Screening for *Sugarcane yellow leaf virus* in quarantine in Mauritius

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Sugarcane yellow leaf virus

Disease: yellow leaf

Virus: +ssRNA genome, family Luteoviridae, genus *polerovirus*

Symptoms: yellowing of midrib - extending to leaf blade, & dwarfing of terminal internodes & producing a fan-like appearance

Transmission: infected cane setts & aphid vectors- *Melanaphis sacchari, Rhopalosiphum maidis & Ceratovacuna lanigera*

Yield loss: up to 25-30% reported in susceptible varieties

Genotypes reported: REU, BRA-PER, CUB, IND, CHN1, HAW-PER





Sugarcane Quarantine in Mauritius

- Mauritius has been importing sugarcane since 1914
- Quarantine facilities exist in the island since 1946
- Import of varieties is subject to: possession of import permit, phytosanitary certificate from exporting country
- Varieties undergo 2 years closed quarantine
- After 2 years clones are released for open quarantine if no problem observed

Year 1

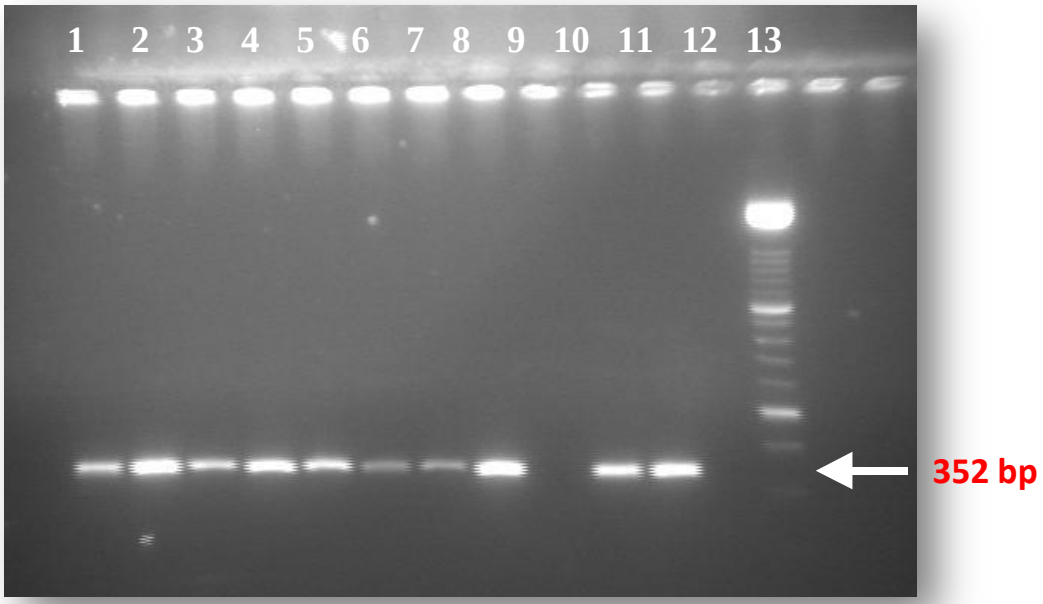
- Plants are observed for disease symptoms; smut, leaf scald, RSD, yellow leaf
- 6 to 8 months old plants screened for SCYLV
- Leaf samples are taken for SCYLV diagnosis

Screening for SCYLV

Conventional RT-PCR

- Total nucleic acids extracted from 1.0 g of leaf including midrib
- cDNA synthesis using reverse primer
- RT-PCR with primers *YLS 462/YLS 111* *

*Irey unpublished



Confirmatory test using Real-Time RT-PCR

Why Real-Time Taqman® RT-PCR?

- Increased sensitivity
- Increased specificity (internal SCYLV probe)
- Amplification of an RNA specific internal positive control based on the intron-exon boundary of caffeic acid 3-o-methyltransfease gene of sugarcane to avoid false -ve

Primers & probes used for SCYLV & plant internal control detections using duplex real-time RT-PCR (Korimbocus *et al., 2002)*

- SCYLV primers/probe designed within conserved regions of coat protein sequence (ORF3)
- Caffeic acid 3-O-Met internal sugarcane positive control

Assay	Name	Sequence 5'-3'	Location on RNA	Product size (bp)
O-MET	501F	AAG GTC CTC ATG GAG AGC TGG TA	501–523	69
O-MET	570R	CCT TGT TGA ACG GGA TGC C	553–570	
O-MET	OMET Probe (Hex-labeled)	CAA GGA CGC GGT GCT TGA CGG	530–551	-
SCYLV	CPF	CCA AAC AAC AAC AGG CTC CAA	386–406	67
SCYLV	CPR	GGG CCG GGA AGA CTT TCT T	438–453	
SCYLV	ScYLV Probe (FAM-labeled)	ATT ACT AGC TTC CCC GTC AAG AGG AAC GC	408–437	-

