Recovery Plan – Draft—
Cotton leaf curl disease

(
): A whitefly-transmitted virus causing leaf curl disease of cotton, vegetable, and ornamental crops

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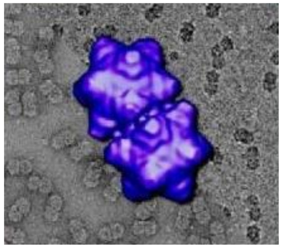


Economics of cotton

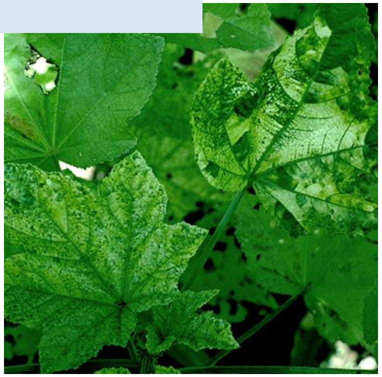
- Cultivated cotton *Gossypium* species (L.) have been a major source of food, feed and fiber worldwide for over 7000 years.
- Globally ~32.6 million hectares are devoted to cotton cultivation with production estimated at 27.6 million tons for 2011/2012 (Anonymous, 2011).
- Cotton is placed sixth on the *List of Economically Important Crops* for the U.S.

Virus-like disorders of cotton

Disease	Vector/Transmission	Geographical Distribution
Acromania	unknown	USA (Arizona), China, Haiti,
Cotton (common) yellow mosaic	*Bemisia tabaci*	Sudan
	B. tabaci	Brazil
Cotton mosaic	unknown	
		Central America
	B. tabaci	
Cotton leaf curl		USA (Louisiana)
	grafting	
Cotton leaf mottle	*Aphis gossypii*	Philippines
Cotton leaf roll /leaf curliness	*A. gossypii*	Sudan,USSR Thailand, Paraguay
Infectious variegation	grafting, *B. tabaci*	India
Indian leaf crumple	grafting, *B. tabaci*	India
Murcha vermelha /purple wilt	*B. tabaci*	South America
		USA (Texas)
Terminal stunt	*A. gossypii*	
Tobacco streak		Brazil
	possibly thrips	

Diverse symptoms: whitefly-associated virus-like diseases


SW US


Caribbean


Central America


Sudan


Pakistan


Guatemala


Puerto Rico


Egypt

Three major cotton-infecting viruses identified and characterized

New World:

: AZ, CA,

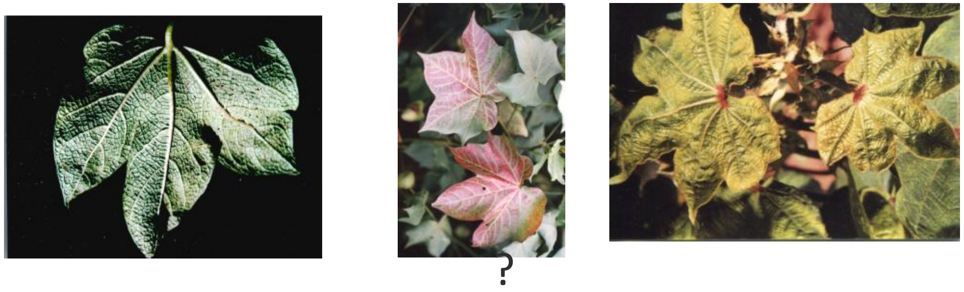
TX, Mexico, Guatemala,
Caribbean, Brazil

Asia

complex (7+ species)

Africa

Cotton leaf curl
Gezira virus
Nile Basin



Specific cotton begomovirus genomes & satellites

New World

- Cotton leaf crumple virus

Africa

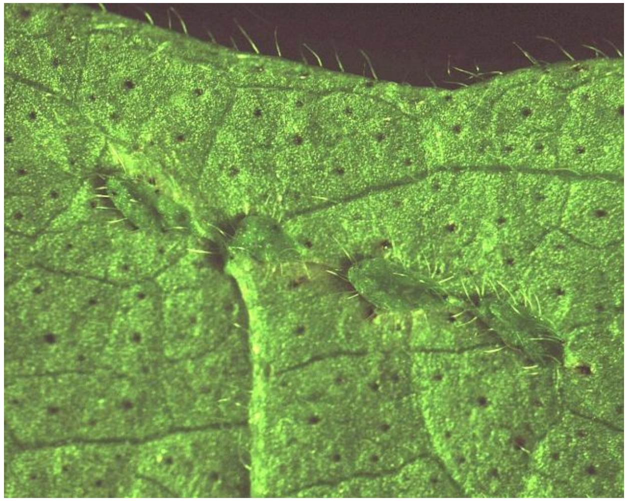
- Cotton leaf curl Gezira virus

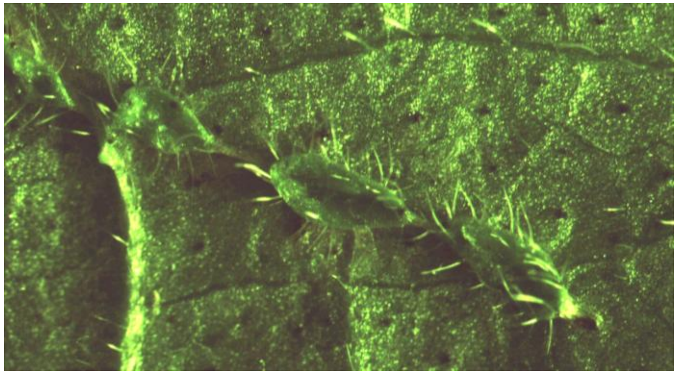
Asia

- 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
-

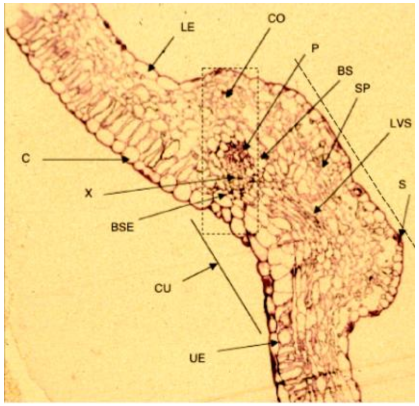
Proliferation of spongy parenchyma and bundle sheath extension cells, and hyperplastic cells (transverse sections) = Enations are a unique feature of OW leaf curl begomoviruses

BS: bundle sheath, BSE: bundle sheath extension, C: cuticle, CO: collenchyma cells, CU: leaf curvature or vein groove, LE: lower epidermis, LVS: secondary vein, UE: upper epidermis, P: phloem, S: stoma, SP: spongy parenchyma, X: xylem.

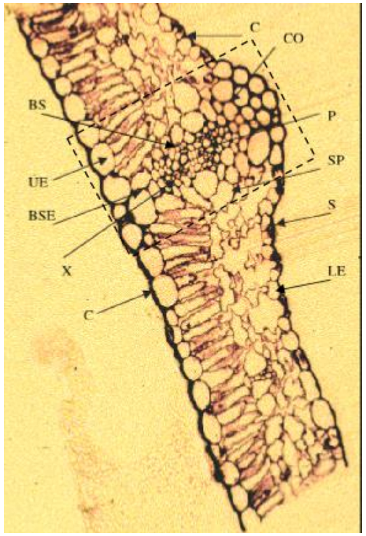




Box = main vein



healthy



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**.

