OCCURRENCE OF THREE GENOTYPES OF SUGARCANE YELLOW LEAF VIRUS IN A VARIETY COLLECTION IN MAURITIUS

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Abstract

SUGARCANE yellow leaf virus (SCYLV) is widely distributed in Mauritius, but so far the characterisation of genotypes of the virus has not been determined. RT-PCR primers were used to identify genetic diversity of the virus in a variety collection plot. Total nucleic acids were extracted from leaves of thirteen introduced varieties infected by SCYLV and a two-step RT-PCR was optimised for each of the three genotype-specific primer pairs CUB-F/CUB-R, REU-F/B-REU, and PER-F/PER-R tested. The presence of three SCYLV genotypes namely the BRA-PER, CUB and REU was confirmed in the germplasm collection. Genotype REU was observed in 10 varieties while 4 varieties were infected by genotype BRA-PER. Mixed infection with genotypes CUB and REU was observed in variety Co6304 while Q88 was co-infected by genotypes BRA-PER and REU. PCR fragments of 363 bp (BRA-PER), 452 bp (CUB) and 905 bp (REU) amplified from varieties PR67245, Co6304 and S17 were cloned and sequenced. Blast analysis of these three sequences showed high homologies with the corresponding genotype sequences from GenBank – 100% similarity with isolate Taiwl (AJ491144, BRA genotype), and > 99% similarity with isolates CUB-YL1 (AM083988) and REU-YL2 (AM072756) respectively. Recent screening of some local commercial cultivars revealed infection by REU and BRA-PER genotypes. Further intensive surveys are being carried out to assess the distribution of the three SCYLV genotypes in sugarcane fields in Mauritius.

Introduction

Yellow leaf disease of sugarcane (previously called yellow leaf syndrome) occurs in all major sugarcane producing countries of the world (Lockhart and Cronjé, 2000). The causal agent, *Sugarcane yellow leaf virus* (SCYLV), belongs to the genus *Polerovirus* of the family *Luteoviridae* (D'Arcy and Dormier, 2005).

SCYLV is transmitted mainly by infected cane setts and aphid vectors, *Melanaphis sacchari* and *Rhopalosiphum maidis*.

The disease is characterised by an intense yellowing of the midrib on the abaxial surface of the leaf, extending to the whole leaf as the disease progresses. Necrosis of leaves, accumulation of sucrose in leaves and shortening of terminal internodes resulting in a fan-like appearance can also be observed in diseased plants.

These symptoms, nevertheless, can also be associated with other biotic and abiotic factors including water stress. Plants may also be infected without exhibiting outward symptoms.

Leaf yellows disease caused by sugarcane yellows phytoplasma is also responsible for similar symptoms in sugarcane in some countries, including Mauritius (Cronjé *et al*., 1998; Aljanabi *et al*., 2001; Arocha *et al*., 2005).

SCYLV infection has been reported to result in yield losses. In Brazil up to 20% yield loss has been observed in cultivar SP71-6163 (Vega *et al*., 1997). Yield losses may occur even in the absence of visible symptoms (Rassaby *et al*., 2003; Grisham *et al*., 2009).

Various studies have shown that SCYLV is quite a variable virus (Abu-Ahmad *et al*., 2006a,b; Moonan and Mirkov, 2002). Phylogenetic studies by Abu-Ahmad *et al*. (2006b) identified four genotypes of SCYLV of different geographical origins (genotypes BRA from Brazil, PER from Peru, CUB from Cuba and REU from Réunion). Specific reverse-transcription PCR was developed for these genotypes, but due to the close similarity between BRA and PER genotypes, they were aggregated into a single group termed BRA-PER. Variations in infection capacity and virulence have also been reported among the different genotypes (Abu-Ahmad *et al*., 2007a,b).

The presence of SCYLV in Mauritius was confirmed in 1996 (Saumtally and Moutia, 1997). Recent studies on the prevalence of the virus in commercial sugarcane cultivation showed infection rates of more than 50% in certain varieties (MSIRI, 2008). However, there has not been any extensive study regarding the occurrence of genotypes present in Mauritius. From only a few samples screened by Abu-Ahmad *et al.* (2006b), REU and BRA-PER genotypes were observed in Mauritian cultivars. However, the diversity of SCYLV in Mauritius may be much higher since SCYLV isolate MUS1 from Mauritius (variety M 99/48) could not cluster with genotypes BRA, PER and REU (Abu-Ahmad *et al*., 2006a).