Real-Time Taqman RT-PCR for SCYLV detection

Denature total nucleic acids in presence of reverse primers CPR (for virus) and 570R (plant +ve control) or oligo (dT) primers

95 ºC/ 1 min

Add cDNA mix (including RNase Inhibitor, M-MuLV RT, nucleotides & water) for cDNA synthesis

42 ºC/ 1 hour

95 ºC/ 3 mins

Add PCR mix containing forward primers (CPF and 501F) and probes (SCYLV-Fam labelled and *O-Met* Hex- labelled), *Taq* polymerase to cDNA mix

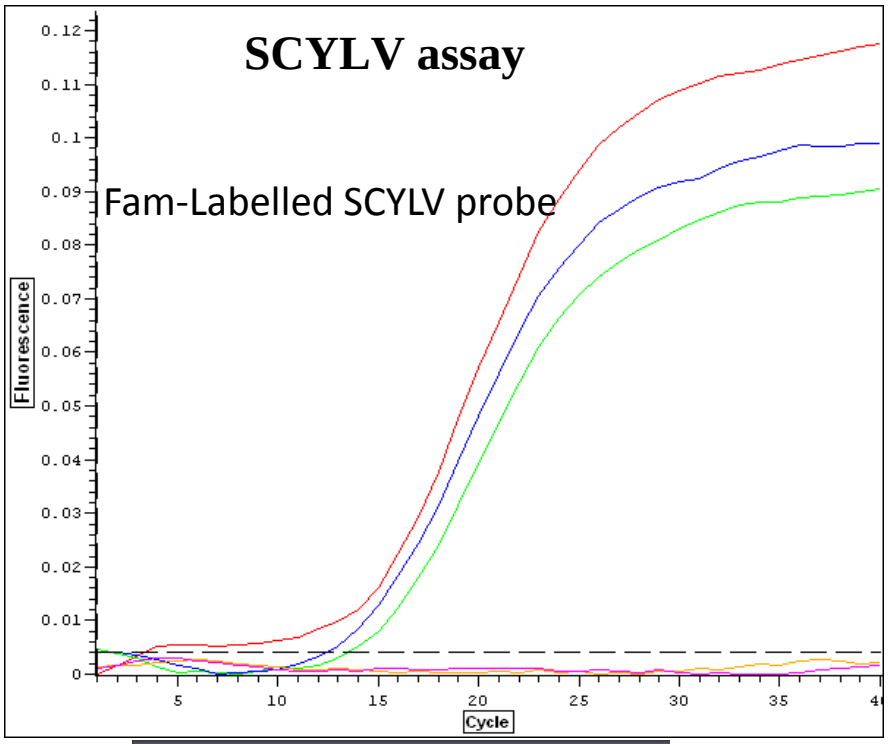
95 ºC/ 3 min

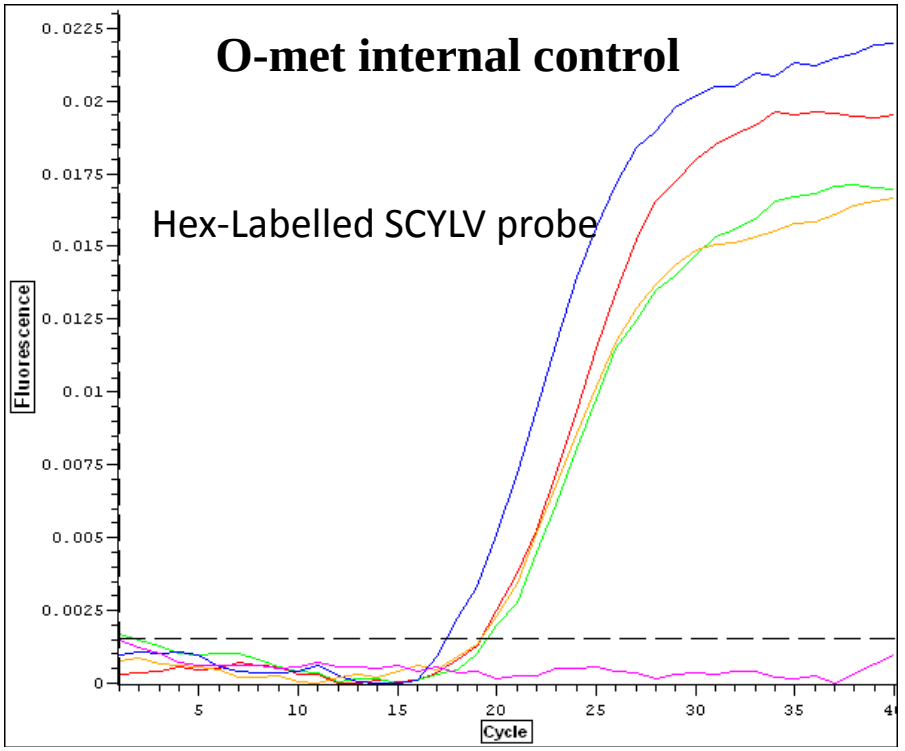
94 ºC/ 15 s

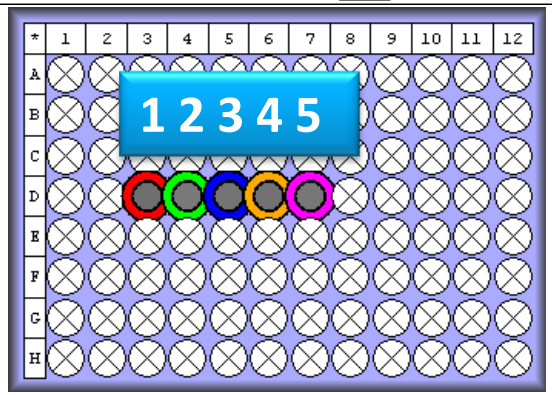
60 ºC/1 min } X 40

Plate read after each extension step

SCYLV & *O-met* detection using duplex Real-Time RT-PCR







1-3: SCYLV +ve plants
4: -ve plant control
5: water control

Diagnosis for SCYLV

- Both conventional & Real Time RT-PCR are reliable to test 6 month old plants
- Real -Time RT-PCR is applied to test tissue culture plants as it is more sensitive

SCYLV detection in quarantine 1999-2011

Country	Quarantine cycle	No of clones introduced/germinated	No of varieties infected by SCYLV
Argentina	2003-2005	4	0
	2006-2008	6	0
Australia	2001-2003	5	0
	2003-2005	7	1
	2008-2010	9	0
	2010-2012	5	0
Barbados	2004-2006	58 (mainly *Spontaneum*)	2
	2008-2010	5	1
Brazil	1999-2001	4	3
	2001-2003	8	5
	2003-2005	4	1
	2006-2008	5	1
Colombia	2001-2003	5	3
Dominican Rep	2006-2008	5	0
Ecuador	2006-2008	3	0

