CSSV – transmission and detection

Andy Wetten

School of Biological Sciences
University of Reading

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Summary

- CSSV characteristics
- Transmission routes
- Universal PCR-based screening
- CSSV DNA sequencing
- ‘Cryotherapy’
- Mealy bug screening

Cacao Swollen Shoot Virus

- Family – *Caulimoviridae*
- Genus – *Badnavirus*
- x 2 stranded circular DNA genome of 7 to 7.3 Kb

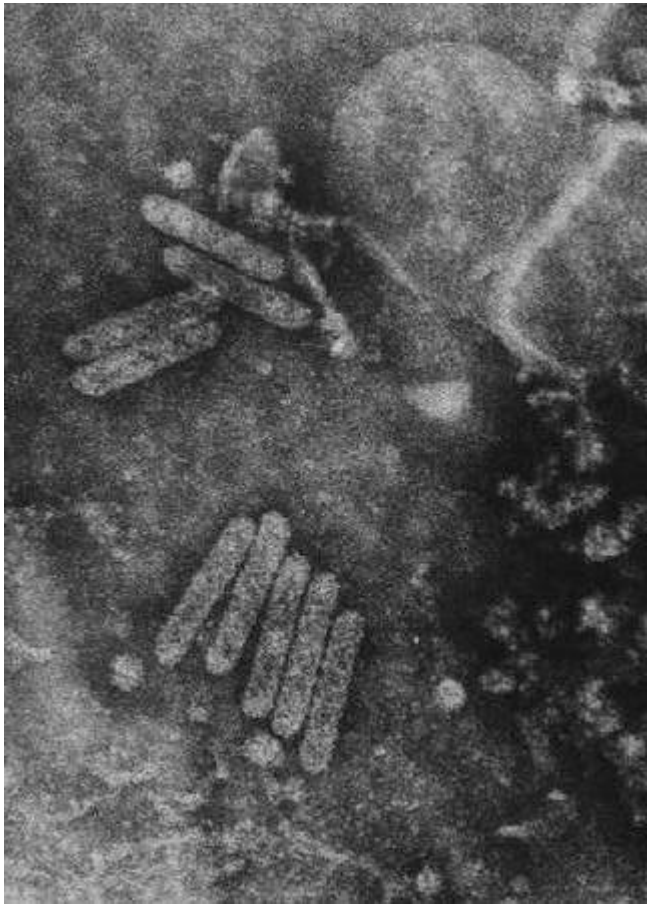
CSSV symptoms

- red vein banding

- swellings on chupons

CSSV virion characteristics

- Bacilliform DNA virus
- non-enveloped (ie no lipid membrane)
- 130 x 30 nm capsid (protein shell)



Transmission

- At least 14 spp. of mealy bugs known to transmit

- Virus retained when vector moults

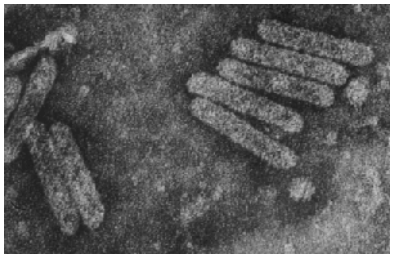
- does not replicate in the vector

- not transmitted congenitally to progeny of the vector

Formicococcus njalensis

Badnaviruses

Seed transmission?



• *Aucuba bacilliform virus*	
• *Banana streak virus*	
• *Cacao swollen shoot virus*	
• *Canna yellow mottle virus*	
• *Citrus mosaic virus*	
• *Commelina yellow mottle virus*	Scott *et al* 1992
• *Dioscorea bacilliform virus*	
• *Kalanchoe top-spotting virus*	
• *Mimosa bacilliform virus*	
• *Pineapple bacilliform virus*	Thomson *et al* 1996
• *Piper yellow mottle virus*	de Silva *et al* 2002
• *Rice tungro bacilliform virus*	
• *Schefflera ringspot virus*	
• *Sugarcane bacilliform virus*	
• *Taro bacilliform virus*	Macanawai *et al* 2005
• *Yucca bacilliform virus*	

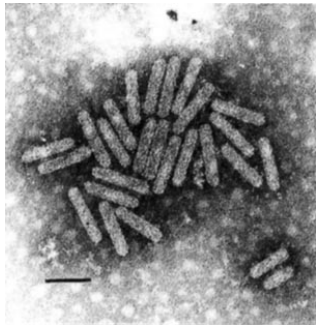
CSSV seed transmission – results summary

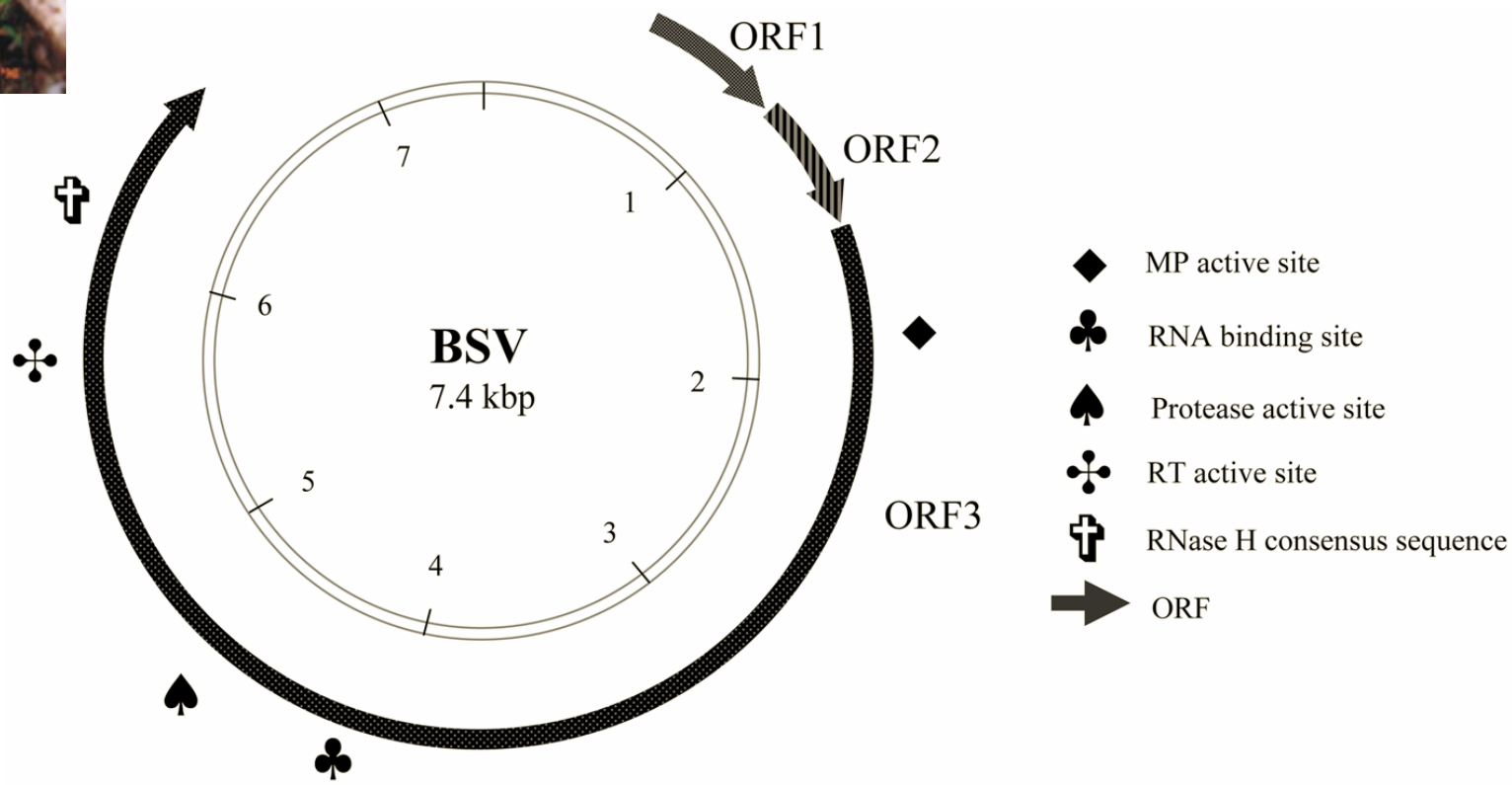
- CSSV DNA can be transferred persistently from maternal parent to seedlings following self-pollination (7–34%)

