

Endemic cotton-infecting viruses from Australia, and detection and studies of the exotic *Cotton leafroll dwarf virus*

Murray Sharman¹, Lewis Wilson², Tanya Smith², Matt Webb¹, Fiona Filardo¹, Jane Ray³, Siwilai Lapbanjob⁴, Safaa Kumari⁵, Marc Giband⁶, Nelson Suassuna⁷, Jean-Louis Belot⁸ Valente Quintao⁹

¹ Queensland Department of Agriculture and Fisheries

² CSIRO

³ Northern Australian Quarantine Strategy

⁴ Department of Agriculture, Thailand

⁵ International Centre for Agricultural Research in the Dry Areas, Tunisia

⁶ CIRAD, Brazil

⁷ EMBRAPA, Brazil

⁸ Mato Grosso Institute of Cotton (IMAmt)

⁹ National Directorate of Quarantine and Biosecurity, Timor Leste

Virus diseases in Australian cotton

Cotton bunchy top virus (CBTV)

- Sporadic outbreaks cause localized losses
- Major outbreaks in 1998-9 and 2010-11

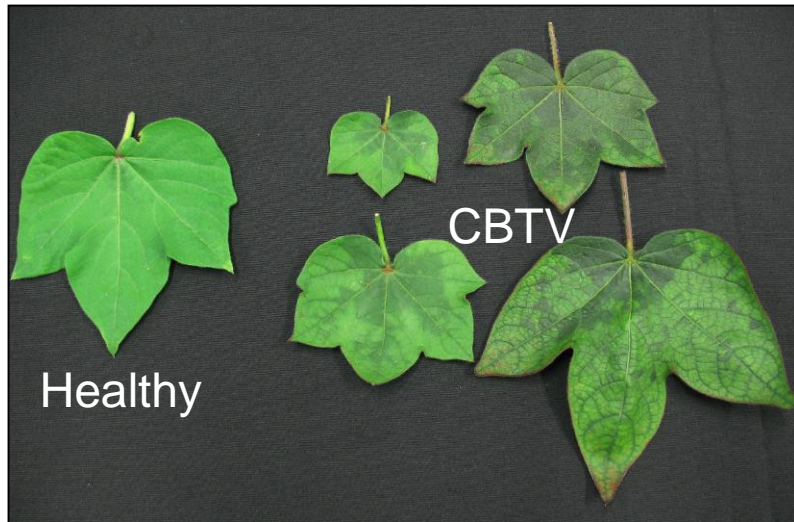
Tobacco streak virus (TSV)

- Common in Central Queensland but no yield or quality affect



Cotton bunchy top virus (CBTV)

CBTV-1 and -2



- Transmitted by cotton aphid (semi-persistent)
- Pale green angular patterns on leaves with dark green centres
- Small leathery, brittle leaves
- Reduced internodes and petioles – bunchy appearance
- Reduced boll size, number and shorter fibres



