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.

<table>
<thead>
<tr>
<th>ORF #</th>
<th>Amino acid % identity</th>
</tr>
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<tbody>
<tr>
<td>ORF 0</td>
<td>51 %</td>
</tr>
<tr>
<td>ORF 1</td>
<td>65 %</td>
</tr>
<tr>
<td>ORF 1+2</td>
<td>75 %</td>
</tr>
<tr>
<td>ORF 3</td>
<td>70 %</td>
</tr>
<tr>
<td>ORF 4</td>
<td>59 %</td>
</tr>
<tr>
<td>ORF 3+5</td>
<td>60 %</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Plant Family</th>
<th>Host species</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvaceae</td>
<td><em>Abutilon theophrasti</em></td>
<td>Velvet leaf</td>
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<tr>
<td></td>
<td><em>Anoda cristata</em></td>
<td>Spurred anoda</td>
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<tr>
<td></td>
<td><em>Gossypium australe</em></td>
<td>Desert rose</td>
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<td></td>
<td><em>Gossypium hirsutum</em></td>
<td>Cotton</td>
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<td></td>
<td><em>Hibiscus sabdariffa</em></td>
<td>Rosella</td>
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<td></td>
<td><em>Hibiscus trionum</em></td>
<td>Bladder ketmia</td>
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<td></td>
<td><em>Malva parviflora</em></td>
<td>Marshmallow</td>
</tr>
<tr>
<td></td>
<td><em>Malva sp.</em></td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td><em>Malvastrum coromandelianum</em></td>
<td>Malvastrum</td>
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<tr>
<td></td>
<td><em>Sida rhombifolia</em></td>
<td>Paddy’s lucerne</td>
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<tr>
<td>Euphorbiaceae</td>
<td><em>Gossypium sturtianum</em></td>
<td>Sturt’s desert rose</td>
</tr>
<tr>
<td></td>
<td><em>Euphorbia hirta</em></td>
<td>Asthma plant</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Lamium amplexicaule</em></td>
<td>Deadnettle</td>
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<tr>
<td>Fabaceae</td>
<td><em>Cicer arietinum</em></td>
<td>Chickpea</td>
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<td><em>Medicago polymorpha</em></td>
<td>Burr Medic</td>
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<td></td>
<td><em>Vicia faba</em></td>
<td>Faba bean</td>
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<tr>
<td>Aizoaceae</td>
<td><em>Trianthema portulacastrum</em></td>
<td>Black or Giant pigweed</td>
</tr>
</tbody>
</table>
Localised CBT disease outbreak, 2018

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

CLRDV3675F

Pol3982R

Q3513
Q3514
M
Q3515
Q3518
Q3519
Q3523
Q3524
Q3526
Q4625
Q4631
M
CBTV 1/2
CBTV 1/2
Healthy cotton

Thailand CLRDV

Brazil CLRDV
Detection of CLRDV

- Non-specific reactions are a problem with species other than cotton
Improving detection of CLRDV

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

Pol3628F → CLRDV3675F → Pol3982R
Pol4021R

1st PCR

2nd (nested) PCR

Malvaceae sample 29
Malvaceae sample 30
Malvaceae sample 31
Malvaceae sample 32
CLRDV + Malvaceae sample 30
CLRDV Brazil isolate Q4620
CLRDV East Timor JR2843
Healthy cotton
PCR negative
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• Grains Research and Development Corporation (GRDC)
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