Recovery Plan – Draft—
Cotton leaf curl disease

*Caused by Cotton leaf curl virus complex (Begomovirus, Geminiviridae): A whitefly-transmitted virus causing leaf curl disease of cotton, vegetable, and ornamental crops*

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Economics of cotton

• Cultivated cotton *Gossypium* species (L.) have been a major source of food, feed and fiber worldwide for over 7000 years.
• Globally ~32.6 million hectares are devoted to cotton cultivation with production estimated at 27.6 million tons for 2011/2012 (Anonymous, 2011).
• Cotton is placed sixth on the *List of Economically Important Crops* for the U.S.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Vector/Transmission</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acromania</td>
<td>unknown</td>
<td>USA (Arizona), China, Haiti, Sudan</td>
</tr>
<tr>
<td>Cotton (common) yellow mosaic</td>
<td><em>Bemisia tabaci</em></td>
<td>Brazil</td>
</tr>
<tr>
<td>Cotton mosaic</td>
<td><em>B. tabaci</em></td>
<td>Central America</td>
</tr>
<tr>
<td>Cotton leaf curl</td>
<td><em>B. tabaci</em></td>
<td>USA (Louisiana)</td>
</tr>
<tr>
<td>Cotton leaf mottle</td>
<td>grafting</td>
<td>Philippines</td>
</tr>
<tr>
<td>Cotton leaf roll/leaf curliness</td>
<td><em>Aphis gossypii</em></td>
<td>Sudan, USSR, Thailand, Paraguay</td>
</tr>
<tr>
<td>Infectious variegation</td>
<td>grafting, <em>B. tabaci</em></td>
<td>India</td>
</tr>
<tr>
<td>Indian leaf crumple</td>
<td>grafting, <em>B. tabaci</em></td>
<td>India</td>
</tr>
<tr>
<td>Murcha vermelha/purple wilt</td>
<td><em>B. tabaci</em></td>
<td>South America</td>
</tr>
<tr>
<td>Terminal stunt</td>
<td><em>A. gossypii</em></td>
<td>USA (Texas)</td>
</tr>
<tr>
<td>Tobacco streak</td>
<td>possibly thrips</td>
<td>Brazil</td>
</tr>
</tbody>
</table>
Diverse symptoms: whitefly-associated virus-like diseases
Three major cotton-infecting viruses identified and characterized

**New World:**
*Cotton leaf crumple virus*: AZ, CA, TX, Mexico, Guatemala, Caribbean, Brazil

**Asia**
*Cotton leaf curl virus complex* (7+ species)

**Africa**
*Cotton leaf curl Gezira virus*
*Nile Basin*
Specific cotton begomovirus genomes & satellites

**New World**
- Cotton leaf crumple virus

**Africa**
- Cotton leaf curl Gezira virus

**Asia**
- Cotton leaf curl Allahabad virus
- Cotton leaf curl Bangalore virus
- Cotton leaf curl Burewala virus
- Cotton leaf curl Kokhran virus
- Cotton leaf curl Multan virus
- Cotton leaf curl Rajasthan virus
- Cotton leaf curl Shadadpur virus
**Cotton leaf curl Gezira virus:** Proliferation of spongy parenchyma and bundle sheath extension cells, and hyperplastic cells (transverse sections) = Enations are a unique feature of OW leaf curl begomoviruses.

Cotton leaf curl disease (CLCuD) is a debilitating disease of cotton that results in leaf curling, development of leaf-like enations on the undersides of leaves, overall stunting of the plant, and reduced yield and quality. CLCuD occurs in Africa, Pakistan, and northwestern (Punjab region) India. Caused by one or more whitefly-transmitted geminiviruses (family Geminiviridae, genus Begomovirus) that serve as the ‘helper virus’ for to different kinds of DNA satellite molecules of non-viral origin: betasatellites and alphasatellites.

The satellites contribute to host defense suppression, leading to increased virulence of the helper virus that leads to systemic infection and severe disease symptoms; the promiscuity of the beta/alpha satellites may confer versatility to helper viruses. The host range of the CLCuD virus-satellite complexes has been found to be astoundingly broad, spanning multiple plant families, albeit, it was initially thought to be restricted to cotton and several wild malvaceous hosts.