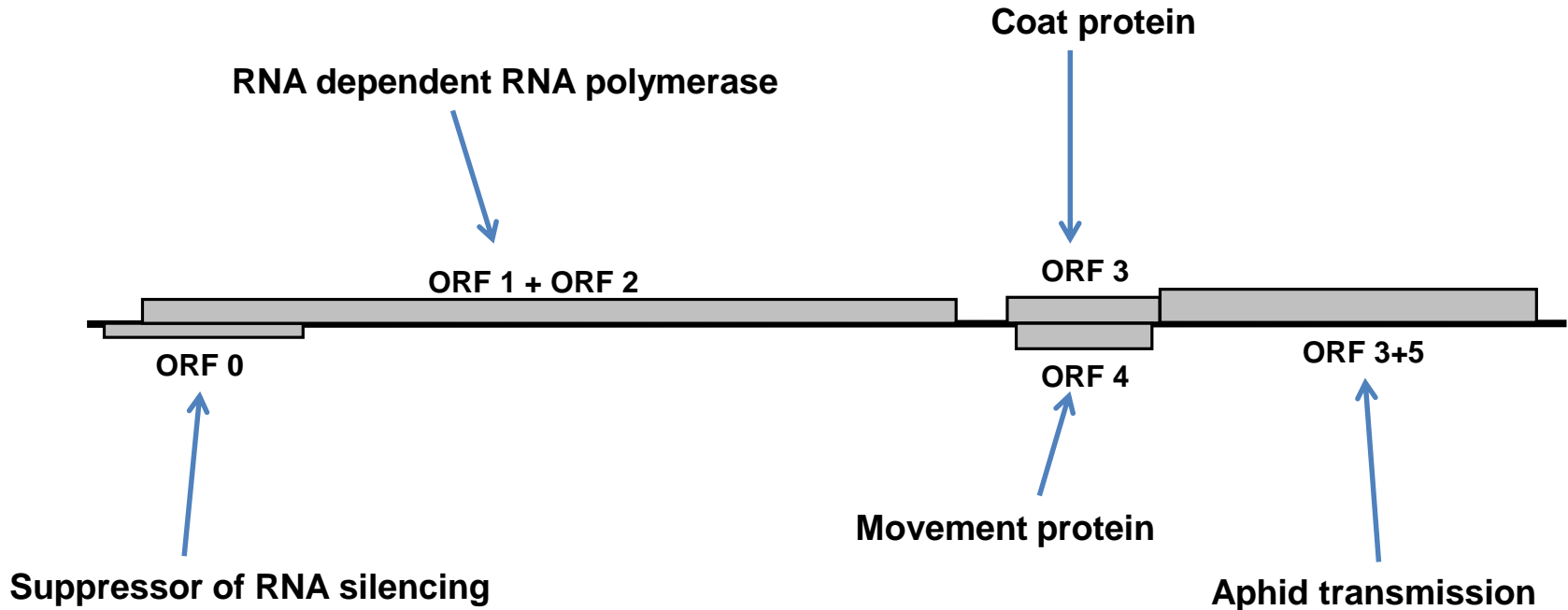
Edge infection and spread



Severe widespread damage

Polerovirus genome

- ssRNA genome (~5900nt). Several open reading frames (ORF) code for proteins that allow virus to infect, replicate and transmit



CBTV-1 and -2 are distinct species

- Distinct virus species have less than 90 % aa identity for one or more open reading frames (ORFs).

CBTV-1 and -2 have less than 90 % aa identity for all ORFs

- Distinct species don't cross protect.