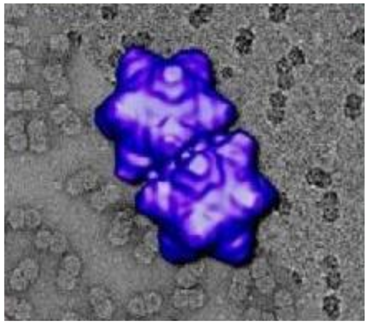
The satellites contribute to host defense suppression, leading to increased virulence of the helper virus that leads to systemic infection and severe disease symptoms; the promiscuity of the beta/alpha satellites may confer versatility to helper viruses.
-**The host range** of the CLCuD virus-satellite 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.

Brief history: Leaf curl viruses of cotton

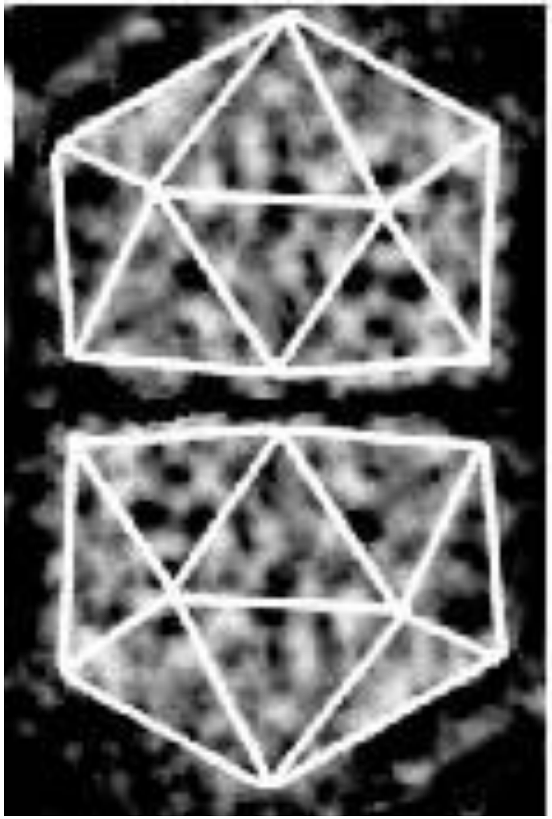
1912-1960	**Nigeria (1912-*G. barbadense*), Sudan (1924) –Leaf curl Gezira virus,Tanzania (1926), USA-CA/AZ leaf crumple virus (1951), Philippines (1959). In all instances - symptoms associated with whitefly *Bemisia tabaci* presence.**
1967-1973	**Leaf curl symptoms first reported in Pakistan in Multan, Punjab Provinces; by 1973 'Cotton leaf curl' disease; Cotton leaf crumple occurs annually in cotton, US/Mexico.**
1983-1993	**CLCD outbreak in cotton near Multan –resulting in the first epidemic (80% incidence), spreading throughout Pakistan and into western India. The disease emerged again during 1989 and 1992-1994 (entire cotton belt).**
1994-1999	**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.
1997	**Leaf curl symptoms reported in cotton fields in the Sindh Province.**
2001	**First appearance of resistance breaking in cotton in western India.**
2001-2004	**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)
2009-12	**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**

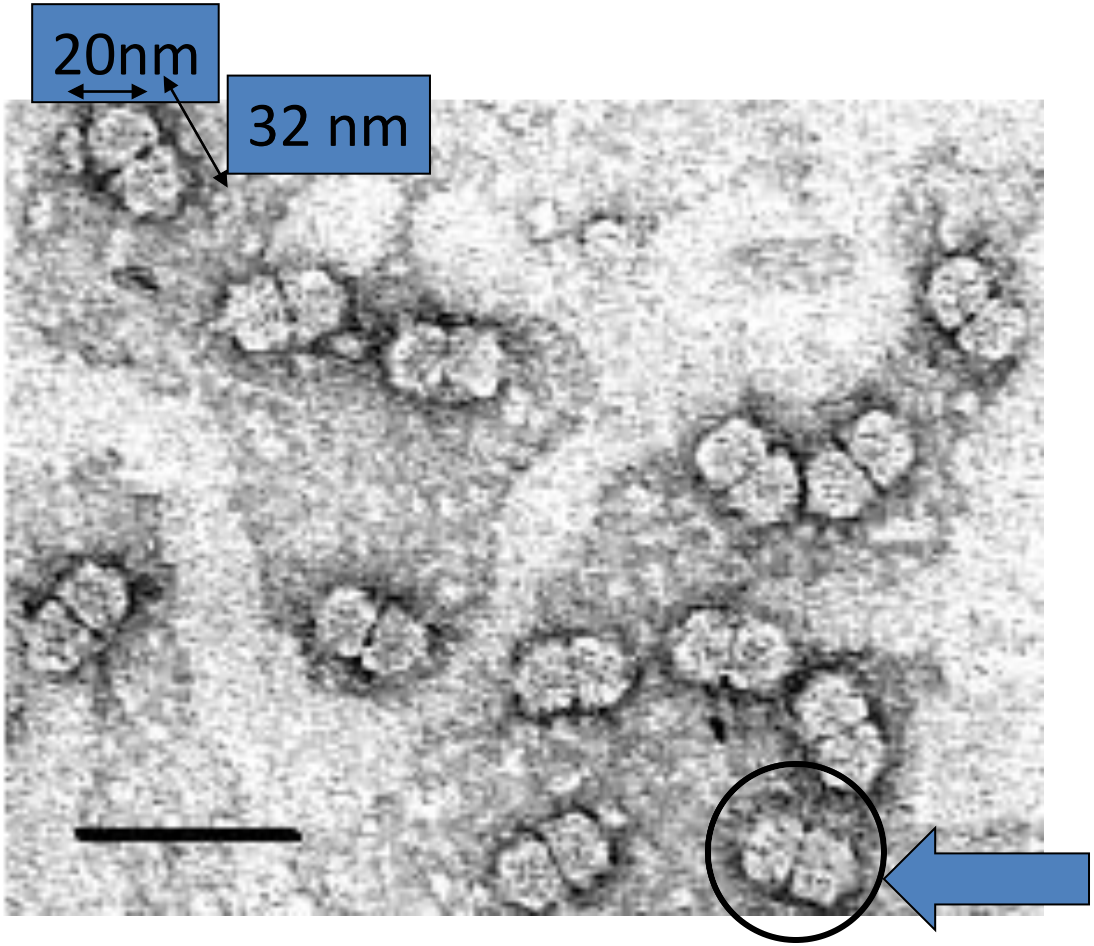
Primary causal agents:
Whitefly-transmitted geminiviruses