Since 1990 Pakistan and India have experienced two epidemics: - the most recent is the result of an emergent, resistance breaking strain (2001-present) - overcame host-plant resistance in varieties developed to combat the first outbreak occurring in about 1990.
<table>
<thead>
<tr>
<th>Year Range</th>
<th>Event Description</th>
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<tbody>
<tr>
<td>1967-1973</td>
<td>Leaf curl symptoms first reported in Pakistan in Multan, Punjab Provinces; by 1973 ‘Cotton leaf curl’ disease; Cotton leaf crumple occurs annually in cotton, US/Mexico.</td>
</tr>
<tr>
<td>1994-1999</td>
<td>Common Fund-Cotton project initiated with NIBGE (Zafar-NIBGE and Brown-UA); first effort to characterize the causal agent; identified a monopartite begomoviral genome and beta satellite complex. Cotton viruses in Sudan and American Tropics studied; causality proven for two main species (Brown et al) + Leaf curl in Pakistan. Breeding yielded resistant cotton varieties – production returned to pre-epidemic levels.</td>
</tr>
<tr>
<td>1997</td>
<td>Leaf curl symptoms reported in cotton fields in the Sindh Province.</td>
</tr>
<tr>
<td>2001</td>
<td>First appearance of resistance breaking in cotton in western India. Evidence for more than one viral genome: Cotton leaf curl Alalabad virus (CLCuAV) and Cotton leaf curl Khokhran virus (CLCuKV); Papaya leaf curl virus (Mansoor et al., 2003b) and Tomato leaf curl Bangalore virus (Kirthi et al 2004); Cotton leaf curl Rajasthan virus in tomato and cotton India and Pakistan (Kirthi, Rehman, Shahid)</td>
</tr>
<tr>
<td>2001-2004</td>
<td>All cotton varieties susceptible – tolerance in some lines; presence of a recombinant virus + Multan beta satellite: Cotton leaf curl Burewala virus (Amrao et al., 2010b) = parental viruses: CLCuMuV and CLCuKoV; subsequently spread to India = Current Project</td>
</tr>
</tbody>
</table>
Primary causal agents: 
Whitefly-transmitted geminiviruses

- Family: Geminiviridae
- Genus: *Begomovirus*
- Distributed worldwide
- Infect dicotyledonous hosts
- Monopartite begomoviruses - one circular ssDNA component ~2.8 kb
- Bipartite begomoviruses - two ss circular DNA components ~2.6 kb each
Particle Morphology

One ssDNA molecule per particle
**Structure of DNA $\beta$**
- A single conserved ORF
- A region rich in adenine (approx. 300bp)
- A region conserved between all DNA $\beta$s (approx. 300bp)
- A hairpin structure (ori)

**Properties of DNA $\beta$**
- **Trans-replicated** by begomovirus encoded Rep
- Requires **helper** virus CP for transmission
- Essential for **symptom development** by helper virus
- Enhances **helper virus DNA accumulation**
**Kingdom:** Animalia  
**Phylum:** Arthropoda  
**Class:** Insecta  
**Order:** Hemiptera  
**Family:** Aleyrodidae  
**Genus:** Bemisia  
**Species:** tabaci

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**Bemisia tabaci sibling species group**

**Characteristics**
- Polyphagous insect (collectively, 500 host plant species)
- Haplodiploid reproduction
- Exclusively, phloem feeding
- Phylogenetically divergent mtCOI (0-26%)
- Variants referred to as haplotypes (mtCOI), or biotypes (mtCOI divergence + biological data)
- mtCOI phylogeographic distribution
- Causes billions of dollar losses annually worldwide
- Highly fecund
- Only insect vector, genus: *Begomovirus* (family, Geminiviridae)
- Recent examples invasive movement: TYLCV - worldwide owing to dispersal via ornamentals, seedlings, whitefly; *Cotton leaf curl virus* - Pakistan, India, China
Cotton leaf curl virus timeline

First report: 1967
- Sporadic isolated infections of cotton

Start of the CLCuV epidemic: 1988
- Introduction of an American cotton variety (S12)
- $5 billion losses from 1992-1997 due to CLCuD

Resistance breaking: 2001
- Second Epidemic: recombinant virus + CLCuMB

Resistance attributed to 3 genes: two R genes and “suppressor”

Introduction of resistant varieties late 1990s

Courtesy R. Briddon
The “Multan” strain of CLCuD

- *Cotton leaf curl Multan virus*
- *Cotton leaf curl Kokhran virus*
- *Papaya leaf curl virus*
- *Tomato leaf curl Karnataka virus*
- *Cotton leaf curl Alabad virus*
- *Cotton leaf curl Rajasthan virus*
- *Cotton leaf curl Bangalore virus*
- One betasatellite (CLCuMB)