CBTV-1 and -2 can co-infect same plant

- Distinct species have differences in host range

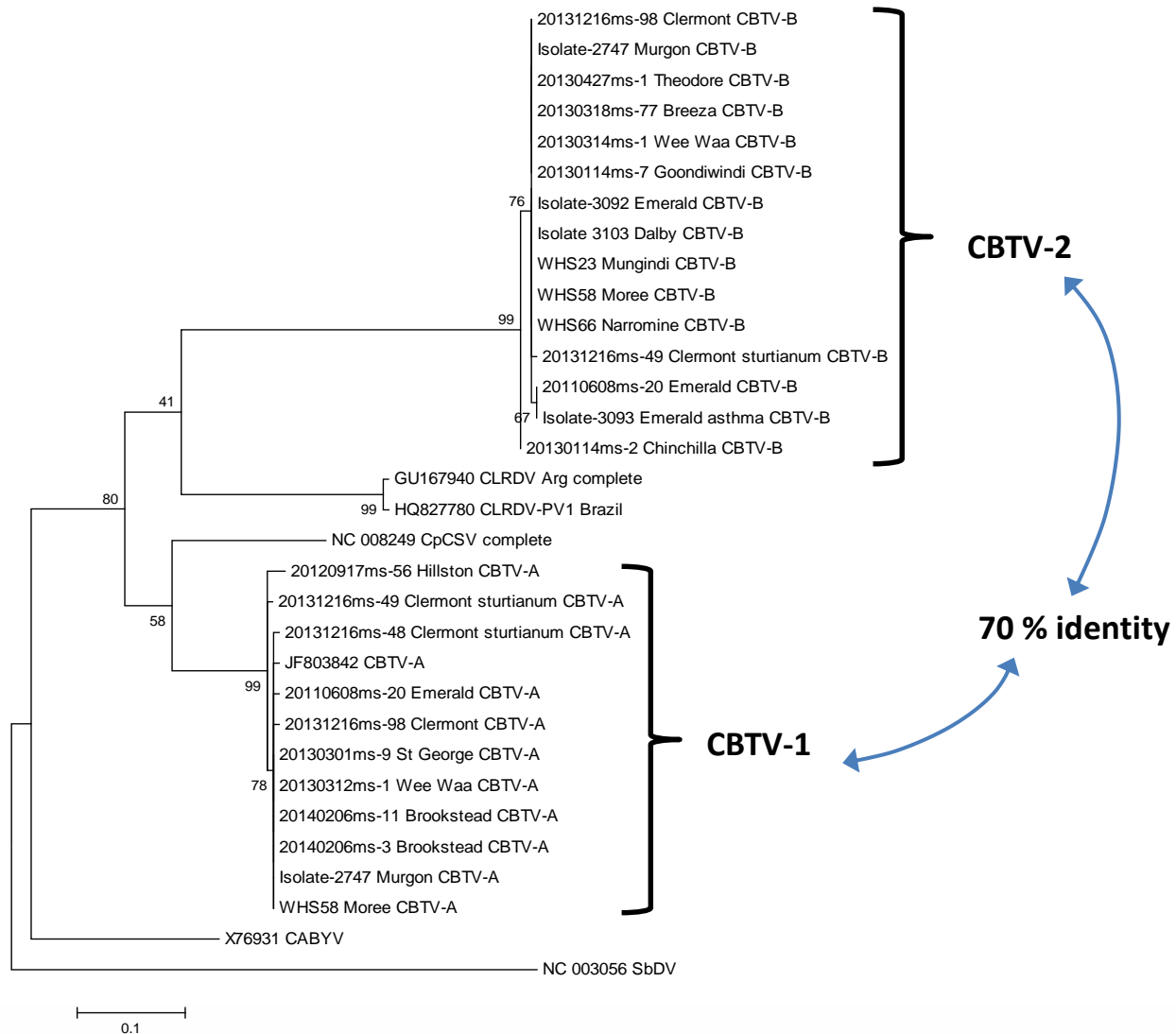
CBTV-1 and -2 appear to have some unique hosts. CBTV-2 is causing CBT.

CBTV-1 compared to CBTV-2

ORF #	Amino acid % identity
ORF 0	51 %
ORF 1	65 %
ORF 1+2	75 %
ORF 3	70 %
ORF 4	59 %
ORF 3+5	60 %

CBTV-1 and -2 have little variation within each species

- Phylogenetic tree of 201aa complete coat protein (ML analysis)