- **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





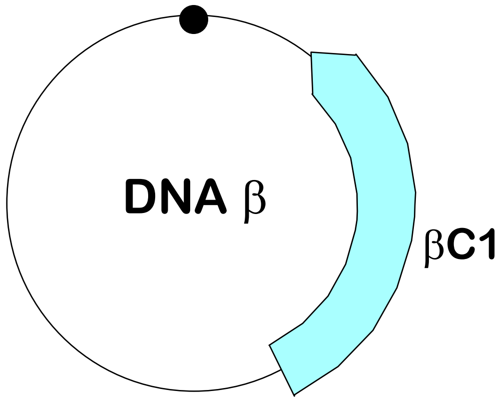
20nm

32 nm

One ssDNA molecule per particle

DNA β

satellite

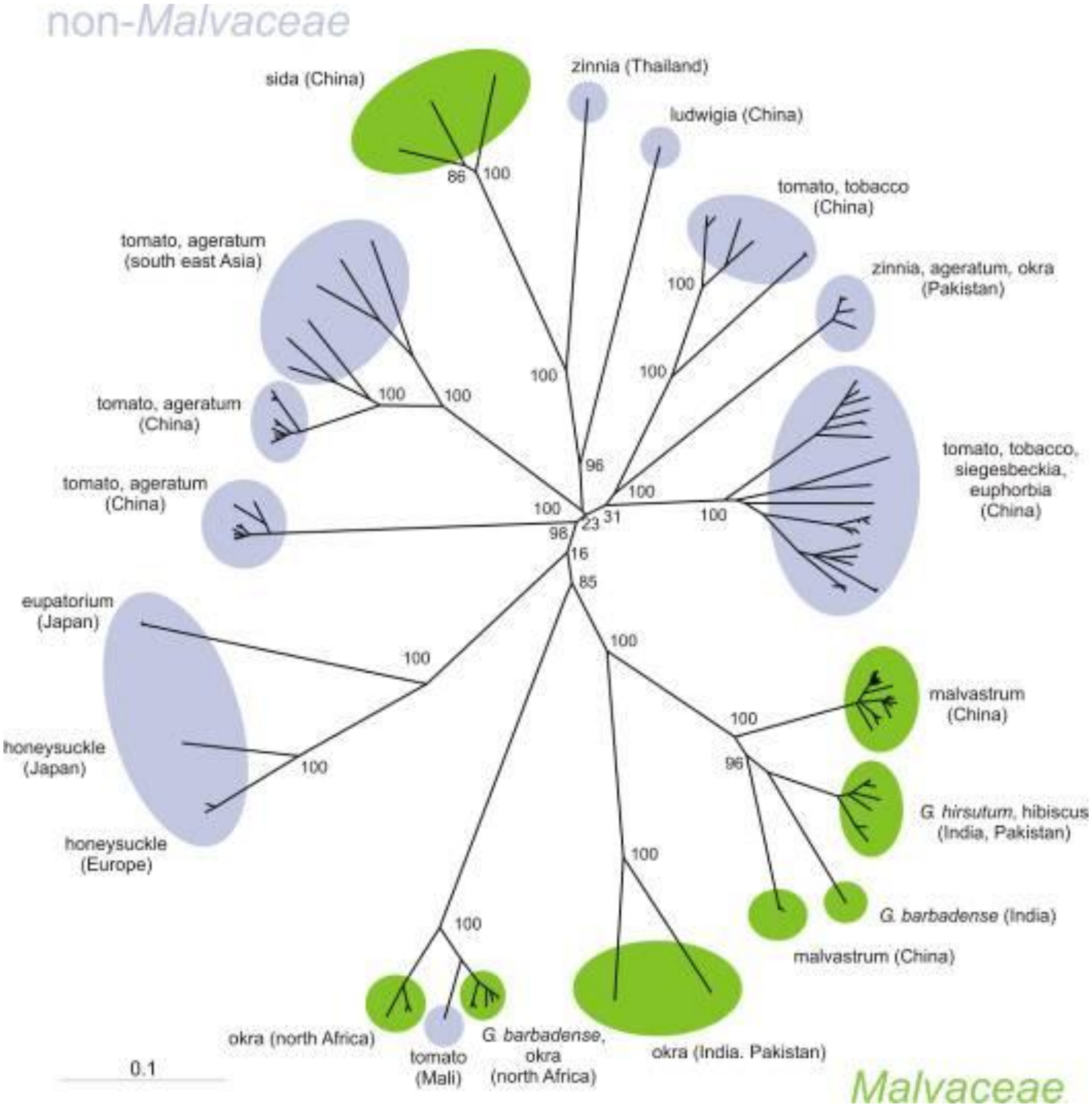


Structure of DNA β

- A single conserved ORF
- A region rich in adenine (approx. 300bp)
- A region conserved between all DNA βs (approx. 300bp)
- A hairpin structure (ori)

Properties of DNA β

- Trans-replicated by begomovirus encoded Rep
- Requires helper virus CP for transmission
- Essential for symptom development by helper virus
- Enhances helper virus DNA accumulation


non-Malvaceae
sida (China)
zinnia (Thailand)
ludwigia (China)
tomato, tobacco (China)
zinnia, ageratum, okra (Pakistan)
tomato, ageratum (south east Asia)
tomato, ageratum (China)
tomato, ageratum (China)
tomato, tobacco, siegesbeckia, euphorbia (China)
eupatorium (Japan)
honeysuckle (Japan)
honeysuckle (Europe)
malvastrum (China)
G. hirsutum, hibiscus (India, Pakistan)
G. barbadense (India)
malvastrum (China)
okra (north Africa)
tomato (Mali)
G. barbadense, okra (north Africa)
okra (India. Pakistan)
Malvaceae
0.1
86
100
100
100
100
100
100
96
100
100
100
23
31
98
16
85
100
100
100
100
96
100
100

The vector

sibling species group

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Hemiptera
Family:	Aleyrodidae
Genus:	Bemisia
Species:	tabaci

Characteristics

- Polyphagous insect (collectively, 500 host plant species)
- Haplodiploid reproduction
- Exclusively, phloem feeding

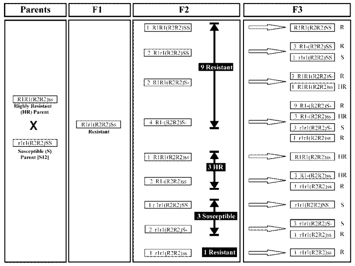
- Phylogenetically divergent mtCOI (0-26%)
- Variants referred to as haplotypes (mtCOI), or biotypes (mtCOI divergence + biological data)
- mtCOI phylogeographic distribution

- Causes billions of dollar losses annually worldwide
- Highly fecund
- Only insect vector, genus: *Begomovirus* (family, Geminiviridae)

- <u>Recent examples invasive movement</u>: TYLCV - worldwide owing to dispersal via ornamentals, seedlings, whitefly; *Cotton leaf curl virus*- Pakistan, India, China

Cotton leaf curl virus timeline

Resistance attributed to 3 genes: two R genes and "suppressor"



First report

1967

Sporadic isolated infections of cotton

Start of the CLCuV epidemic

1988

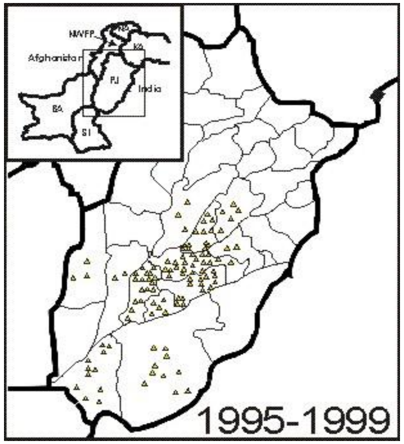
Introduction of resistant varieties late 1990s

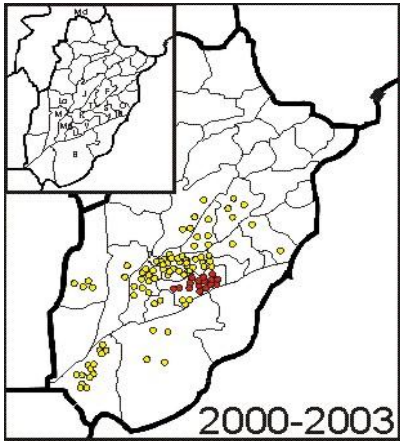
Introduction of an American cotton variety (S12)