- CSSV DNA transmission via pollen is rare (only 1 testa PCR+ out of 172 seeds tested, all seedlings negative) following cross pollination

- Lack of RNA transcripts – all RT PCR tests on 25 PCR+ seedlings have been negative
- No symptom expression on any of 66 PCR+ seedlings 24 months post germination ie no evidence of CSSV disease transmission
- Evidence of methylation of seedling CSSV DNA
- CSSV DNA integration into cocoa genome?

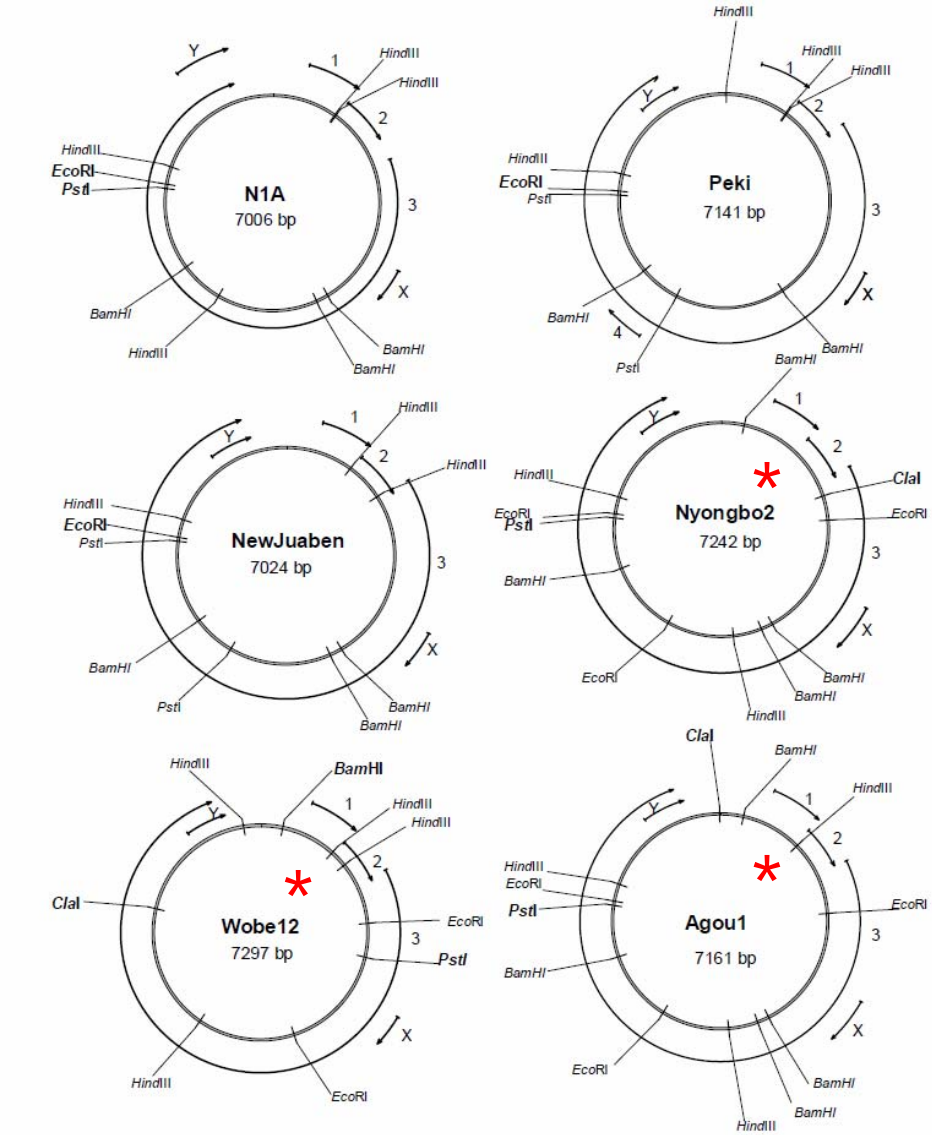
Homology among Badnavisuses





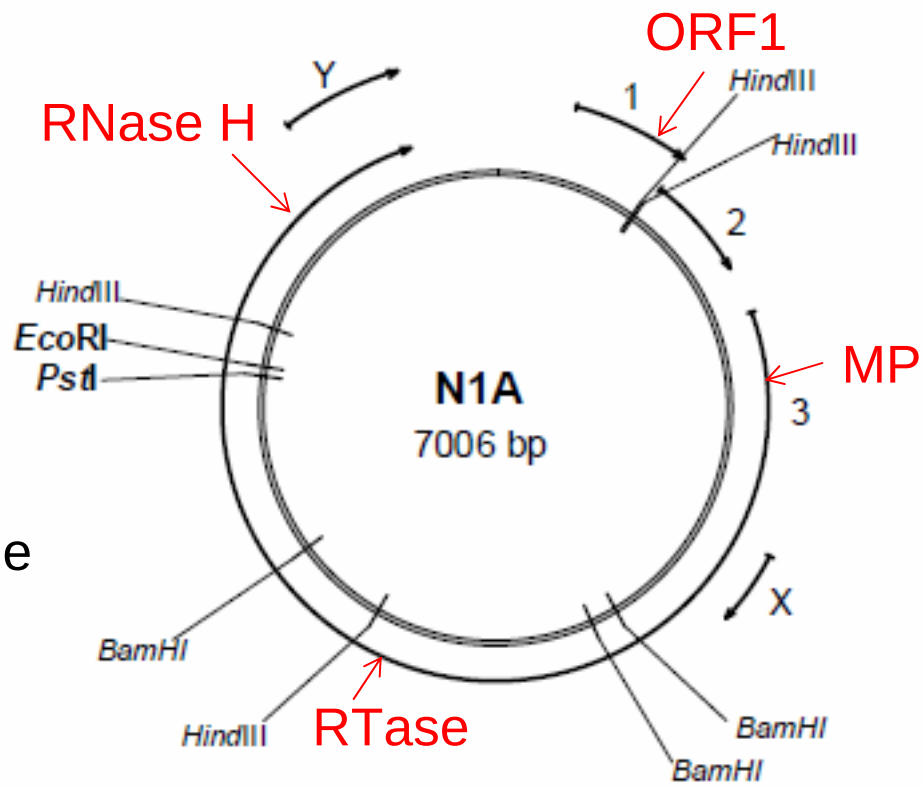
CSSV sequences

- 6 complete available
- 3 from Togo * & 3 from Ghana



Degenerate primers designed for:

- ORF1 – Open Reading Frame 1
- MP – Movement Protein
- RTase – Reverse Transcriptase
- RNase H – Catalyzes RNA cleavage

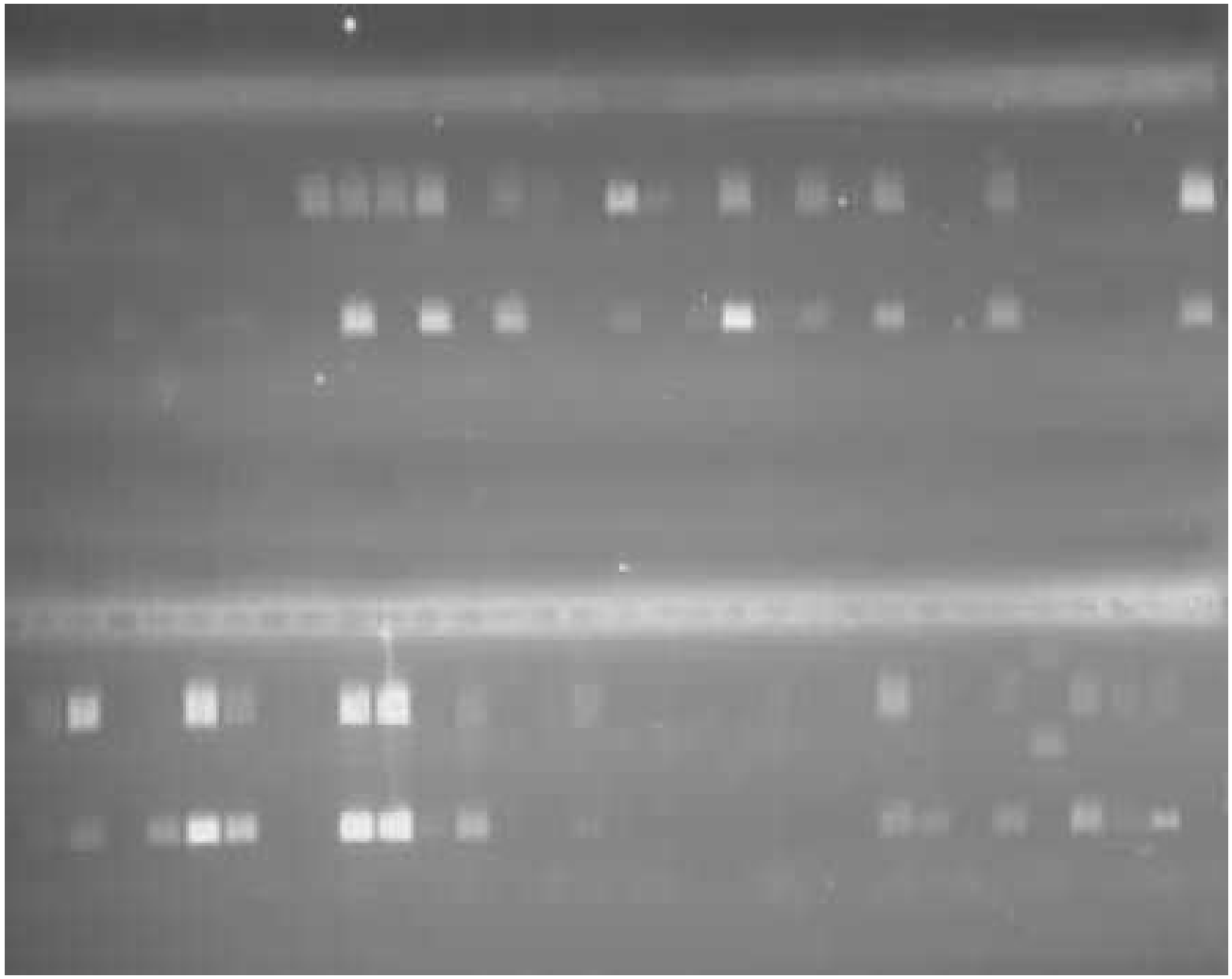


RTase

Rnase

RTase

Rnase