The current study was initiated to verify the occurrence of genotypes of SCYLV in a variety collection plot.

Material and methods

Total nucleic acid extractions

Leaf samples were collected from 13 introduced varieties in a collection at Réduit (Table 1) and total nucleic acids were extracted using the CTAB protocol. 1.0 g of leaf tissue was ground in liquid nitrogen and transferred to 15 mL tube with 5 mL CTAB buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 8.0, 0.2% w/v β-mercaptoethanol). After incubation at 60°C for 1 h, an equal volume of chloroform:isoamylalcohol (24:1) solution was added and centrifuged at 9000 rpm for 10 min. The extraction step was repeated once more and the nucleic acids were precipitated using two-third volume of isopropanol. Following two washes in 70% ethanol, the pellets were recovered, allowed to dry and resuspended in 500 µL of sterile distilled water.

Table 1—List of varieties tested.

	Variety	Imported from	Year of introduction
1	BJ 6732	Barbados	1983
2	B 69337	Barbados	1982
3	CP 722086	USA	1983
4	Co 6304	India	2003
5	PR 67245	Puerto Rico	1983
6	N 13	South Africa	1982
7	J 593	South Africa	1982
8	H 746418	Hawaii	1983
9	Q 88	Australia	1969
10	R 534040	Fiji	1972
11	SP 701423	Brazil	1982
12	VMC 67611	Philippines	1982
13	S 17	US World collection	1962

Reverse transcription PCR

(i) Primers YLS 462/111

The presence of SCYLV in the samples was confirmed first using RT-PCR with SCYLV specific primers YLS 462/111 (Irey, unpublished)

(ii) Genotype specific primers (BRA-PER, CUB and REU)

RT-PCR for each of the BRA-PER, CUB and REU genotypes was optimised separately. Sequences of primers used (Abu-Ahmad *et al*., 2006b) are detailed in Table 2.

Table 2—Primers for specific amplification of SCYLV genotypes BRA-PER, CUB and REU.

Genotype	Primer	Sequence (5'-3')	Expected Product size (bp)
BRA-PER	PER-F	AAC TGC TGC GTC AGG CCC A	362
	PER-R	GAC GAG CTT GCG TTG TTT TTC T	
CUB	CUB-F	GTG CTT CTC CCG GCG GTT CAC T	450
	CUB-R	ATT CGA GAA CAA CCT CCG CCT C	
REU	REU-F	CAA GCT TCT AGC GGG AAT C	905
	B-REV	CAG TTG CTC AAT GCT CCA CG	

1.5 µL of total nucleic acids was mixed with 1.0 µL of reverse primer (10 mM) RT-PCR and made to 10 µL using sterile distilled water. Denaturation was performed at 95°C for 1 min followed by immediate quenching on ice for at least 2 min.

The reverse transcription mix (10 µL) was then added. It comprised 4 µL of 5X RT buffer (Roche), 1.0 µL of dNTPs (10 mM each), 0.5 µl of RNase Inhibitor (20 U/µL), 1.0 µL of 40 U/µL M-MuLV RT (Roche) and sterile distilled water to 10 µL. The RT reaction mix was incubated at 42°C for 1 h and then denatured at 95°C for an additional 3 min.

1 µL of cDNA was used as template for PCR including 1X PCR buffer (including Mg), 200 nM of each of forward and reverse primers, 0.2 mM of each nucleotide, 1 U of *Taq* polymerase (Roche) and sterile distilled water to a final volume of 50 µL.

The amplification consisted of a denaturation phase of 95°C for 3 min, followed by 35 cycles (94°C for 30 s, 57°C for 1 min- genotype REU (or 61°C for genotypes CUB and BRA-PER), and 72°C for 1 min and 15 s), followed by a final cycle at 72°C for 10 min in a thermal cycler (Veriti, Applied Biosystems). PCR products were separated by electrophoresis in a 1% agarose gel, stained using ethidium bromide and visualised under UV light.