$5 billion losses from 1992-1997 due to CLCuD

Resistance breaking

2001





Second Epidemic:

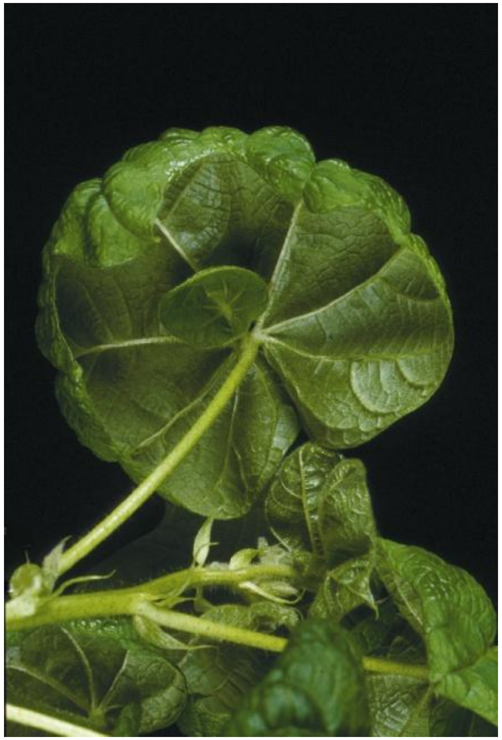
recombinant virus + CLCuMB

Bur

Courtesy R. Briddon)

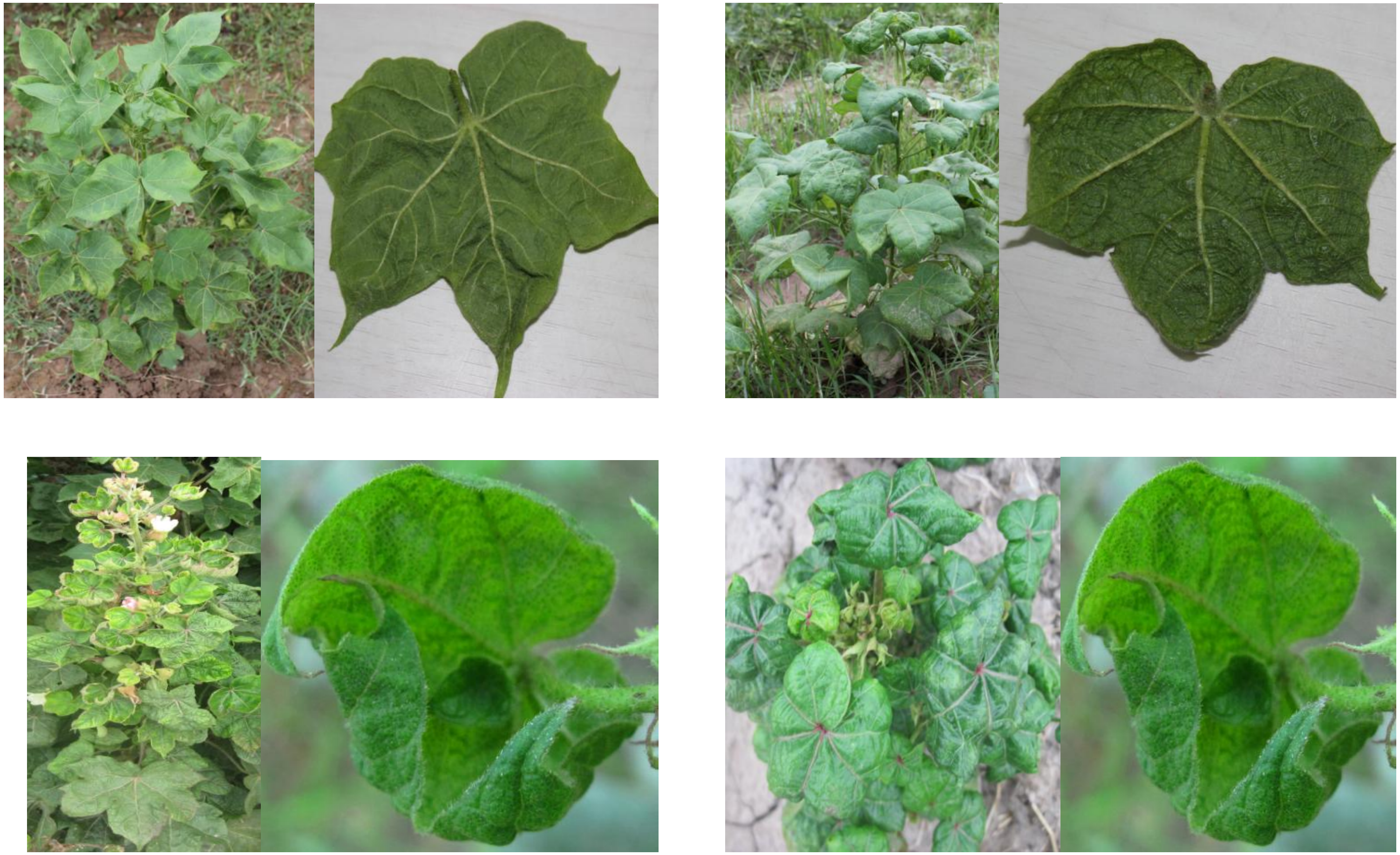
The “Multan” strain of CLCuD

- **One betasatellite (CLCuMB)**



- Pre-resistance breaking - 7 viruses one betasatellite
- Post -resistance breaking - 1 virus one betasatellite