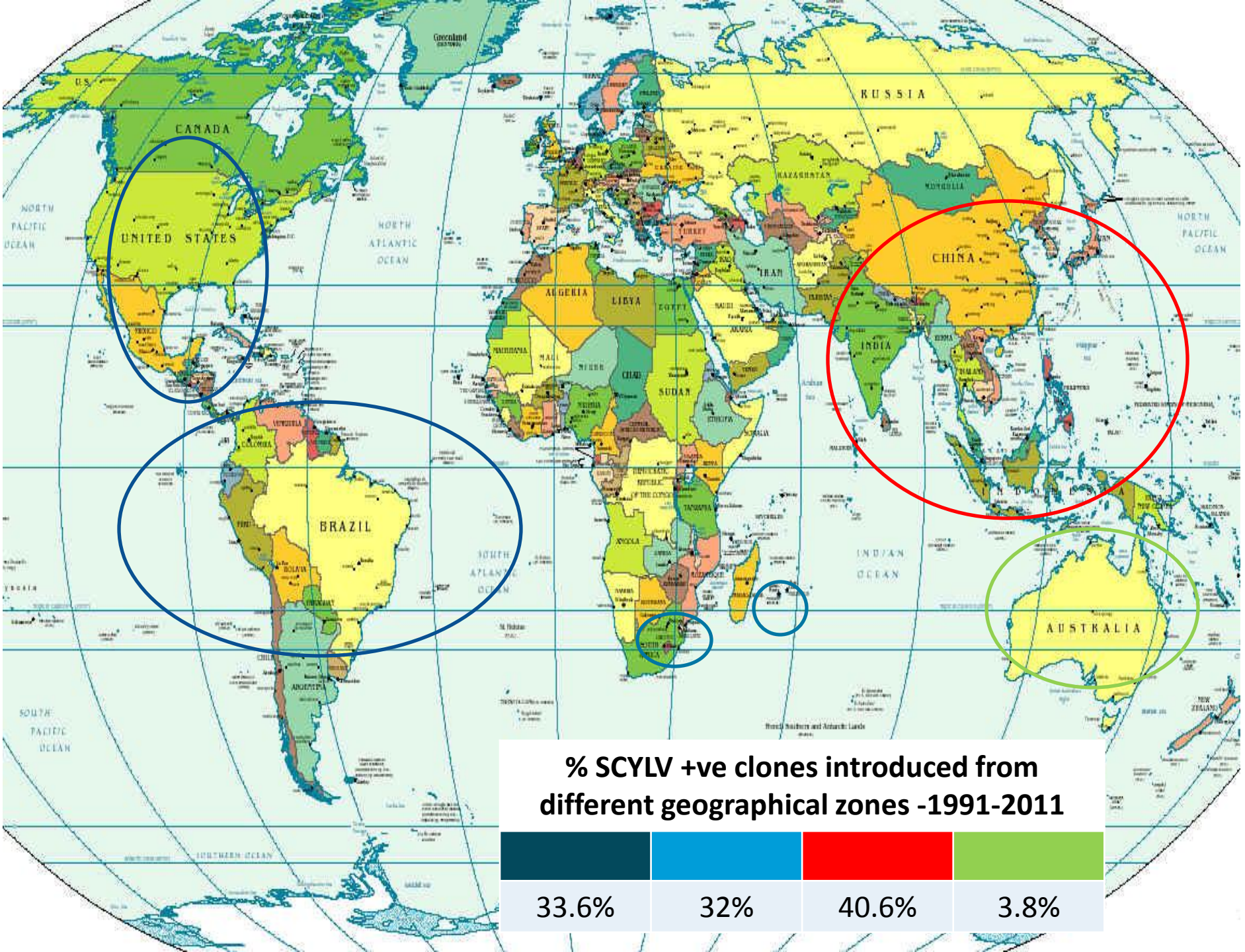
SCYLV detection in quarantine 1999-2011

Country	Quarantine cycle	No of clones introduced/germinated	No of varieties infected by SCYLV
Fiji	2010-2012	3	0
France (Intermediate)	2001-2003	5	1
	2003-2005	4	0
	2006-2008	8	0
	2008-2010	1	0
Guatemala	2003-2005	6	6
	2006-2008	14	9
	2008-2010	6	5
	2010-2012	11	6
India	2003-2005	1	0
Japan	2003-2005	10	6
Mexico	2001-2003	5	4
Philippines	2006-2008	7	2

SCYLV in Quarantine 1999-2011

Country	Quarantine cycle	No of clones introduced/germinated	No of varieties infected by SCYLV
Reunion	1999-2001	9	5
	2001-2003	8	6
	2006-2008	7	0
	2008-2010	7	3
South Africa	1999-2001	6	1
	2003-2005	2	0
	2006-2008	8	0
	2008-2010	6	2
Sri Lanka	2006-2008	6	0
Taiwan	1999-2001	2	2
	2001-2003	2	2
	2003-2005	1	1
USA	1999-2001	7	7
	2001-2003	22	18
	2008-2010	21	1
	2010-2012	3	2
Total introduced		**331**	**106**

country	Infected varieties
Argentina	0/10
Australia	1/26
Barbados	3/63
Brazil	10/21
Colombia	3/5
Dominician Republic	0/5
Ecuador	0/3
Fiji	0/3
France (Intermediate)	1/18
Guatemala	26/37
India	0/1
Japan	6/10
Mexico	4/5
Philippines	2/7
Reunion	14/31
South Africa	3/22
Sri Lanka	0/6
Taiwan	5/5
USA	28/53


% SCYLV +ve clones introduced from different geographical zones -1991-2011
33.6%
32%
40.6%
3.8%

Concerns!!!!