Cloning and sequencing

The pGEM T Easy Vector system (Promega) was used to clone three PCR products (corresponding to each genotype- REU from S17, CUB from Co6304 and BRA-PER from PR67245) according to manufacturers' recommendations. Following plasmid extraction with the QIAprep Spin Miniprep kit (Qiagen), sequencing was performed using an ABI 310 Genetic Analyser.

Analysis of cloned sequences was performed using Bioedit software and alignment with ClustalW. All sequences from this study were deposited in the Genbank database. Additionally, sequences of SCYLV genotypes were retrieved from Genbank (Table 3) and used for phylogenetic analysis.

Table 3—Isolates of SCYLV used for comparison.

Isolate	Variety	Origin	Genbank Accession nos
BRA-PERmu	PR67245	Mauritius	GQ907000*
CUBmu	Co6304	Mauritius	GQ907001*
REUmu	S17	Mauritius	GQ907002*
CUB-YL1	C132-81	Cuba	AM083988
REU-YL3	SP71-6163	Réunion	AM085306, AM085307
REU-YL2	R490	Réunion	AM072756
REU-YL1a	R570	Réunion	AM072754
REU-YL1b	R570	Réunion	AM072755
REU42	SP71-6163	Réunion	AJ621159
SCYLV-F	CP65-357	Florida	AJ249447
MUS1	M 99/48	Mauritius	AJ606085
PER-YL1a	H50-7209	Peru	AM072752
PER-YL1b	H50-7209	Peru	AM072753
PHL1	VMC76-16	Philippines	AM072628, AM072628
BRA2	RB83-5054	Brazil	AM072623
BRA1	SP83-5073	Brazil	AJ606086
SCYLV-A	CP65-357	Texas	AF157029
SCYLV-Ind	–	India	AY236971
CHN-YL1	CGT63-167	China	AM072751
Taiw1	ROC11	Taiwan	AJ491144, AJ491127
MYS1	TC4	Malaysia	AJ606084

* This study

Results and discussion

Sugarcane yellow leaf virus was present in all 13 varieties tested by RT-PCR with general primer pair YLS 462/111. Subsequently, separate RT-PCRs were optimised for each of the three genotypes (BRA-PER, CUB and REU). Using the 61°C annealing temperature as utilised by Abu-Ahmad *et al*. (2006), only the BRA-PER and CUB genotypes could be amplified with their respective primers. Further optimisation was required for the REU fragment and the final annealing temperature chosen was 57°C for successful amplification of the REU fragment using REU-F/B-REV.

Using the optimised RT-PCR, the presence of all three genotypes of SCYLV was confirmed in the variety collection plot, with amplification of a fragment of 362 bp, 450 bp and 905 bp representing the BRA-PER, CUB and REU genotypes respectively (Figure 1a,b,c). Genotype REU was observed in 10 varieties (Figure 1c), 4 varieties were infected with BRA-PER genotype (Figure 1b), and CUB genotype was present only in variety Co6304 (Figure 1c). Two varieties were co-infected with two genotypes of SCYLV, notably REU/BRA-PER (variety Q88, Lane 9) and CUB/REU (variety CO6304, Lane 4).

The PCR fragments from the three genotypes were cloned and sequenced (BRA-PERmu from PR67245-363 bp, CUBmu from CO6304, 452 bp and REUmu from S17-905 bp). Blast sequence analysis confirmed the occurrence of the BRA, CUB and REU genotypes in Mauritius. The BRA-PERmu fragment shared highest homology with isolate Taiw1 (AJ491144, BRA genotype), while the CUBmu fragment shared > 99% similarity with isolate CUB-YL1 (AM083988) and similarly for REUmu and REU-YL2 (AM072756).

Sequences of the genotypes from this study and those retrieved from Genbank were compared and a phylogenetic tree constructed for fragment CUB (Figure 2). The CUBmu genotype clustered with the CUB-YL1 genotype (Var C132-81) from CUBA and diverged from other genotypes of SCYLV. Abu-Ahmad *et al*. (2006a) analysed the amino acid identity of part of the open reading frame I of this genotype and compared with BRA, PER and REU. The CUB-YL1 genotype shared only 77–80% amino acid sequence identity with other genotypes prompting the suggestion of occurrence of a new virus.