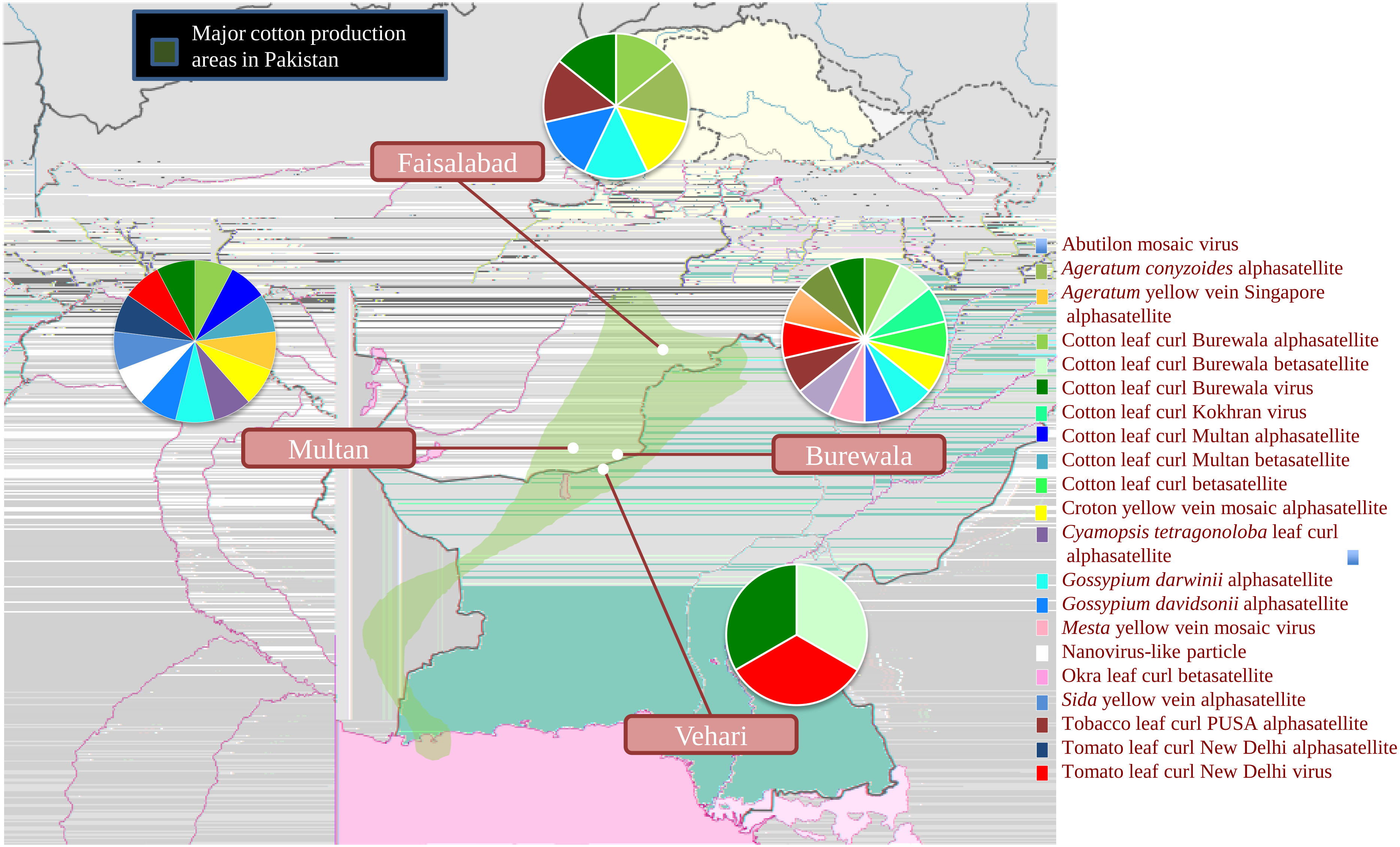
Disease Severity Ratings 0-4



Estimated losses due to CLCVD

Year of infestation	Estimated crop damage			Estimated Financial Losses	
	(Partial)	(Complete)	(Total)	(Mill.Bales)	(Bill.PKR)
2006-07	1686.40	25.21	1711.63	1.23	14.063
2007-08	1432.80	2.50	1435.29	0.95	13.778
2008-09	1440.10	40.25	1480.35	1.11	16.079
2009-10	1693.50	43.39	1956.62	1.84	44.160
2010-11	1341.80	28.33	1370.12	1.16	48.457
2011-12	569.60	0.00	569.60	0.48	11.815