- Pre-resistance breaking - 7 viruses one betasatellite
- Post-resistance breaking - 1 virus one betasatellite
Disease Severity Ratings 0-4
## Estimated losses due to CLCVD

<table>
<thead>
<tr>
<th>Year of infestation</th>
<th>Estimated crop damage</th>
<th>Estimated Financial Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Partial)</td>
<td>(Complete)</td>
</tr>
<tr>
<td>2006-07</td>
<td>1686.40</td>
<td>25.21</td>
</tr>
<tr>
<td>2007-08</td>
<td>1432.80</td>
<td>2.50</td>
</tr>
<tr>
<td>2008-09</td>
<td>1440.10</td>
<td>40.25</td>
</tr>
<tr>
<td>2009-10</td>
<td>1693.50</td>
<td>43.39</td>
</tr>
<tr>
<td>2010-11</td>
<td>1341.80</td>
<td>28.33</td>
</tr>
<tr>
<td>2011-12</td>
<td>569.60</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Viruses and satellites in cotton, Pakistan 2012

Brown Lab: SNP detection in CLCuV and associated satellites
CLCuMV distribution in China-2012

Only in South China presently
DNA sequencing of RCA products reveals presence of one isolate:

**CLCuMV:**
- Multan strain +
- CLCuMB
Virus detection to populations.....

- Entire venue for virus detection and identification of species and variants has evolved from:
  - No molecular techniques to DNA hybridization – to Polymerase chain reaction to genome sequencing – Rolling circle amplification – to New Generation sequencing
- Enables high throughput vs single samples
- Facilitating views of diversification and population structure in a new light
from…..PCR strategies for ID & diversity studies

**core Cp**
quick ID: universal primers & determine seq in a single run
(576-579 bp)

**full CP**
1.1 kbp fragment; two sequencing rxns; provisional ID and relationships = DNA-A)

+ **2/3 DNA-A overlap** w/ CP
  CP & CR
  (2.1 kbp)

**Top Half B**
**CR** - cognate component
(1.5 kbp)

Core CP

= DNA-A
Cloning sites

to..
Virus or satellite alignment

Recombination and sites-under-selection detection

Model of evolution
Bayesian phylogenetic analysis

SNP detection using software tools

Association of discovered SNPs and phenotypes (symptoms)

Trace character (nucleotide) history based on phylogeny to detect useful SNPs

SNP macro array & other genotyping platforms

.... High throughput processing and DNA sequencing

New Generation sequencing to explore evolutionary space in relation to host genetics
Global Concerns:
- Spread of diverse species to cotton-growing regions and widespread losses could create a global economic crisis and shortage of supply
- CLCuMV and satellite already found in China-moved on ornamental plants
- Invasive whitefly biotypes, also Re-distributed via trade
- Further threat due to **high diversity / broad host range**
Thank you
Phylogeny of OW begomoviruses infecting cotton, hollyhock, and okra
Alphasatellites

HYVB, HYVKoB, ToYDV, EpYVB, SgYVB, EmYVB, LaYVB
(JP, CN, VN)

AYVB, ToLCJJa, ZLCuB, SiYVVNB, MaLCuB, SiYVCNB, ToLCPB, ToLCCNB, ToLCB
(CN, VN, LA, ID, TH, JP, MY)

CLCuGeB
(BF, CM, EG, MA, NE, SD)

Africa

OLCuB/ BYVB
(IN, PK)

LuLDB
(IN)

MaYVVyB
(IN)

KLCuB
(IN)

CLCuMuB
(IN, PK, CN)

ChLCB, ToLCBB, ToLCB, ToLKaB, TLCRaB, AYLCuB, ToLCJoB, ToLCPaB, CroYVMB, PaLCuB ToLCMaB
(PK, IN, BD, OM)
Enations (box) developing on a main vein of cotton leaf inoculated with CLCuGV and sat DNA - ‘vein thickening’

Swelling of veins results from proliferation of spongy parenchyma and bundle sheath cells, with SP being more swollen than bundle sheath cells.

This asymmetric proliferation of veins causes the formation of veinal grooves.

Transverse sections of cells associated with enations that form on CLCuGV-infected leaves contain parenchyma instead of collenchyma with very thin cell walls.