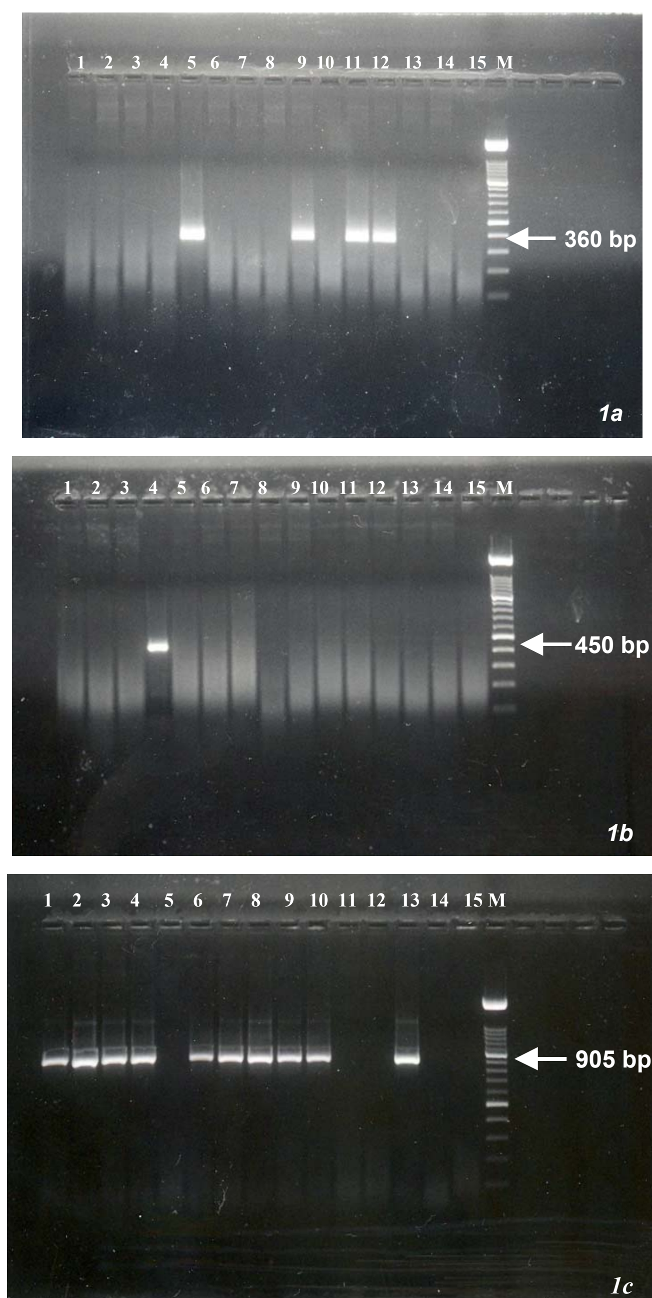

Fig. 1—Amplification of fragments of three genotypes of SCYLV. (a- BRA-PER, b- CUB and c-REU genotypes). Lanes 1–13 are sugarcane varieties as per Table 1, lane 14 is an extract from a disease free sugarcane plantlet, lane 15 is water control and M is 100 bp molecular weight marker, Roche).

Variety Co6304 (infected with CUB genotype) was introduced in 2003 in Mauritius from India and recent studies have confirmed the presence of isolate CUB in India (Viswanathan *et al*., 2008). Studies of Abu-Ahmad *et al*. (2006b) showed the geographical grouping of SCYLV genotypes and implied different virus introduction and evolution histories in the respective environments.

SCYLV diversity in Mauritius may be more complex as a Mauritian isolate, MUS1, was previously found not to cluster with the three groups (Abu-Ahmad *et al*., 2006a). This is also supported by the existence of the three genotypes in the variety collection plot observed in the present study. In recent tests of local cultivars, only isolates BRA-PER and REU have been recorded, but there is a need to establish more precisely the diversity of SCYLV present by

screening a larger number of samples. Variations in virulence and infection capacity of SCYLV genotypes exist, as well as reaction of different varieties to the virus. It is necessary to investigate further the nature of the variation of SCYLV and its impact on sugarcane cultivation in Mauritius. For instance, SCYLV has also been detected in the aphid *Melanaphis sacchari* (MSIRI, 2008), and it would be of epidemiological interest to characterise the genotype(s) harboured by this vector.