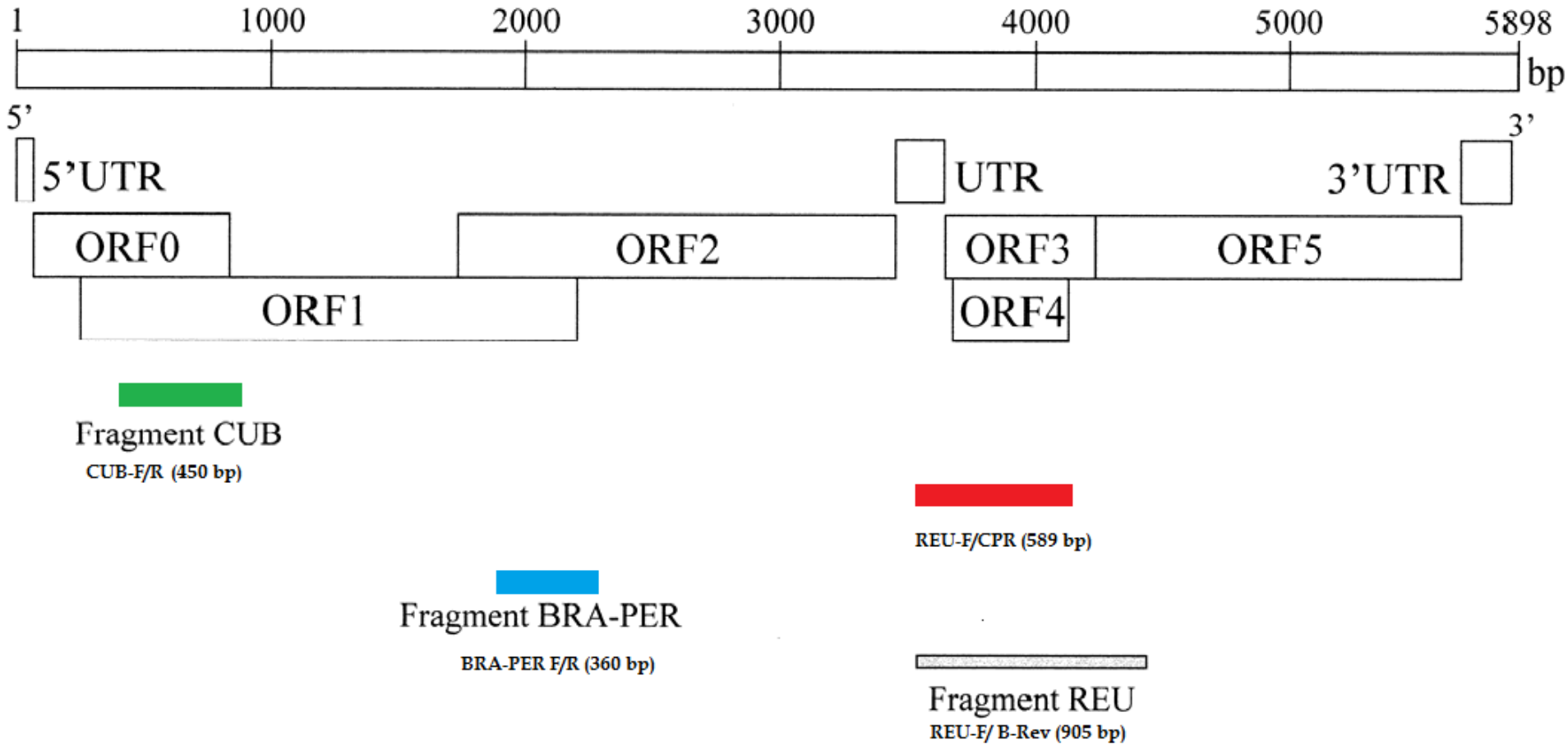
- 32% of germplasm introduced infected by SCYLV
- Although no yield loss observed in Mauritius due to SCYLV, concern is raised if more aggressive genotypes introduced
- Only one Mauritian variety (M 1176/77) identified as resistant to yellow leaf disease
- Genotyping of virus introduced
- Infected material being cleaned by tissue culture