Alternative hosts for cotton bunchy top

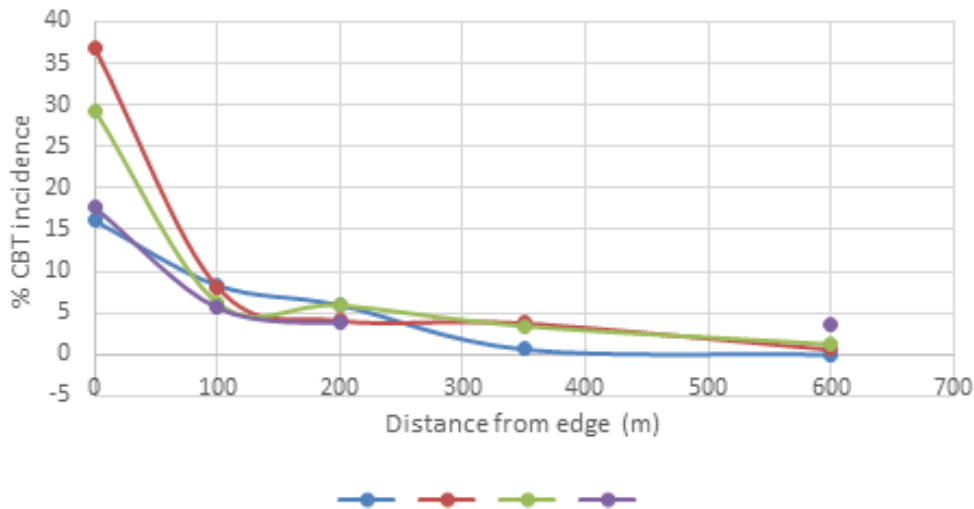
- Host range determined by field surveys, field trials or lab tests

Plant Family	Host species	Common name
Malvaceae	<i>Abutilon theophrasti</i>	Velvet leaf
	<i>Anoda cristata</i>	Spurred anoda
	<i>Gossypium australe</i>	Desert rose
	<i>Gossypium hirsutum</i>	Cotton
	<i>Hibiscus sabdariffa</i>	Rosella
	<i>Hibiscus trionum</i>	Bladder ketmia
	<i>Malva parviflora</i>	Marshmallow
	<i>Malva sp.</i>	unknown
	<i>Malvastrum coromandelianum</i>	Malvastrum
	<i>Sida rhombifolia</i>	Paddy's lucerne
Euphorbiaceae	<i>Gossypium sturtianum</i>	Sturt's desert rose
	<i>Euphorbia hirta</i>	Asthma plant
Lamiaceae	<i>Lamium amplexicaule</i>	Deadnettle
Fabaceae	<i>Cicer arietinum</i>	Chickpea
	<i>Medicago polymorpha</i>	Burr Medic
	<i>Vicia faba</i>	Faba bean
Aizoaceae	<i>Trianthema portulacastrum</i>	Black or Giant pigweed

Localised CBT disease outbreak, 2018



CBTV incidence across paddock



Neighbours fallow paddock upwind with CBTV-infected volunteers. Sprayed with herbicide. Aphids moved into crop nearby, downwind. Four transect counts shown in graph.

Cotton blue disease – *Cotton leafroll dwarf virus* (**exotic**)

- Similar life cycle to cotton bunchy top but probably more easily transmitted by cotton aphid and more severe disease than CBTV
- Symptoms include severe stunting, down curling of leaves and intense green colour