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

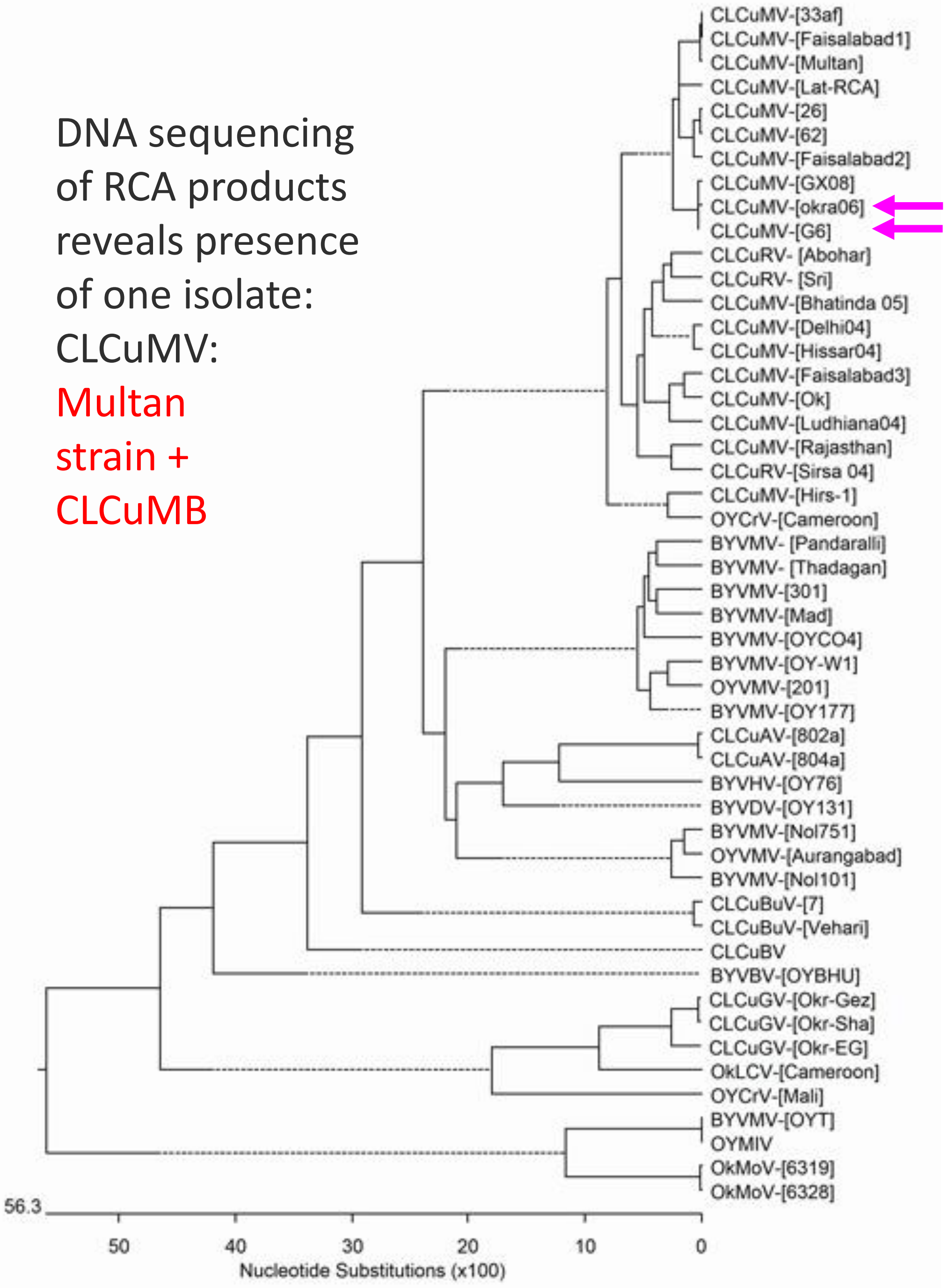
Guangxi

Guangdong

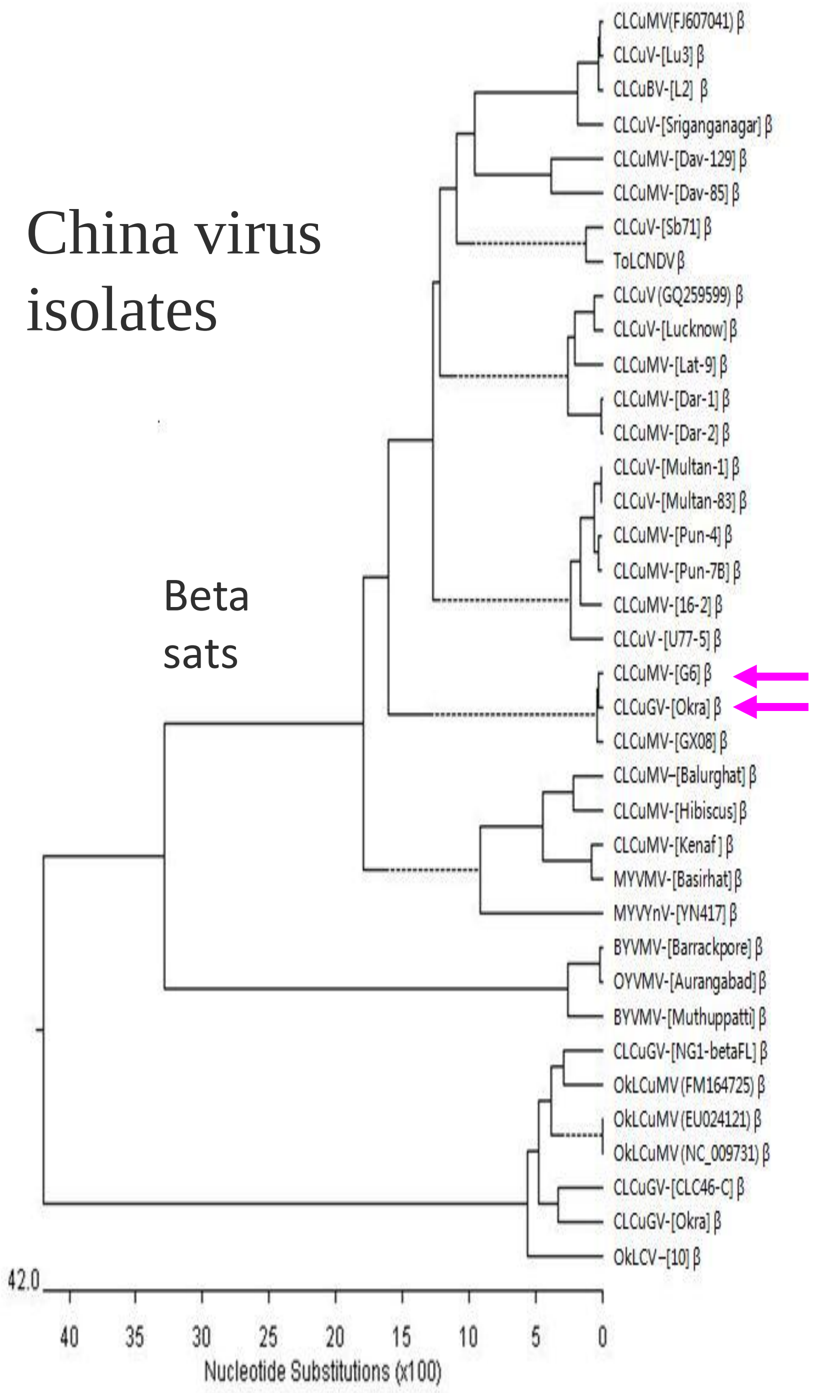
Hainan

DNA sequencing of RCA products reveals presence of one isolate: CLCuMV: Multan strain + CLCuMB

China virus isolates



Beta sats



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

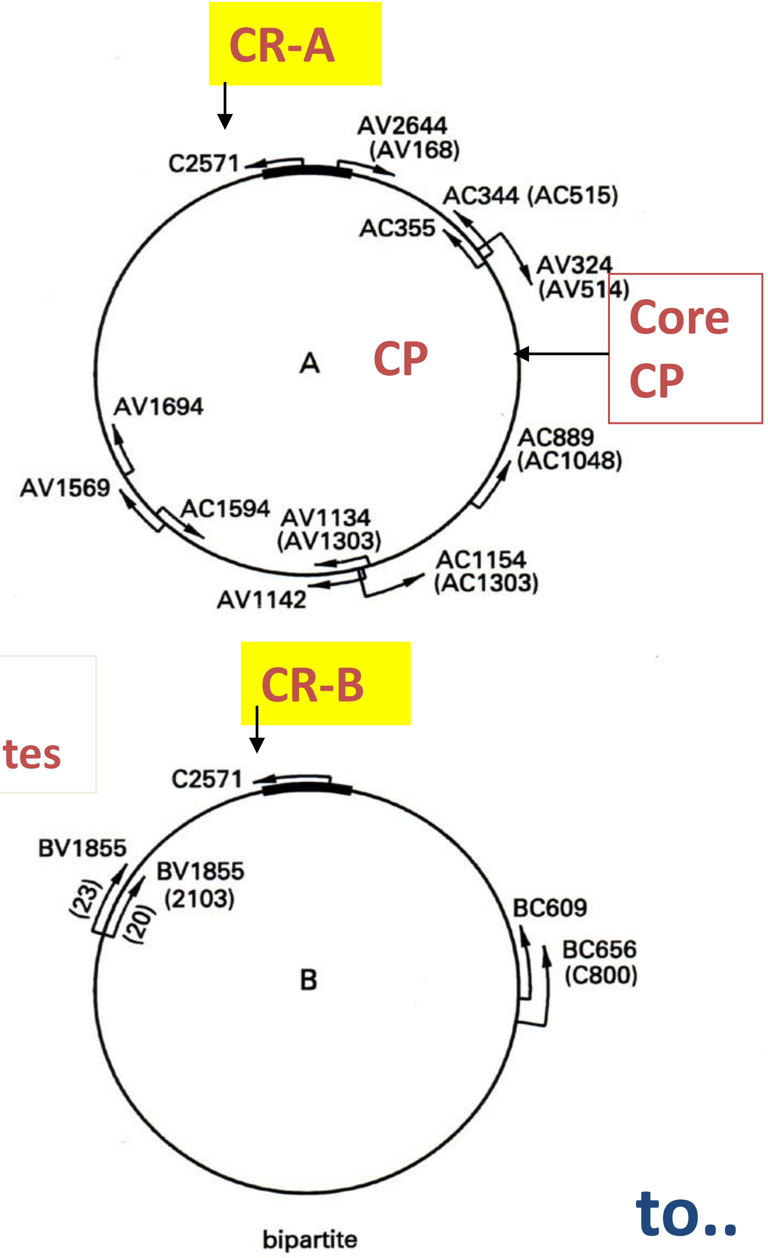
(2.1 kbp)