CRIG CSSV museum field sample codes - 3 12 2010a.xls [Compatibility Mode]

	A	B	C	D	E	F	G	H	I
1	Sample no.	Sample	Collection site	ORF1	Movement	Rtase	Rnase		New movement
2	1	Aboboya (CC) – WR							
3	2	Achechere (CC) - W/R							
4	3	Achiasi W/R		P		P	P		P
5	4	AD 125 ER							
6	5	AD 7 E/R		P		P	P		
7	6	AD 75/ER				P			
8	7	Adienbra / CC WR							
9	8	Agepomaa				P	P		P
10	9	Aiyim (CC) – WR		P		P	P		P
11	10	Amafie W/R		P					P
12	11	Anibil (CC) WR							
13	12	Asamankese Isolate							
14	13	Ayiboso – W/R		P	P	P	P		P
15	14	Bakukrom /CC/W/R		P		P	P		P
16	15	Bechem B/A		P		P	P		P
17	16	Bisa							
18	17	Bobiriso / Juaso 1 ASH				P			
19	18	Bosomtwe /Juaso 1							
20	19	Bosomuoso 2 W/R		P		P	P		
21	20	Datano W/R **							
22	21	Dawa / 1H / ER							
23	22	Dochi /IG/ER				P	P		p
24	23	Enchi E1/A/3 W/R							

Sheet1 Sheet2 Sheet3

Why using 454 sequencing for CSSV screening?