CBD - Brazil



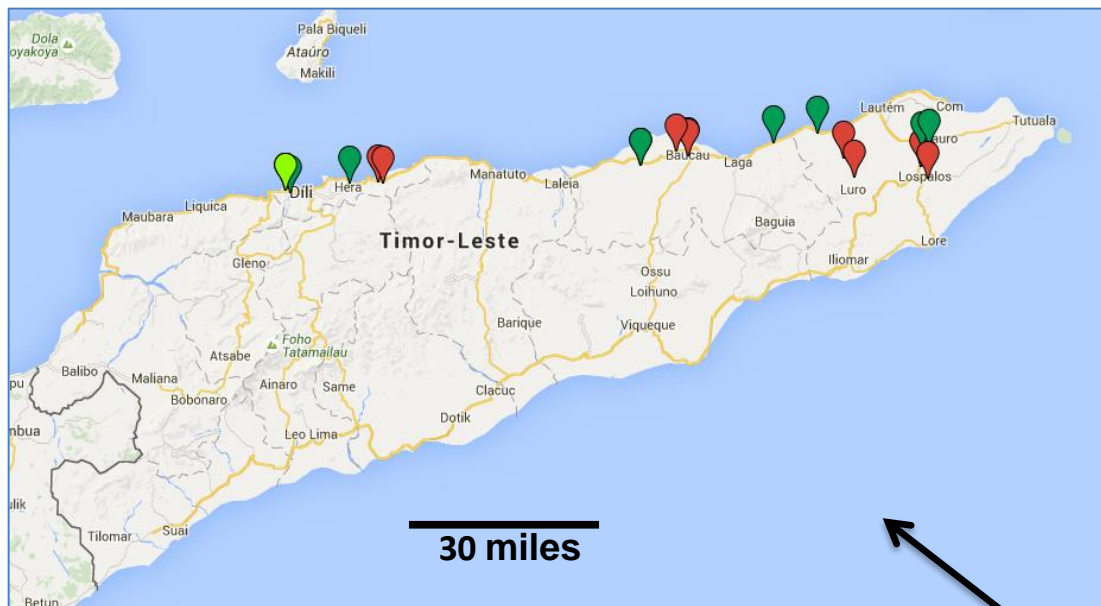
CBD - Thailand



- **Wide geographical distribution**
- **Confirmed by PCR in *G. hirsutum*, *G. barbadense*, chickpea and hibiscus**



Surveys for CLRDV in Timor-Leste



- 69 cotton plants tested from >20 locations
- CLRDV in 10/32 *G. barbadense*, 7/19 *G. arboreum* and 4/18 *G. hirsutum*
- >400 samples from about 30 host species collected for host range studies (continuing).