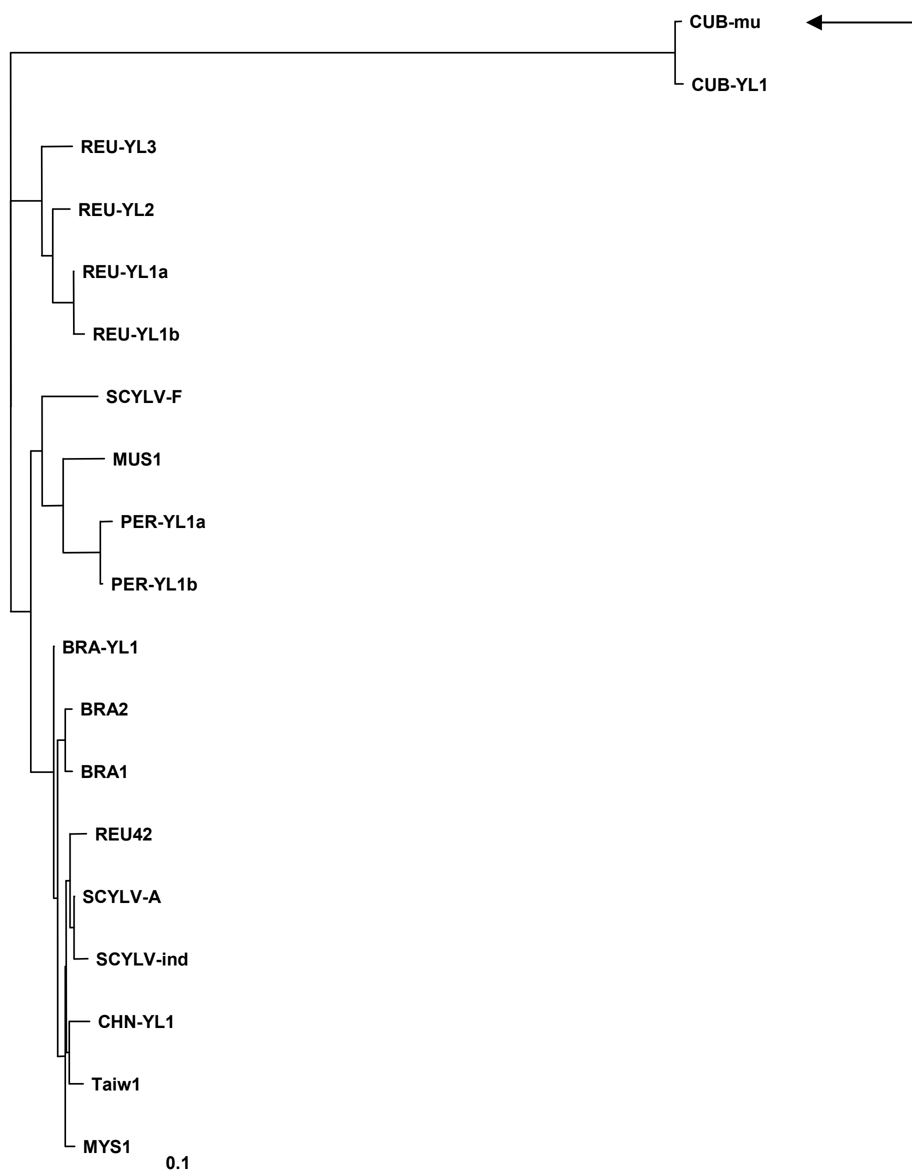

Fig. 2—Neighbour-joining tree of SCYLV isolates. The CUB-mu fragment (This study) cluster with genotype CUB-YL1.

Conclusions

The present study confirmed the occurrence of three genotypes of SCYLV in a sugarcane germplasm collection in Mauritius. During an initial screening of commercial fields, the REU and BRA-PER genotypes have been found predominant while the CUB genotype detected in this study is from a germplasm collection. Further investigations are warranted for a more precise assessment of the distribution of the genotypes, their epidemiology and their impact on yield.

