, Historical and Cultural Aspects of the Origin of the Indian and Chinese Sugarcanes *S. barberi* and *S. sinense*. *Sugarcane Breeding Newsletters*, vol. 36: 4–23. ISSN 1028-1193.

Deren, C.W. 1995. Genetic base of U.S. Mainland sugarcane. *Crop Science* v.35: 1195-1199.

D'Hont, A., G.M. Souza, M. Menossi, M. Vinentz, M.A. Van Sluys, J.C. Glaszmann and E. Ulian. 2008. Sugarcane: A major Source of Sweetness, Alcohol, and Bio-energy, In: *Genomics of Tropical Crop Plants*, eds. Moore, P.H. and R. Ming, 483-513. New York: Springer. ISBN: 9781441924339.

Duarte Filho, L.S.C., P.P. Silva, J.M. Santos, G.V.S. Barbosa, C.E. Ramalho Neto, L. Soares, J.C.F. Andrade, and C. Almeida. 2010. Genetic similarity among genotypes of sugarcane estimated by SSR and coefficient of parentage. *Sugar Tech* 12: 145–149.

Gonçalves, M.C., L.R. Pinto, S.C. Souza and M.G.A. Landell. 2012. Virus Diseases of Sugarcane. A Constant Challenge to Sugarcane Breeding in Brazil. *Functional Plant Science & Biotechnology* 6: 108-116.

Gonçalves, M.C., D.M. Galdeano, I.G. Maia and C.M. Chagas. 2011. Genetic variability of *Sugarcane mosaic virus* genotypes causing maize mosaic in Brazil. *Pesquisa Agropecuária Brasileira* 46: 362-369.

Gonçalves, M.C., A.S. Santos, L.G. Maia, C.M. Chagas and R. Harakava. 2007. Characterization of an isolate of Sugarcane mosaic virus breaking down resistance of commercial sugarcane varieties. *Fitopatologia Brasileira* 32: 32-39.

Gonçalves, M.C., Y.J.C.B Moreira, I.G. Maia, A.S. Santos, G.M. Fantin and A. Chaves. 2004. Identificação e caracterização de isolados pertencentes ao subgrupo do *Sugarcane mosaic virus* no estado de São Paulo. *Fitopatologia Brasileira* 29: 129-129.

Grisham M.P., D.M. Burner and B.L. 1992. Legendre Resistance to the H Strain of *Sugarcane mosaic virus* among wild forms of sugarcane and relatives. *Plant Disease* 76: 360-362.

Grivet, L., J.C. Glaszmann and A. D´Hont,. 2006. Molecular Evidence for Sugarcane Evolution and Domestication, In: *Darwin's Harvest. New Approaches to the Origins, Evolution and Conservation*, eds. Moley, T., N. Zerega and H. Cross, 49-66. West Sussex, UK: Columbia University Press. ISBN 9780231133166.

Grivet L. and P. Arruda. 2002. Sugarcane genomics: depicting the complex genome of an important tropical crop. *Current Opinion in Plant Biology* 5: 122-127.

Irvine, J.E. 1999. *Saccharum* Species as Horticultural Classes. [s.I.]: *Theoretical and Applied Genetics*, vol.98, No.2, 186-194, ISSN 0040-5752.

Jeswiet, J. 1930. The development of selection and breeding of the sugar cane in Java. [s.I.]: *Proc. Int. Soc. Sugar Cane Technol.* 17: 1523-1527.

Koike, H. and A.G. JR. Gillaspie. 1989. Mosaic. In: *Diseases of Sugarcane: Major Diseases*, eds. Ricaud C., B.T. Egan, JR. A.G. Gillaspie and C.G. Hughes, 301-322. Amsterdam, the Netherlands: Science Publishers.

Koike, H. 1980. Evidence of resistance in *Saccharum spontaneum* and Saccharum-related genera to sugarcane mosaic virus strains H and I. [s.I.]: *Proc. Int. Soc. Sugar Cane Technol.* 17: 1523-1527.

Li, W., X. Wang, Y. Huang, H. Shan, Z. Luo, X. Ying, R. Zhang, K. Shen and J. Yin. 2013. *Screening sugarcane germplasm resistant to Sorghum mosaic virus. Crop Protection*, 43: 27-30.

Pinto, L.R., M.C. Gonçalves, D.M. Galdeano, D. Perecin, C.N.F. Medeiros and M.G. Landell. 2013. Preliminary investigation of sugarcane mosaic virus resistance and marker association in a sugarcane family sample derived from a bi-parental cross. São Paulo: *Proceedings Int. Soc. Sugarcane Technol. Brisbane: Int. Soc. Sugarcane Technol.* In: XXVIII ISSCT CONGRESS 28.

SAS Institute. *SAS/STAT user's guide, version 9.2 SAS,* Cary. 2008.

Scortecci, K. C., S. Creste, T. Calsa Jr., M.A. Xavier, M.G.A. Landell, A. Figueira and V.A. Benedito. 2012. Challenges, Opportunities and Recent Advances in Sugarcane Breeding. In: *Plant Breeding*, ed. Abdurakhmonov, I. InTech, available from: <http://www.intechopen.com/books/plant-breeding/challenges-opportunities-and-recent-advances-insugarcane-breeding > ISBN: 978-953-307-932-5.

Summers, E. M., E. W. Brandes and R.D. Rands. 1948. *Mosaic of sugar cane in the United States, with special reference to strains of the virus*, 124. U.S: Dep. Agric. Tech. Bull. 955.