- Screening of multiple viral gene regions
- Screening of multiple accession
- Screening of multiple viruses within accessions -

How?

- Each PCR product is identified by is own DNA Tag

Single PCR using Tagged primers for each CSSV gene/accession combination

Individual PCR Products all mixed together

454

20000 sequences (minimum run)

Bioinformatic

Sequences catalogued according to Tags *in silico*

CRIG museum samples by region

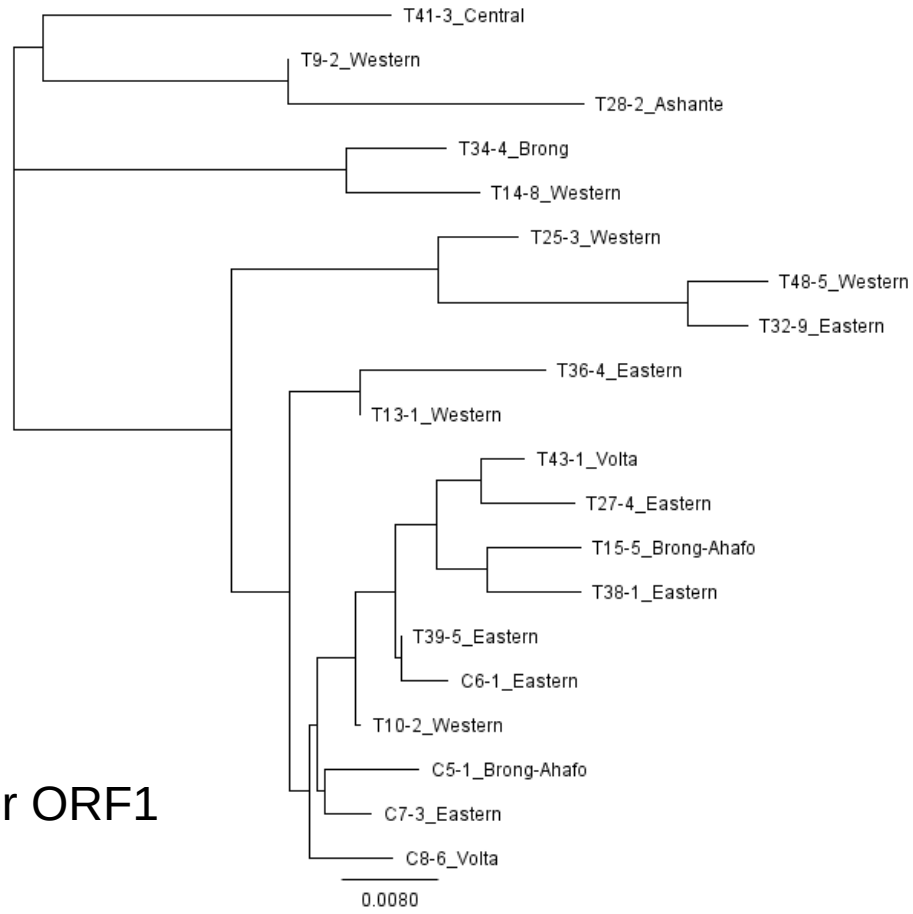
T32	Kwakoko Juansa North A/R ?	Eastern Region	P
T33	Kwaku Anyan T1 B/A	Ashante Region	
T34	Madjeda Nkwanta a gogo F1T2	Brong Ahafo Region	P
T35	Mampong (1M) - ER?	Ashante Region	P
T36	Miaso Isolate	Eastern Region	P
T37	N1 Isolate	Eastern Region	
T38	New Juaben Isolate (1A)	Eastern Region	P
T39	Nkrankwanta Isolate	Eastern Region	P
T40	Nsaba Isolate	Brong Ahafo Region	
T41	Oyimso Agogo 5 ASH	Central Region	P
T42	Pa Men (1e) - ER	Ashante Region	P
T43	Peki - U/R	Volta Region	P
T44	Punekrom – W/R	Western Region	
T45	Sankore T3/3	Brong Ahafo Region	P
T46	SS 365B Isolate	Eastern Region	
T47	SS167 – E/R (mildstrain)	Eastern Region	
T48	Suhuma W/R	Western Region	P
C1	Surowno /WR	Western Region	P
C2	Tafo Yellow	Eastern Region	
C3	Tease Adeakyi	Eastern Region	P
C4	Tease Atomsu-Abuom	Eastern Region	P
C5	Techimantia outbreak 3T-15	Brong Ahafo Region	P
C6	Virus AD 14/ER	Eastern Region	P
C7	Virus AD 196	Eastern Region	P
C8	Worawora	Volta Region	P