Genotyping for SCYLV

A multiplex RT-PCR assay developed for 3 genotypes: REU, BRA-PER, CUB

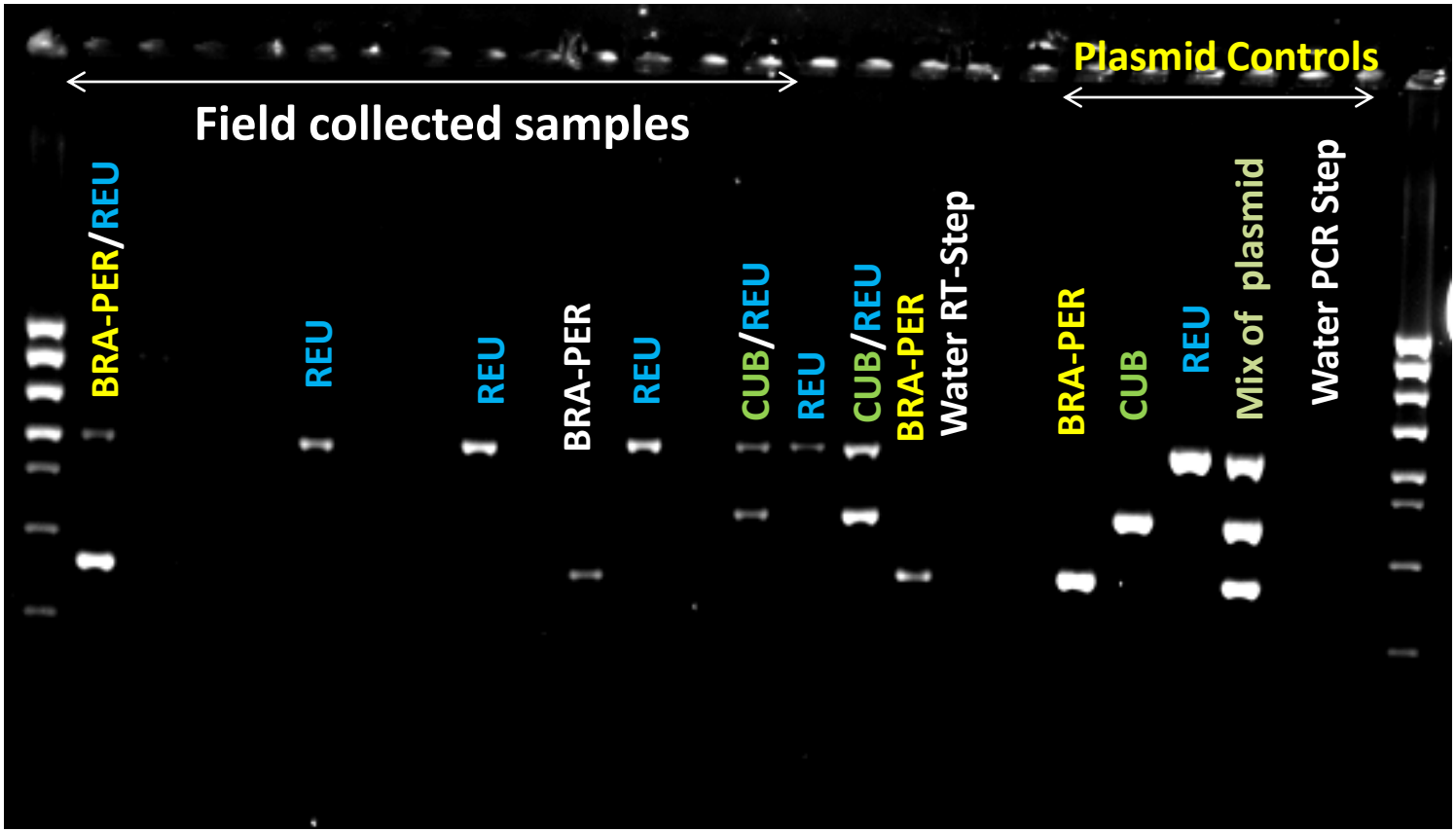
Genotype	Primer	Sequence (5'-3')	Product (bp)	Reference
BRA-PER	**PER-F**	AAC TGC TGC GTC AGG CCC A	**362**	Abu-Ahmad et al., (2006)
	PER-R	GAC GAG CTT GCG TTG TTT TTC T		
CUB	**CUB-F**	GTG CTT CTC CCG GCG GTT CAC T	**450**	
	CUB-R	ATT CGA GAA CAA CCT CCG CCT C		
REU	**REU-F**	CAA GCT TCT AGC GGG AAT C	**905**	
	B-REV	CAG TTG CTC AAT GCT CCA CG		
REU	**REU-F**	CAA GCT TCT AGC GGG AAT C	**589**	
	CPR	GGG CCG GGA AGA CTT TCT T		Korimbocus et al. (2002)

Location of genotype specific fragments



Multiplex RT-PCR for CUB, BRA-PER & REU genotypes

Primers: REU-F/CPR, CUB-F/R & BRA-PER F/R



SCYLV genotypes detected in quarantine clones 2009-2010

- 43 varieties screened
- 13 infected by SCYLV

Genotype	No. of varieties	Country of origin
BRA-PER	9	USA (5), S Africa (2), Guatemala (2)
REU	4	Reunion (3) Barbados (1)