Viswanathan, R. and D. Mohanraj. 2001. Detection of sugarcane viral diseases by serological, techniques. In: *Sugarcane Pathology* vol. 11, eds. Rao, G.P., R.E. Ford; M.S. Tosic and D.S. Teakle, 195-208. USA, Enfield (NH): Virus and Phytoplasma Diseases, Science Publishers.

Yang, Z.N., and T.E. Mirkov. 1997. Sequence and relationships of sugarcane mosaic and sorghum mosaic virus strains and development of RT-PCR-based RFLPs for strain discrimination. *Phytopathology*, 87: 932-939.

Table 1. List of sugarcane clones (accessions) from germplasm collection and origin species

Genotype	Specie	Genotype	Specie
Badila Java	F1 (*S. officinarum*)	IS76-155	F1 (*S. officinarum*)
IJ76-560	F1 (*S. officinarum* x ?)	IJ76-418 red	F1 (*S. officinarum*)
NG7718	F1 (*S. officinarum)*	NG57-50	Hybrid (*S. officinarum*) x ?
NG57-213	F1 (*S. officinarum*)	Ceram red	*S. officinarum*
Caina fita	F1 (*S. officinarum*)	Badila	*S. officinarum* x NG96
Pitu	F1 (*S. officinarum*)	Sac,off. 8276	Hybrid (*S. officinarum* x ?)
Caiana	F1 (*S. officinarum*)	Fiji19	F1 (*S. officinarum*)
Formosa	F1 (*S. officinarum* x ?)	IJ76-313	F1 (*S. officinarum*)
IN84-126	F1 (*S. officinarum*)	NG57-12	*S. robustum*
Manteiga	F2 [(F1) *S. officinarum* x ?]	IM76-229	*S. robustum*
Ajax	F2 (*S. officinarum*)	IJ76-293	*S. robustum* x ?
IJ76-566	F1 (*S. officinarum* x ?)	US 57-141-5	*S. robustum*
IJ76-317	F1 (*S. officinarum* x ?)	SES205A	*S. spontaneum*
NG21-21	F1 (*S. officinarum*)	IN8458	*S. spontaneum* x ?
IJ76-325	F1 (*S. officinarum* x ?)	Krakatau	*S. spontaneum*
Sabura	Hybrid (*S. officinarum* x ?)	US851008	*S. spontaneum* x US60-313
White transp.	*S. officinarum*	IN84-88	*S. spontaneum*
Cana alho	*S. officinarum*	IN84-82	*S. spontaneum* x ?
MZ151	F1 *(S. officinarum)*	Gandacheni	*S. barberi* x ?
IN84-105	F1 (*S. officinarum* x ?)	Chin	*S. barberi* x ?
Caiana risc.	F1 (*S. officinarum*)	Chunee	*S. barberi*
Zopilota	*S. officinarum* x ?		

Table 2. Variance analysis for mosaic symptom grade in clones (accessions) of germoplasm collection.

F.V.	D.F.	Q.M.	F
Block	1	0.038	2.41^{ns}
Genotype (sp)	42	0.036	2.27^{**}
Specie	3	0.102	6.45^{**}
Resídual	38	0.016	
S.barberi	2	0.000	0.00^{ns}
S. officinarum	29	0.045	2.76^{**}
S.robustum	3	0.030	1.83^{ns}
S.spontaneum	5	0.000	0.00^{ns}
CV(%)			7.29

Table 3. Mean comparisons of *Saccharum* species for mosaic symptom grade by LSD test (p<0,05).

Genotype	Mean[a]	Mean[b]	
S. officinarum	0,953	(1,765)	a
S. robustum	0,500	(1,697)	ab
S. spontaneum	0,000	(1,609)	b
S. barberi	0,000	(1,609)	b

a: non transformed data. b: ln (x+5) transformed data.

Table 4. Mean comparisons of genotypes for mosaic symptom grade by LSD test (p<0,05).

Genotype	Sp.[a]	Mean[b]	Mean[c]		Genotype	Sp.[a]	Mean[b]	Mean[c]	
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
NG7718	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	Badila	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
Badila 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	US851008	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	IN8458	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	Chunee	S. bar.	0,000	(1,609)	d
NG57-12	S. rob.	0,414	(1,689)	cd	Gandacheni	S. bar.	0,000	(1,609)	d
US 57-141-5	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: Specie of accession, (S. off.) *S. officinarum*, (S. rob.) *S. robustum*, (S. spo.) *S. spontaneum* and (S. bar.) *S. barberi*. b: non transformed data. c: ln (x+5) transformed data.

Table 5. Symptom grades of mosaic and PTA-ELISA results for SCMV infection of sugarcane genotypes.

Clones de *S. officinarum*							
Genotype	**ELISA[a]**	**Maximum grade[b]**	**Mosaic resp.[c]**	**Genotype**	**ELISA[a]**	**Maximum Grade[b]**	**Mosaic resp.[c]**
Badila Java	+	1	I	Sabura	+	2	S
IJ76-560	+	3	S	White transp.	+	2	S
NG7718	+	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	Badila	-	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
Clones de *S. robustum*							
IJ76-293	+	2	S	IM76-229	+	0	R
NG57-12	+	1	I	US 57-141-5	-	0	R
Clones de *S. spontaneum*							
US851008	+	0	R	Krakatau	-	0	R
SES205A	+	0	R	IN84-88	-	0	R
IN8458	-	0	R	IN84-82	-	0	R
Clones de *S. barberi*							
Chunee	-	0	R	Chin	-	0	R
Gandacheni	-	0	R				

a: PTA-ELISA result, (-) negative and (+) positive. b: higher grade for mosaic symptoms observed by accession.
c: Susceptible (S), Intermediary (I) and Resistant (R).