Timor-Leste surveillance



Gossypium arboreum



G. barbadense

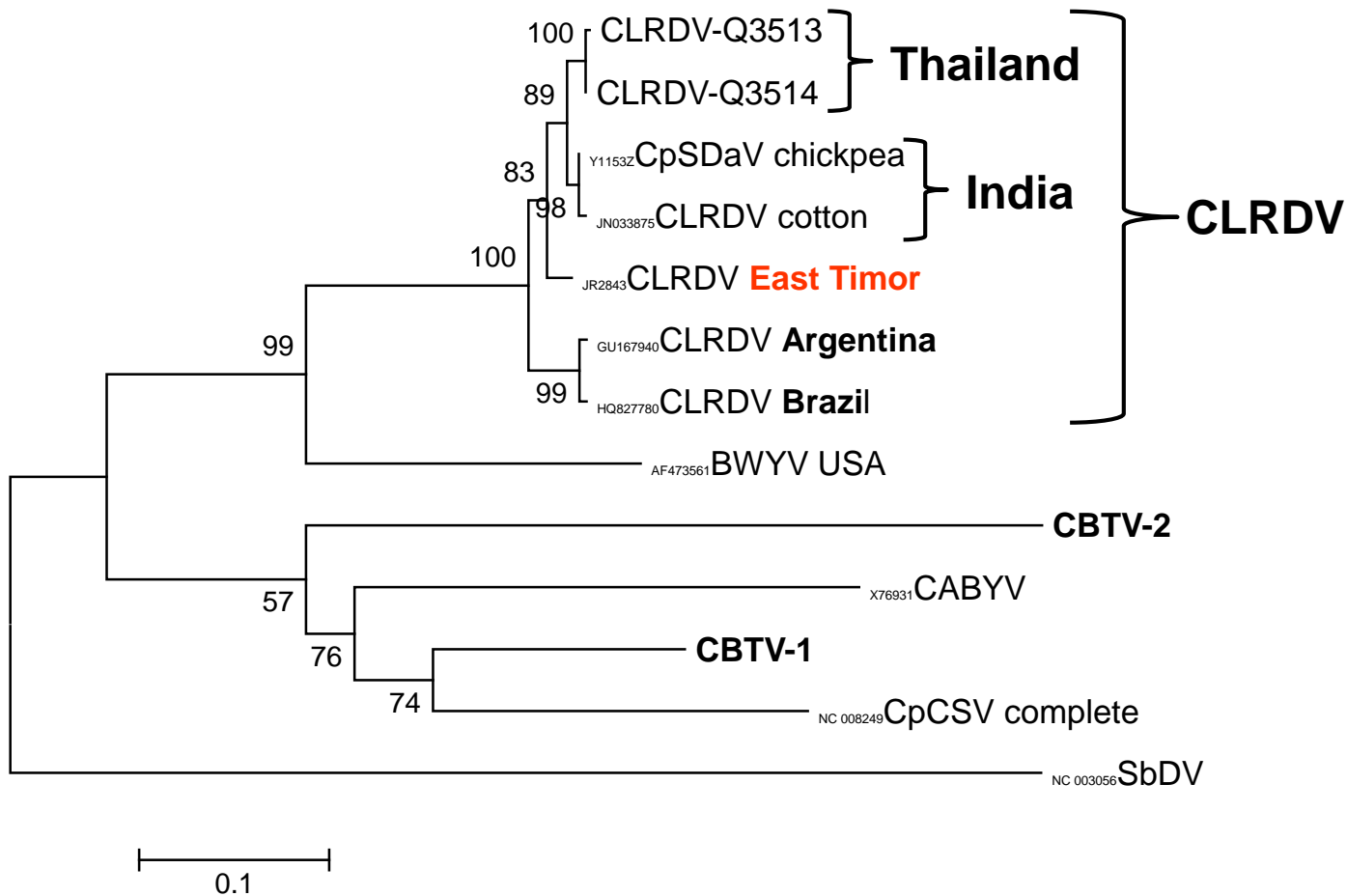


***G. hirsutum* (with CLRDV)**