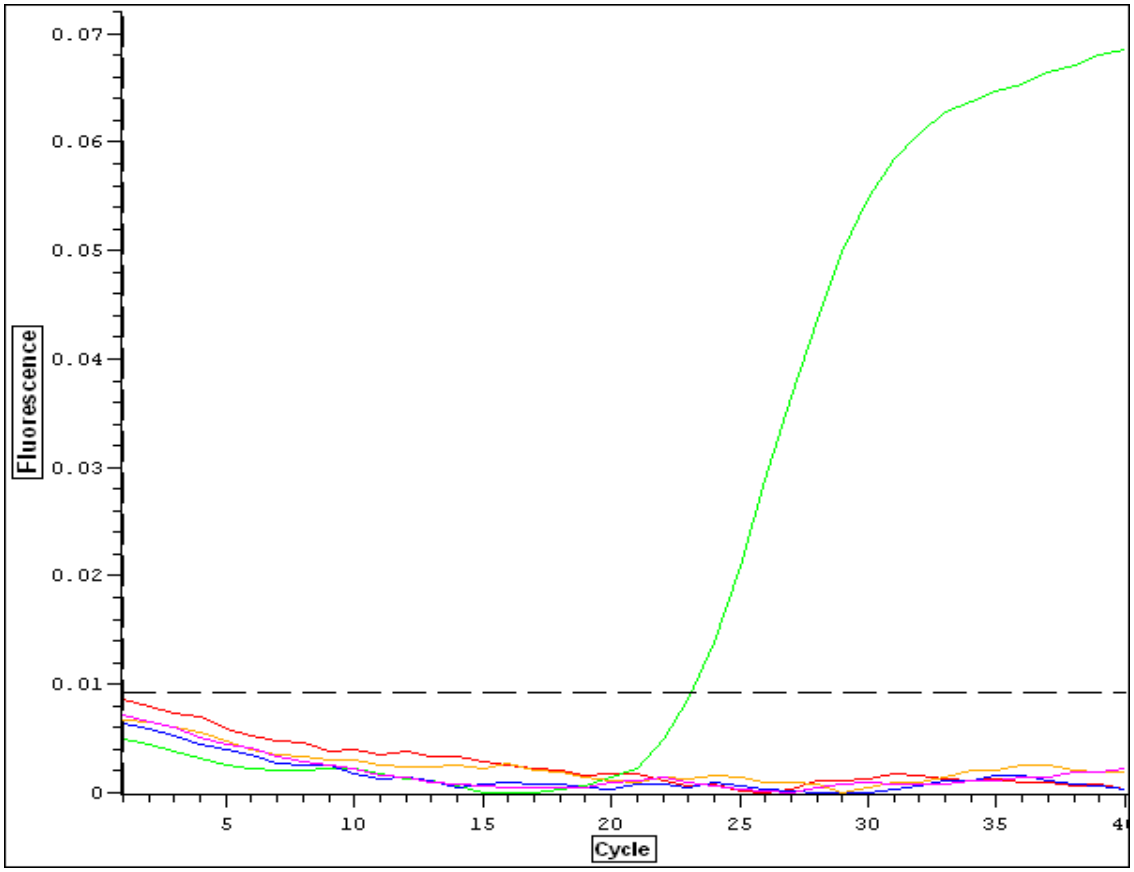
SCYLV genotypes detected in quarantine clones 2011-2012

- 22 varieties screened
- 8 infected by SCYLV

Genotype	No. of varieties	Country of origin
BRA-PER	**8**	USA (2), Guatemala (6)
CUB	**1***	Guatemala