All strong products from museum and 'alternate hosts' submitted to Inqaba Ltd

454 Results - CRIG collection

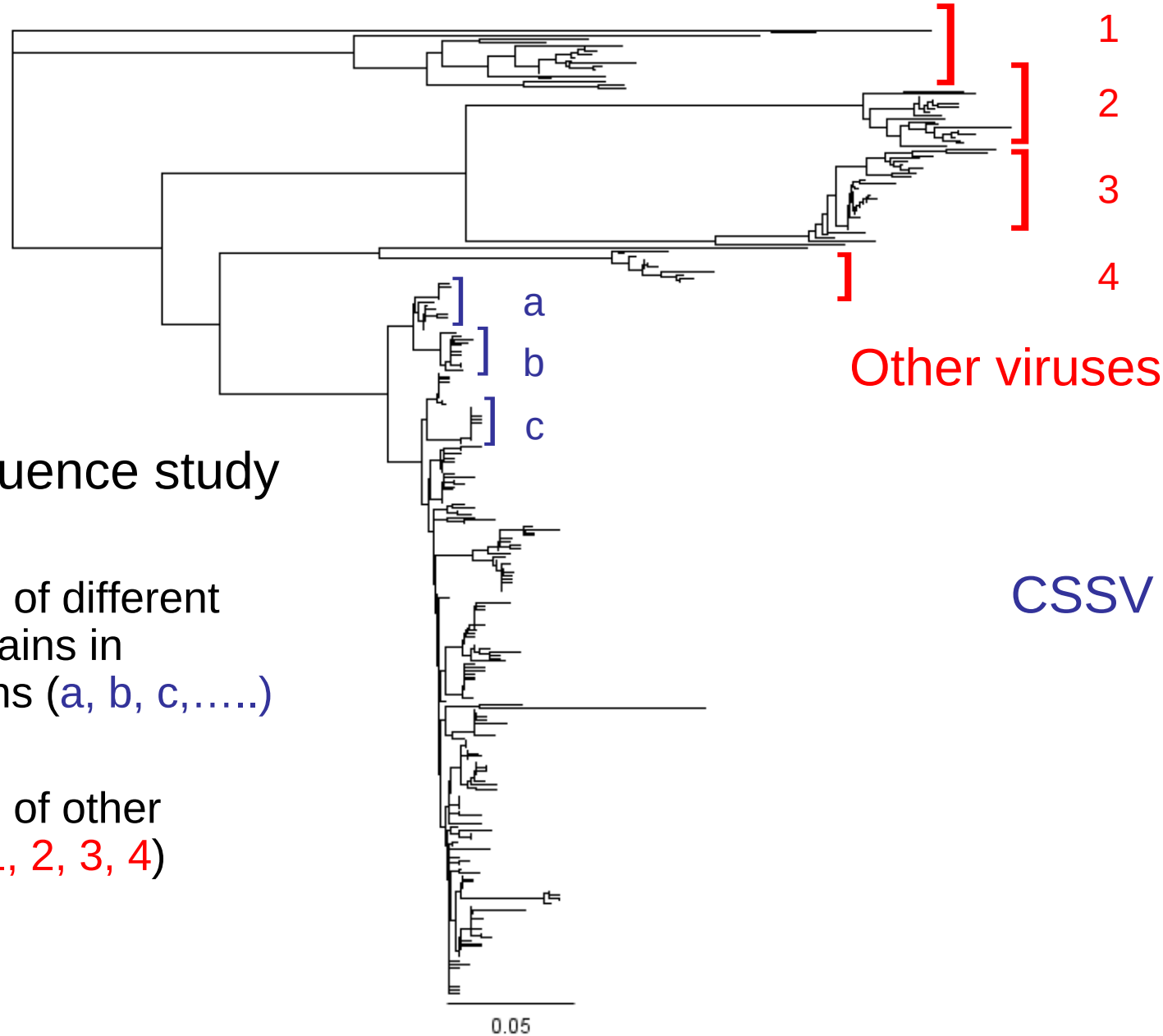
Analysis of ORF1 and three genes from ORF3 (Mvnt, Rtase and Rnase)

CRIG collection valuable as it comprises accessions with distinct CSSV strains (consistancy of the result across all gene regions)



Result for ORF1

CSSV variation within accession



Rnase sequence study

- Detection of different CSSV strains in accessions (a, b, c,…..)
- Detection of other viruses (1, 2, 3, 4)

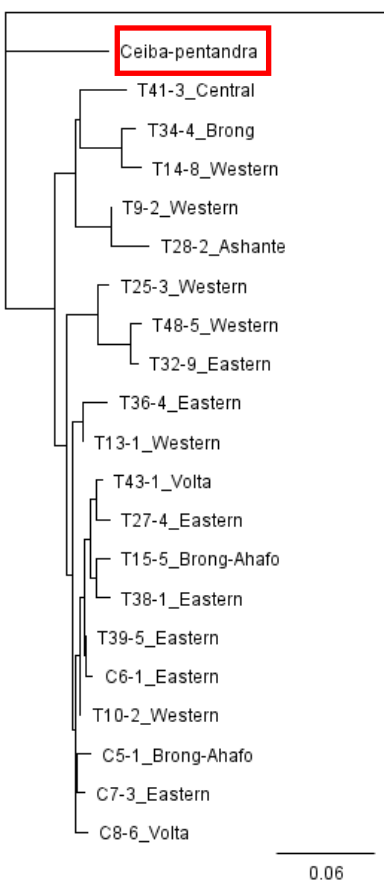
Alternate host species

Preliminary data indicate that the species *Ceiba pentandra* harbours a virus with close resemblance to CSSV (BLAST of ORF1 region)

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AJ609020.1	Cacao swollen shoot virus complete genome, isolate N1A	470	470	86%	4e-129	87%
AJ609019.1	Cacao swollen shoot virus complete genome, isolate Peki	464	464	86%	2e-127	87%
AJ608931.1	Cacao swollen shoot virus ORF1, ORF2, ORF3, ORFX and ORFY	442	442	86%	1e-120	86%
AJ534983.1	Cacao swollen shoot virus ORF1, ORF2, ORF3, ORFX and ORFY, comp	401	401	86%	2e-108	84%
L14546.1	Cacao swollen shoot virus polyprotein gene, complete circular genom	401	401	86%	2e-108	84%

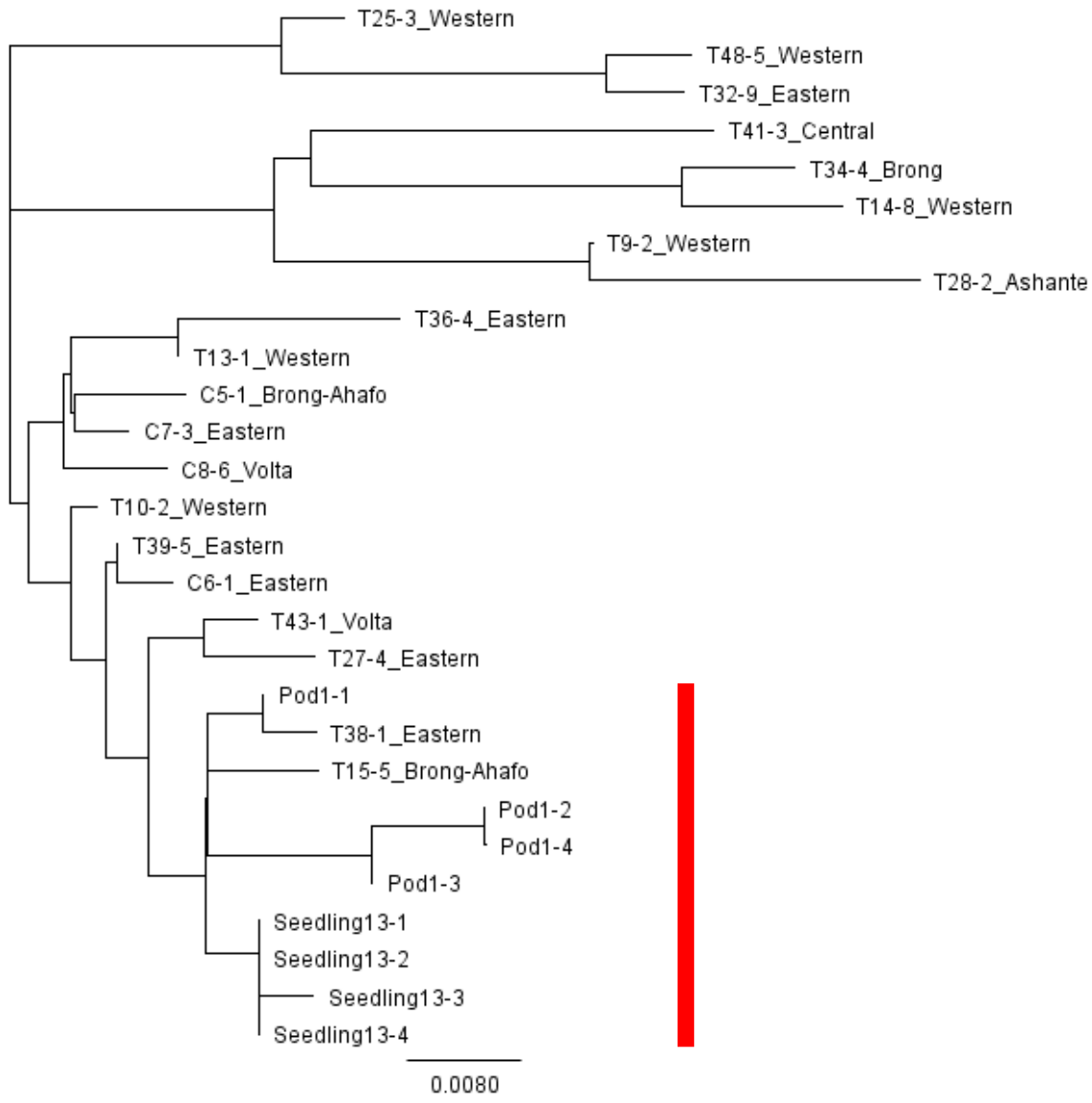
Citrus yellow mosaic virus is used as an out-group