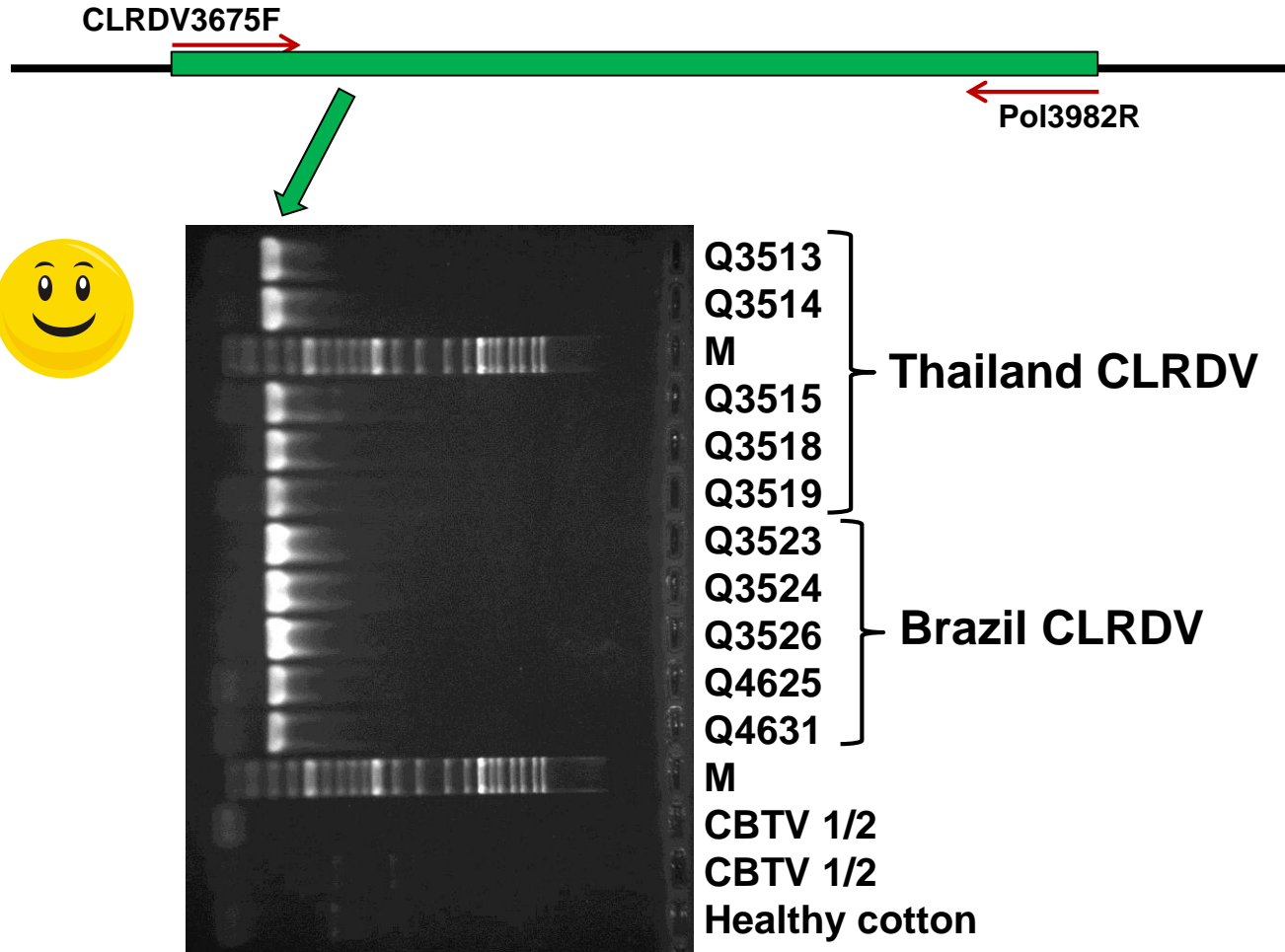


CLRDV diversity

- Phylogenetic tree of 560 bp genome section, partial ORF-3 (ML analysis)