* Mixed infection

Real-Time Taqman® RT-PCR for CUB genotype

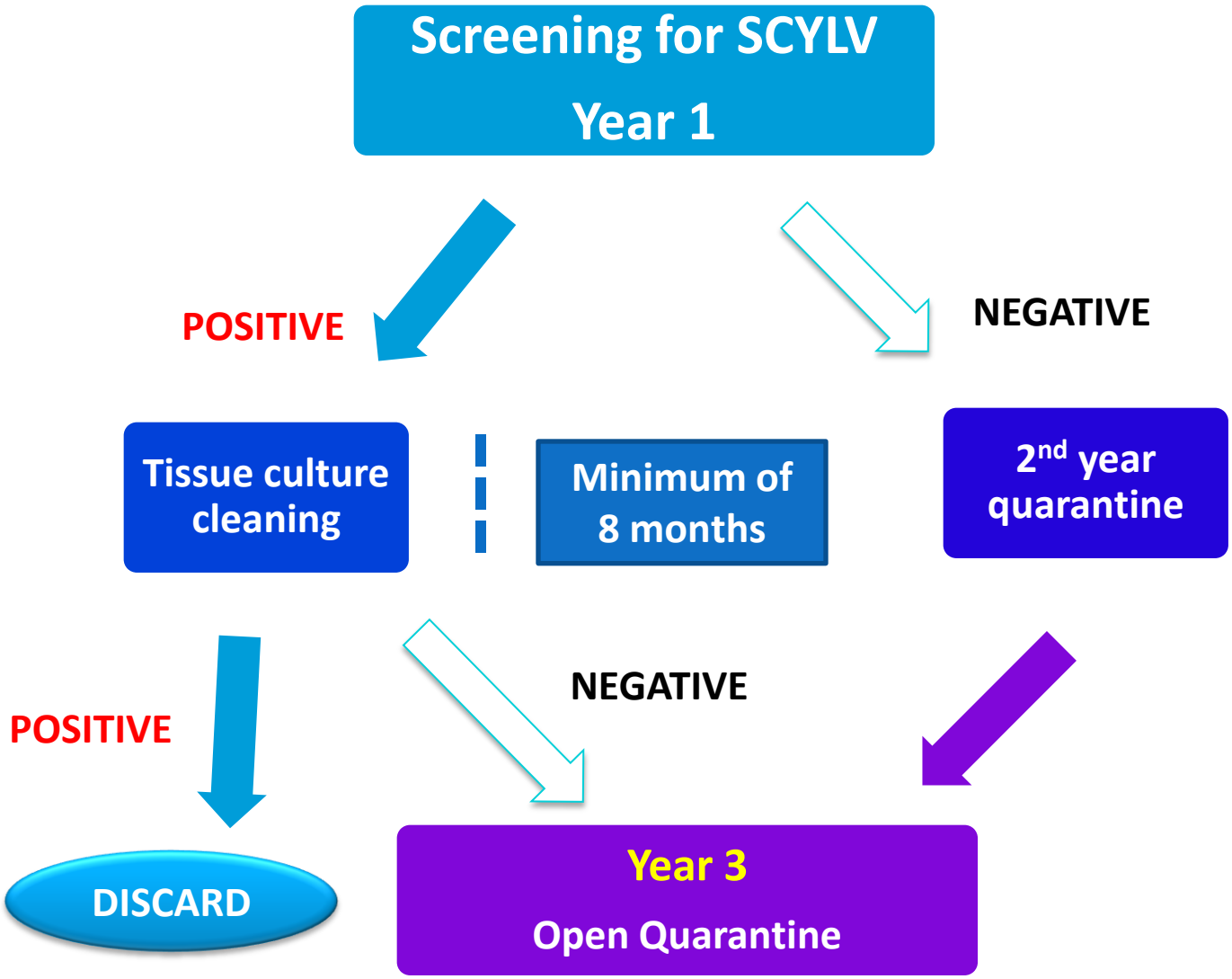


CUB genotype detection indicated by an increase in fluorescence

Extracts from REU & BRA-PER genotypes & water control

- **Specific Primers/probe designed and tested for CUB genotype**
- Evaluation of Real-time RT-PCR for other genotypes in progress

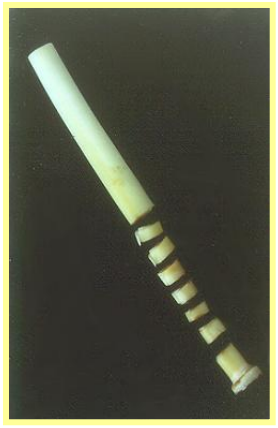
Production of SCYLV-free material

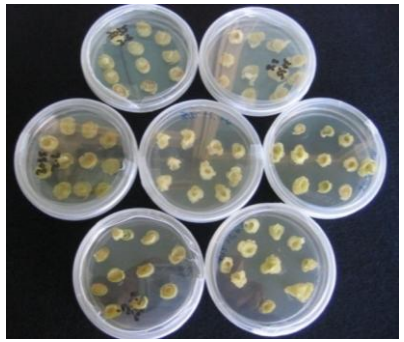


Tissue culture cleaning

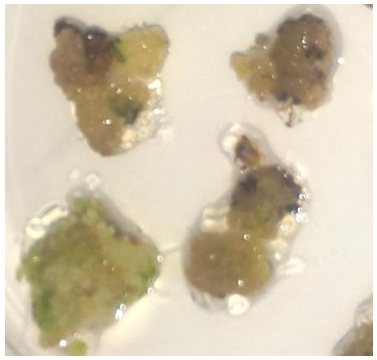


Month 0





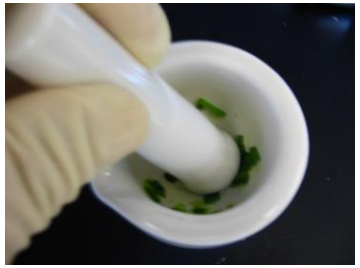
Month 1
Callus formation



Months 2-4

3 subcultures of callus

Months 7-8