Seed transmission

ORF1 CSSV sequence detected in seedling13 group with Pod1

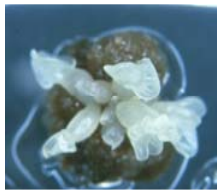
(Multiple sequences from 454 analysis incorporated in the analysis)



Germplasm conservation via cryopreservation

Staminodes harvested from closed flowers

1° somatic embryos initiate after 6 weeks



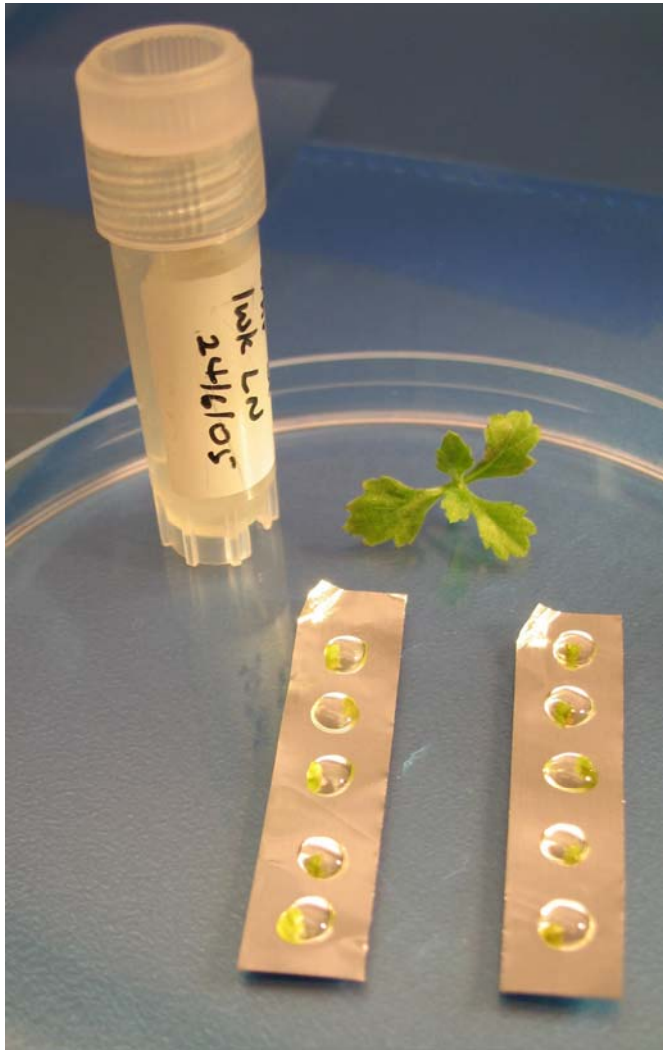
2° embryos initiate on cotyledonary explants from 1° embryos

Dehydrated alginate encapsulated embryos cryopreserved

Embryos weaned after appearance of first true leaves

Encapsulation/dehydration

Fang et al (2004)



Droplet vitrification

PVS2 protocol:

- 2° SEs (2-5mm) precultured on 0.5 M sucrose – 5 days

⇩

- Loading solution (2M glycerol / 0.4M sucrose) – 20 min

⇩

- 10 SEs in 2 ml cryovials + 1 ml PVS2 on ice – 1 h

⇩

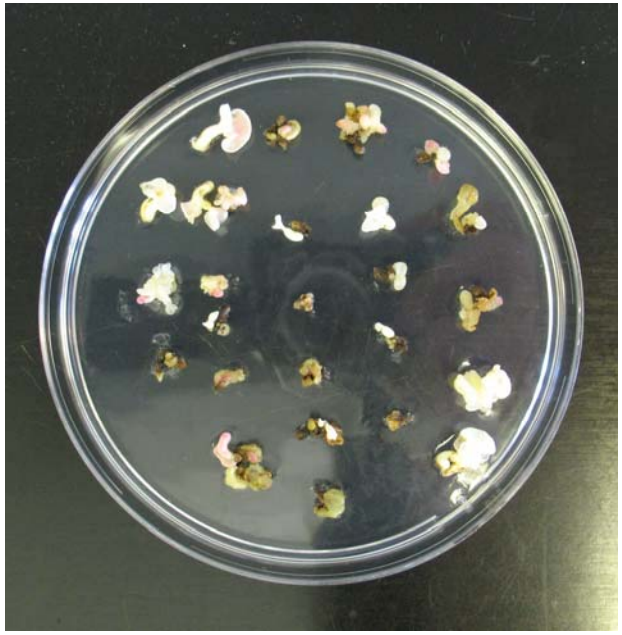
- LN - at least 1 h

⇩

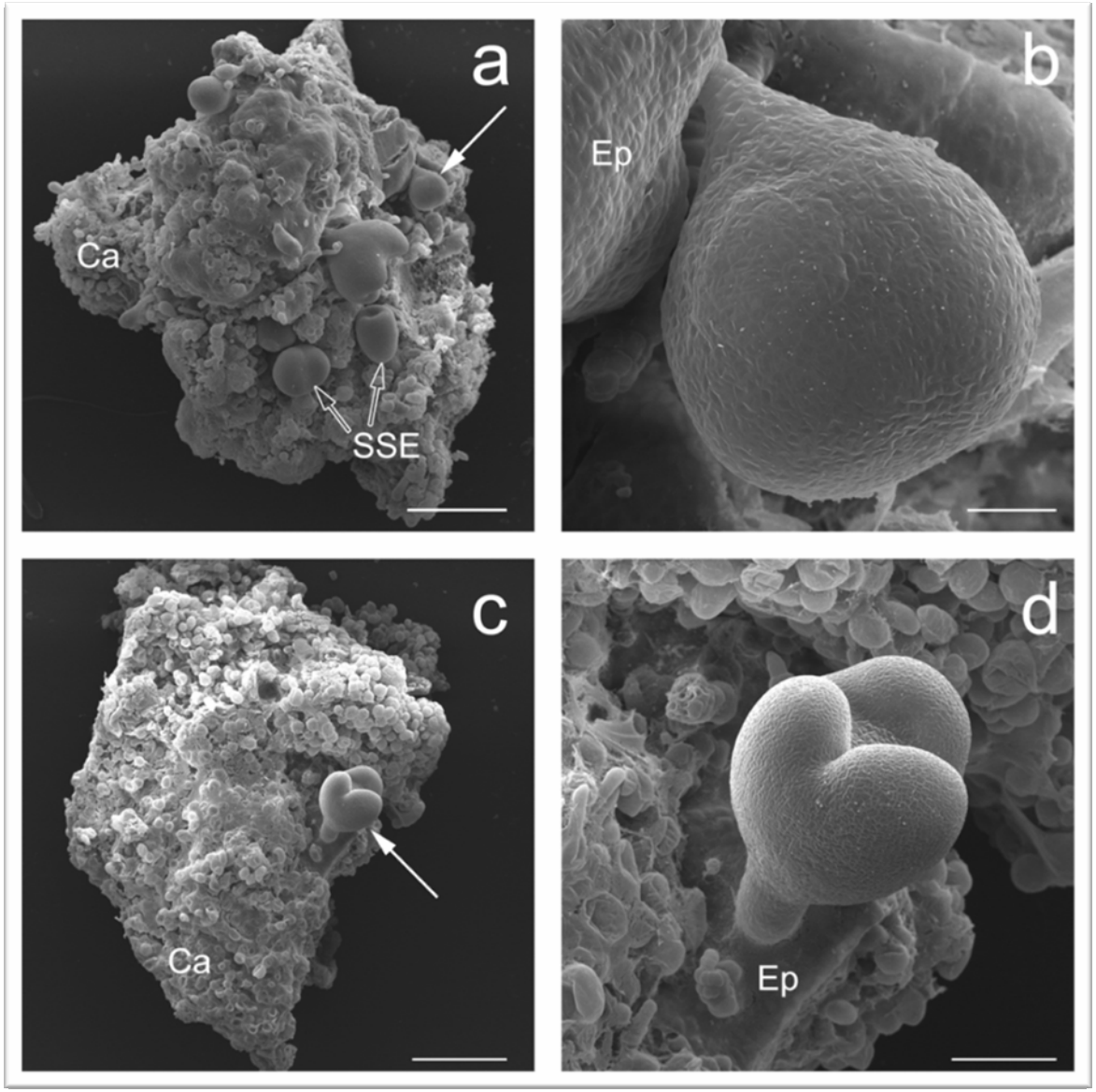
- 42°C water bath

⇩

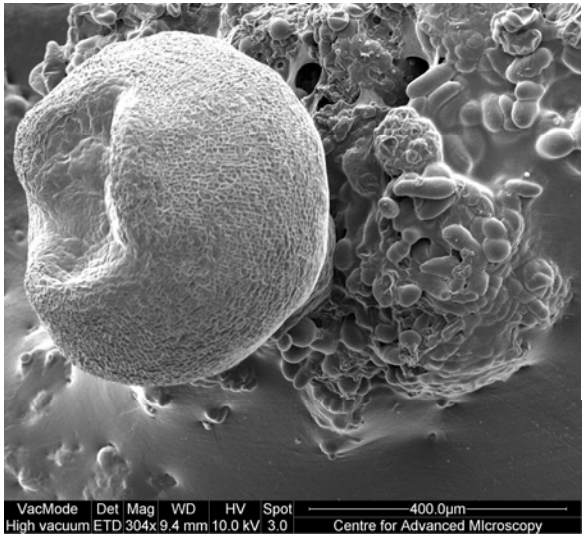
- remove PVS2 and wash twice with liquid ED



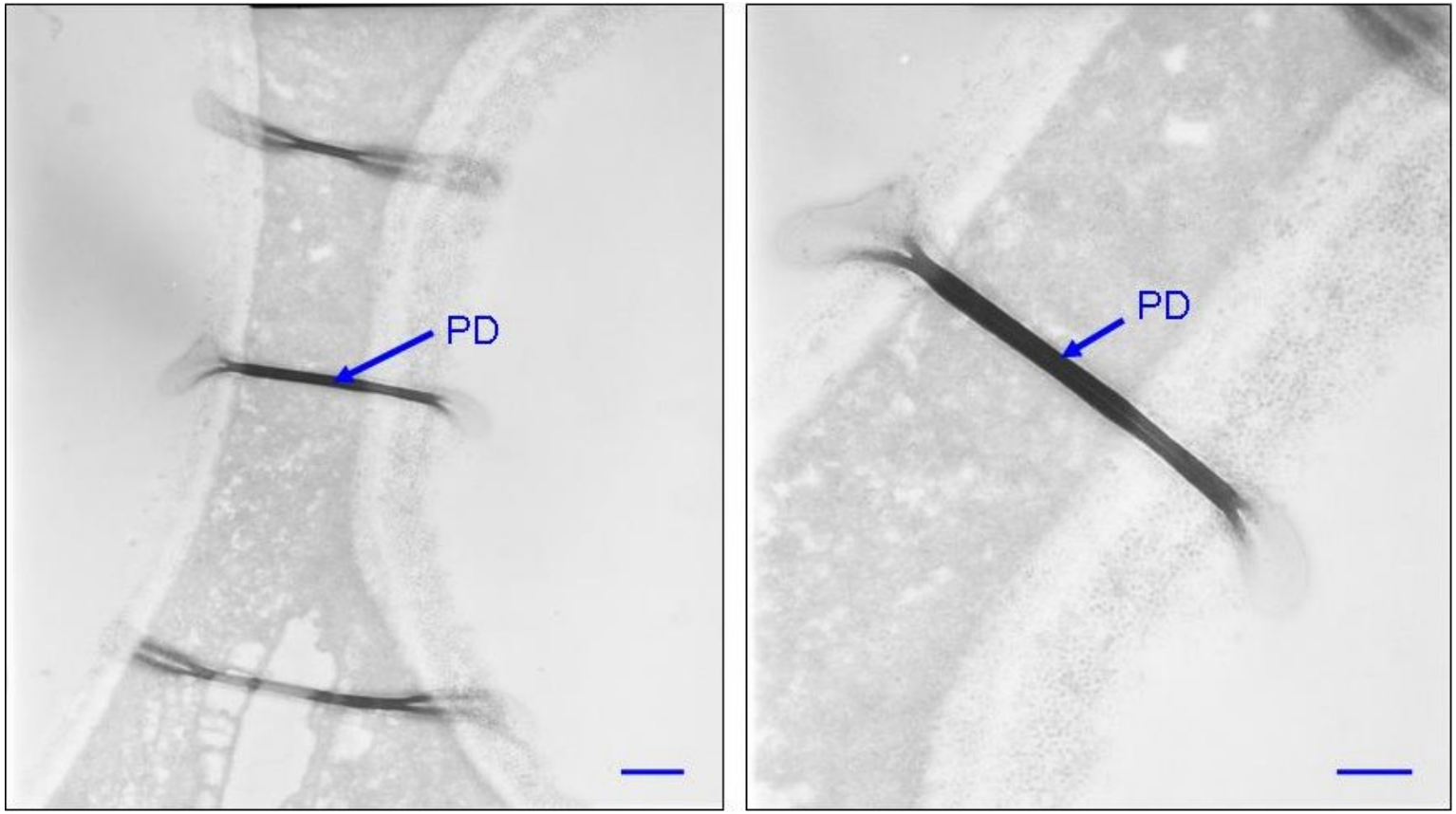
Adu-Gyamfi & Wetten (2011)