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LA PRÉSENCE DE TROIS GÉNOTYPES DU *SUGARCANE YELLOW LEAF VIRUS* (SCYLV) DANS UNE COLLECTION VARIÉTALE À MAURICE

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MOTS- CLÉS: Canne à Sucre, SCYLV, Génotypes, Diversité Génétique.

Résumé

QUOIQUE LE *Sugarcane yellow leaf virus* (SCYLV) est très répandu à Maurice, les génotypes du virus n'ont pas été déterminés. Des amorces RT-PCR ont été utilisées pour identifier la diversité génétique du virus dans treize variétés infectées provenant d'une collection variétale. Les acides nucléiques totaux extraits à partir des feuilles des treize variétés ont été soumis à un test RT-PCR en deux étapes. Le protocole a été optimisé pour chaque génotype avec les paires d'amorces spécifiques CUB-F/CUB-R, REU-F/B-REU et PER-F/PER-R. Trois génotypes de SCYLV notamment BRA-PER, CUB et REU ont été confirmés dans les variétés testées. Le génotype REU a été observé dans 10 variétés tandis que 4 variétés étaient infectées par le génotype BRA-PER. Une infection mixte causée par les génotypes CUB et REU a été observée dans la variété Co6304, tandis que la Q88 était co-infectée par les génotypes BRA-PER et REU. Les produits d'amplification de 363 pb (BRA-PER), 452 pb (CUB) et 905 pb (REU), provenant des variétés PR67245, Co6304 et S17 respectivement, ont été clonés et séquencés. Les trois séquences analysées par l'algorithme Blast démontrent de fortes homologies avec les génotypes correspondants du SCYLV dans Genbank; pour le génotype BRA, 100% de similarité avec Taiwl (AJ491144), et plus de 99% de similarité avec les isolats CUB-YL1 (AM083988) et REU-YL2 (AM072756) respectivement. Récemment les génotypes REU et BRA-PER ont été révélés dans des variétés commerciales, d'où la nécessité de faire des prospections intensives au champ pour évaluer la répartition des différents génotypes de SCYLV à Maurice.

EXISTENCIA DE TRES GENOTIPOS DE VIRUS DE HOJA AMARILLA DE LA CAÑA DE AZÚCAR EN UNA COLECCIÓN DE VARIEDADES DE MAURICIO

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**PALABRAS CLAVE: Caña de Azúcar, SCYLV,
Genotipos de Virus, Diversidad de Virus.**

Resumen

EL VIRUS de la hoja amarilla de la caña de azúcar (SCYLV, por sus siglas en Inglés) está ampliamente distribuído en Mauricio, pero al momento la caracterización de genotipos del virus no ha sido determinada. Iniciadores (*primers*) de RT-PCR fueron usados para identificar la diversidad genética del virus en una parcela con una colección de variedades. Los ácidos nucléicos completos se extrajeron a partir de las hojas de unas trece variedades introducidas, infectadas por el SCYLV, y se evaluaron con una RT-PCR de dos pasos optimizada para cada uno de los pares de iniciadores específicos de los tres genotipos (CUB-F/CUB-R, REU-F/B-REU, y PER-F/PER-R). La presencia de los tres genotipos de SCYLV, en específico BRA-PER, CUB y REU, se confirmó en la colección de germoplasma. El genotipo REU fue observado en 10 variedades, en tanto que 4 fueron infectadas por el genotipo BRA-PER. La infección mixta de genotipos CUB y REU fue observada en la variedad Co6304, mientras que Q88 fue co-infectada con los genotipos BRA-PER y REU. Los fragmentos derivados de la PCR de 363 pares de base (bp) (BRA-PER), 452 pb (CUB), 905 pb (REU) fueron amplificados de las variedades PR67245, Co6304 y S17, clonándoseles y secuenciándoseles. El análisis *Blast* de estas tres secuencias mostró gran homología con las secuencias de genotipo correspondientes en la base de datos "Genbank" - 100% en similitud con el aislado Taiwl (AJ491144, genotipo BRA), y > 99% en similitud con los aislados CUB-YL1 (AM083988) y REU-YL2 (AM072756), respectivamente. La reciente criba de algunos cultivares comerciales reveló una infección por los genotipos REU y BRA-PER. Inspecciones intensivas adicionales serán conducidas para los tres genotipos de SCYLV en campos de caña de azúcar de Mauricio.