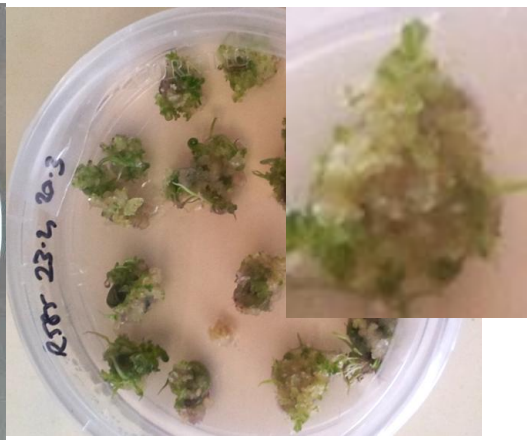
Diagnosis





Months 5-6 Regeneration





Success rate of SCYLV elimination *in vitro*

Period	Infected varieties	Successful regeneration	Virus elimination	% success for virus cleaning
1991-2001	18	16	16	100
2001-2003	38	29	25	86
2003-2005	12	11	11	100
2006-2008	13	10	10	100
2008-2010	13	13	13	100
2011-2012	8	-	In progress	-

Cost of producing SCYLV–free plants in an operational lab: USD 75/variety

Conclusion

- SCYLV can be eliminated from infected material
- Countries introducing varieties should be equipped with diagnostic facilities
- Introduction of new aggressive genotypes of the virus can be avoided



Thank you
for
your attention