Top Half B

CR - cognate component

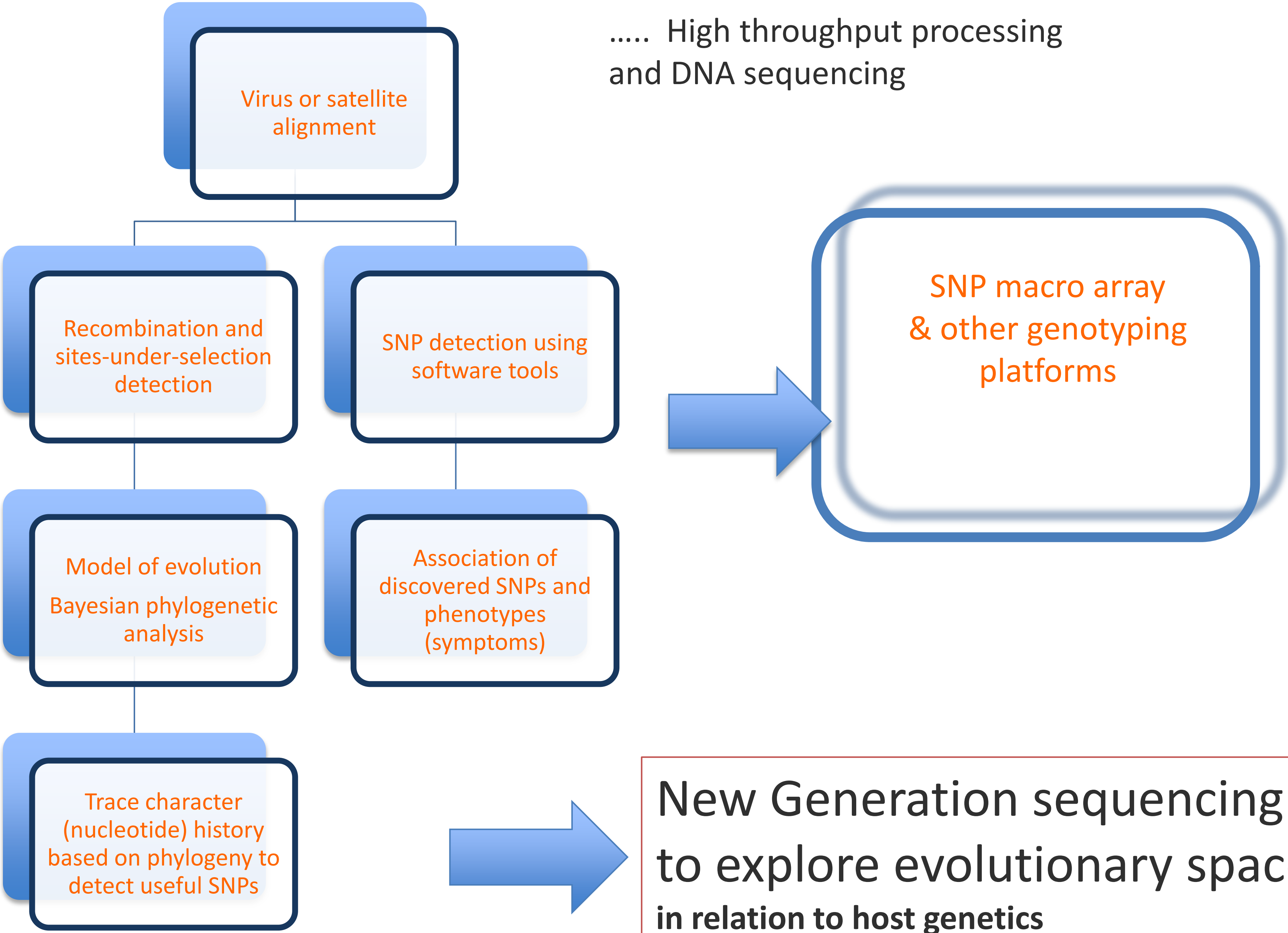
(1.5 kbp)

= DNA-A
Cloning sites



to..

….. High throughput processing and DNA sequencing



Virus or satellite alignment

Recombination and sites-under-selection detection

SNP detection using software tools

Model of evolution

Bayesian phylogenetic analysis

Association of discovered SNPs and phenotypes (symptoms)

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

SNP macro array & other genotyping platforms

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

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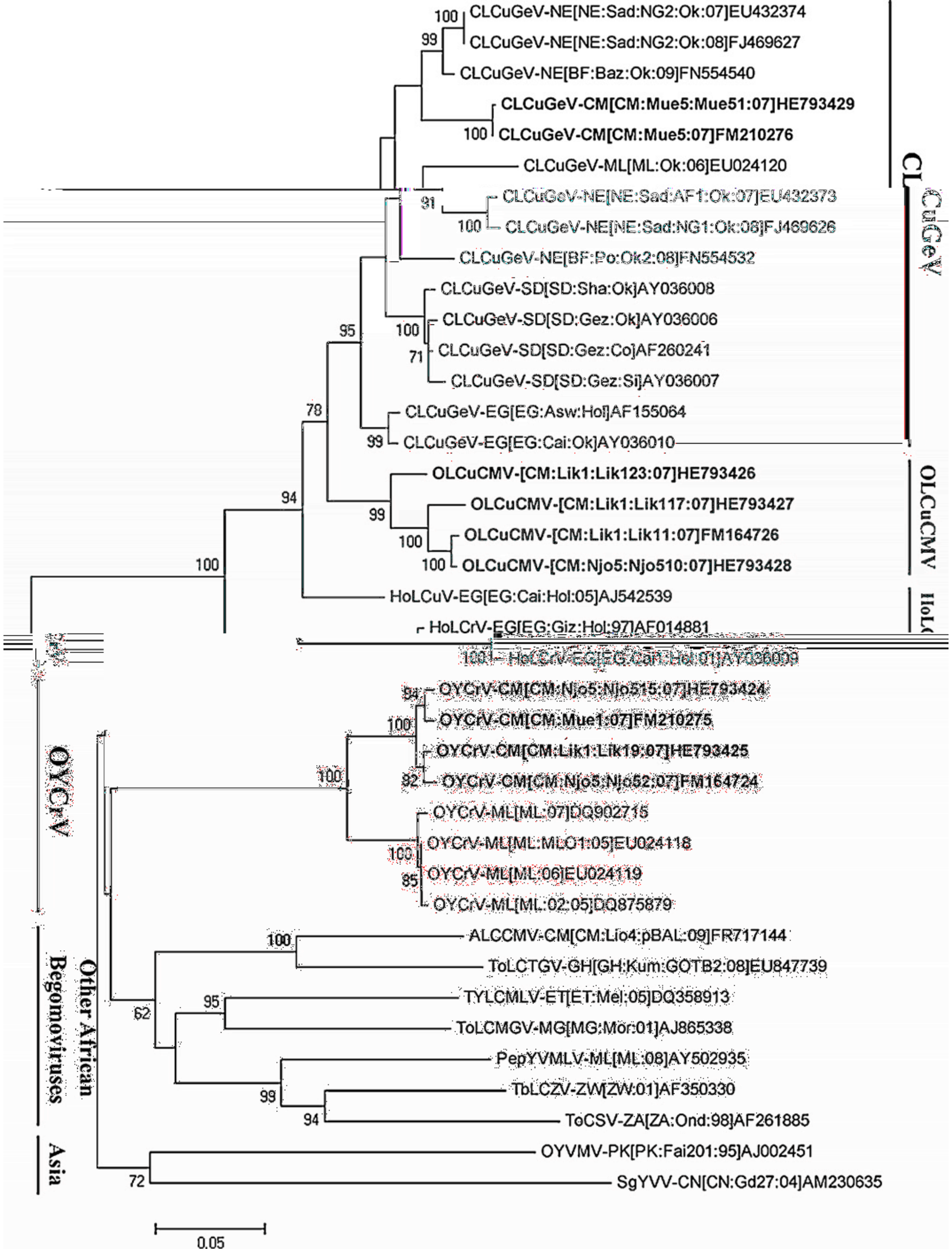
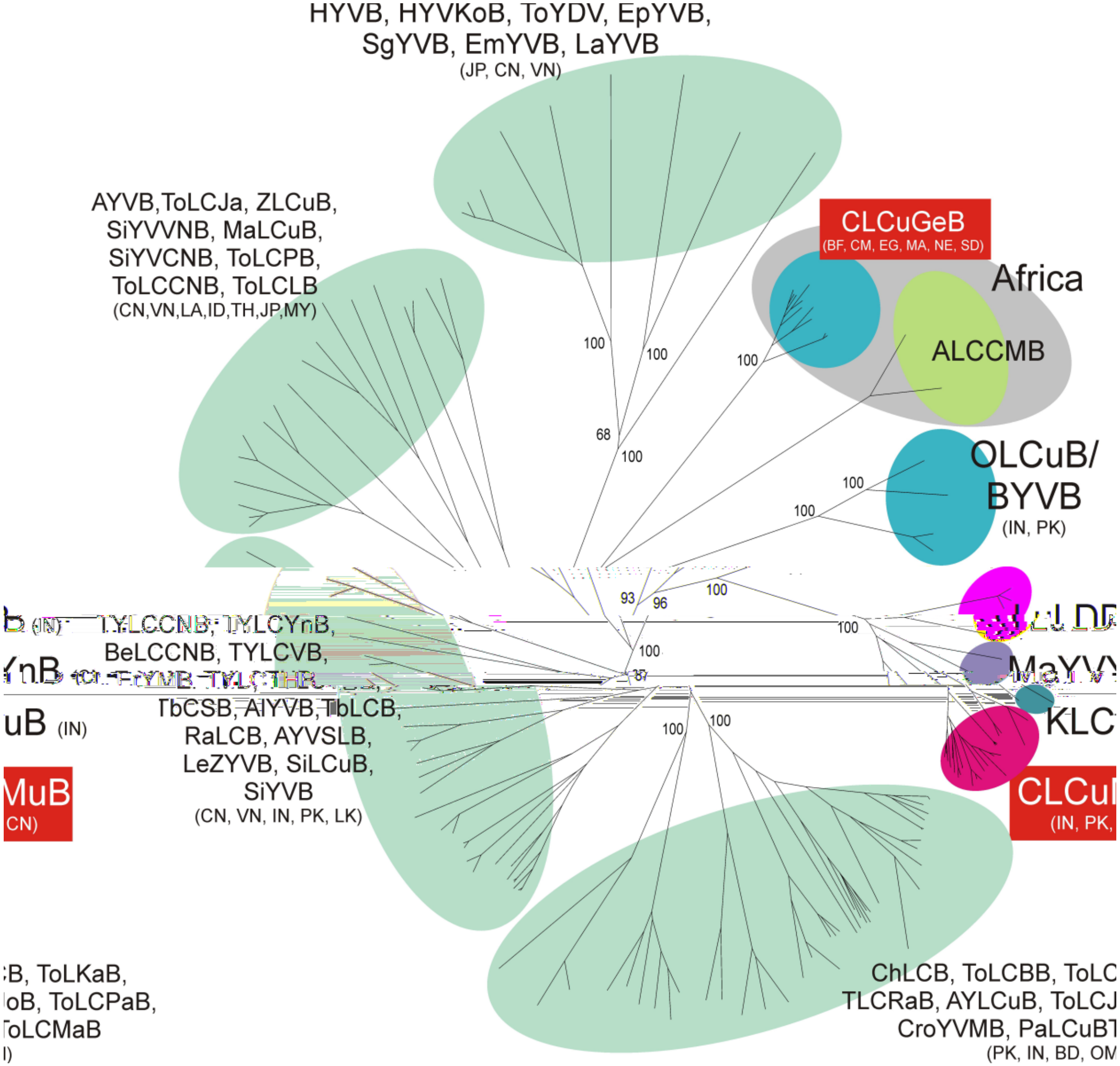
Thank you