a
Ca
SSE
b
Ep
c
Ca
d
Ep

Cryo SEM

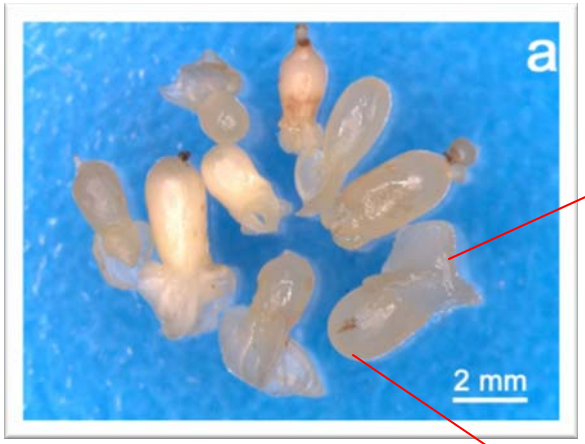

VacMode Det Mag WD HV Spot 400.0µm
High vacuum ETD 304x 9.4 mm 10.0 kV 3.0 Centre for Advanced MIcroscopy

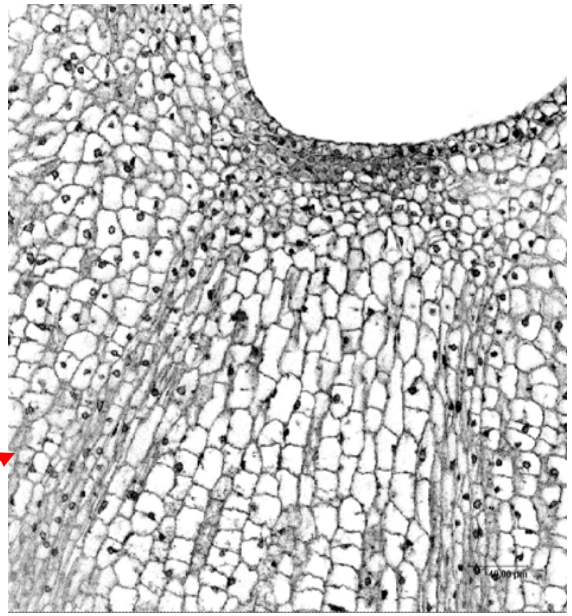
Interruption of CSSV movement via somatic embryogenesis

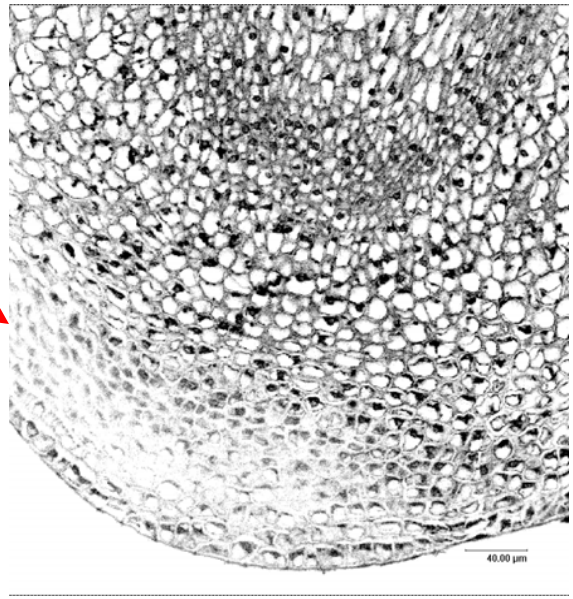


TEM images of plasmodesmata (PD) at intercellular junction of cocoa secondary somatic embryo cells. Bars represent 6 nm (a) and 14 nm (b).

Enhanced CSSV control via
'Cryotherapy'







Confocal microscopy

CSSV screening of mealy bugs

Proposed work: DNA barcoding of CSSV mealy bug vectors

•DNA barcoding used for identification of morphologically cryptic or immature insects

•Cytochrome oxidase 1 mitochondrial (CO1) DNA sequences

•Effective for eg. distinguishing crytic lepidopteran larvae - Emery et al (2009)

Molecular Ecology Resources (2009) **9**:217-223

Follow on work – W African survey

- Utilize 4 gene region-based screening to conduct large scale West Africa CSSV screening exercise
- Tags already available can be used to sample up to 200 more sites
- Now 454 approach proven for CSSV analysis 1/4 run (160K reads) will be sufficient for detailed survey of Ghana and Cote d'Ivoire
- October Ghana trip showed effectiveness of rapid sampling and GPS mapping -
- Alternate hosts and red mistletoe

Cont ..

- Development of screen for non-CSSV pathogens
- Whole canopy sampling to investigate uniformity of CSSV infection
- Epidemiology of alternate hosts eg. *C. pentandra*
- Mealy bug DNA bar coding

Acknowledgements - Collaborators and Funding

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