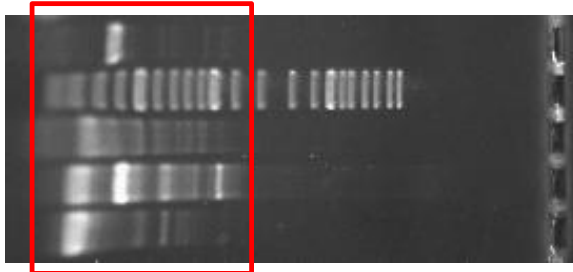


Specific detection of CLRDV

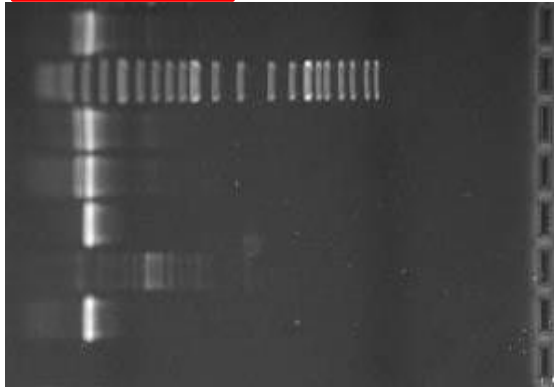


Detection of CLRDV

- Non-specific reactions are a problem with species other than cotton



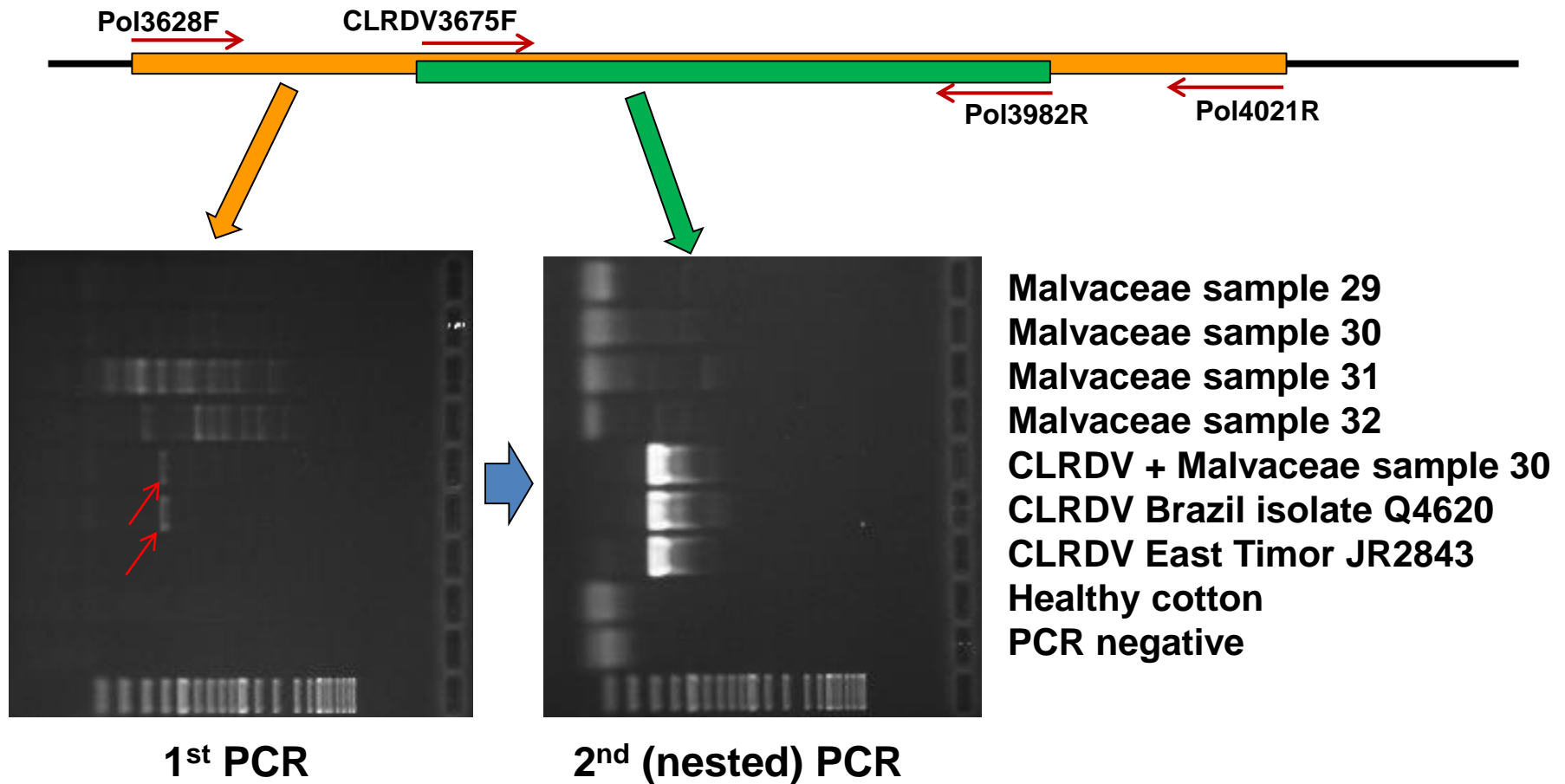
Malvaceae sample 29
M
Malvaceae sample 30
Malvaceae sample 31
Malvaceae sample 32



CLRDV East Timor
M
CLRDV East Timor
CLRDV East Timor
CLRDV Brazil Q4620
Healthy cotton
CLRDV Brazil Q4620
PCR negative

Improving detection of CLRDV

- A nested PCR greatly increases sensitivity and reduces non-specific reactions



Acknowledgments

- Cotton Research and Development Corporation (CRDC)
- Grains Research and Development Corporation (GRDC)
- CSIRO
- Department of Agriculture and Water Resources
- National Directorate of Quarantine and Biosecurity, Timor Leste
- Cotton and Grain growers and agronomists for samples and access to crops