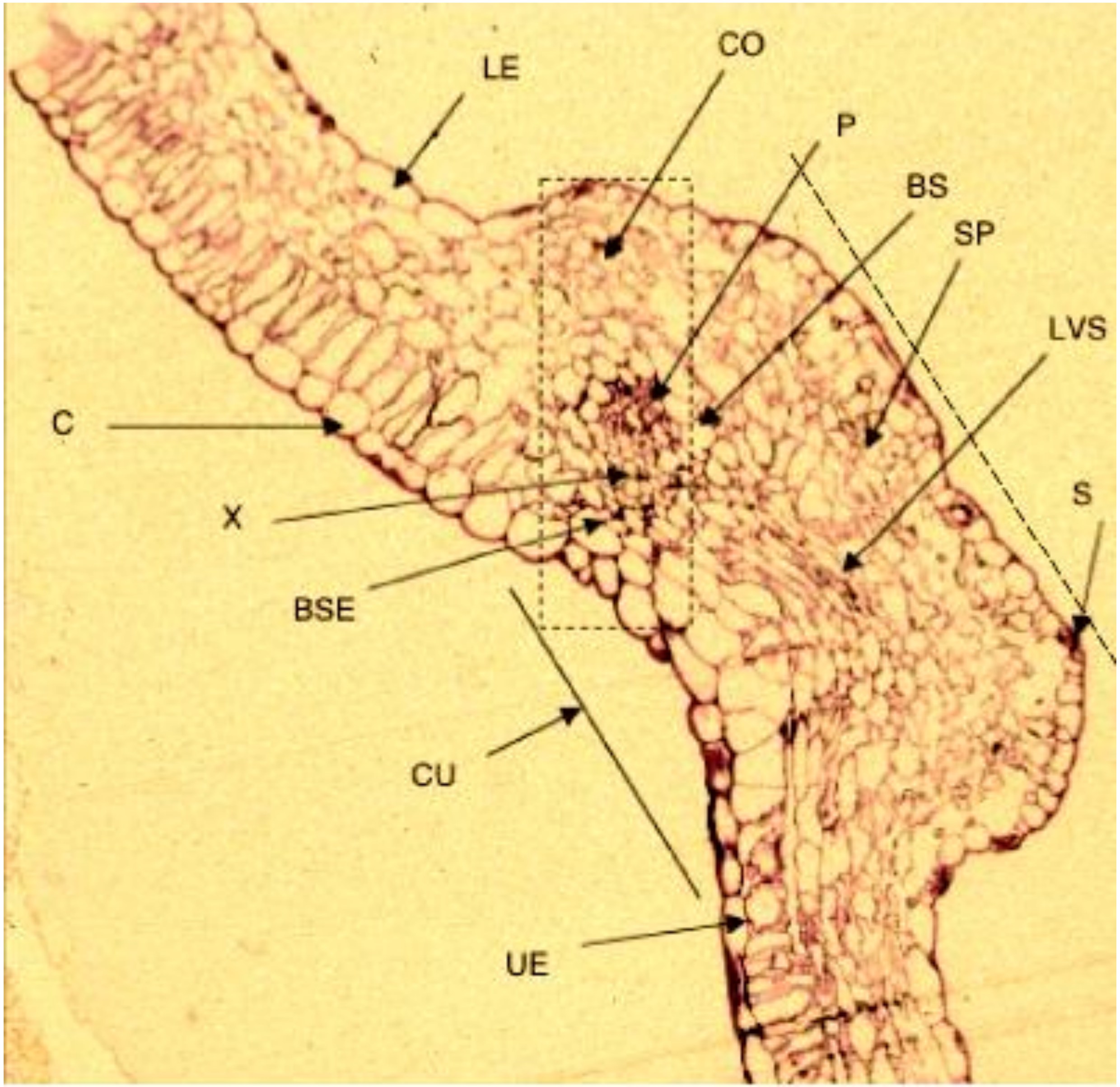
Phylogeny of OW begomoviruses infecting cotton, hollyhock, and okra



Alphasatellites



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.