

“Seminar Report”

International Seminar

on

**Current and Future Prospects of Whitefly and Cotton leaf curl
disease in Pakistan and China**

August, 16, 2018

An International seminar “Current and Future Prospects of Whitefly and Cotton leaf curl disease in Pakistan and China” was organized by the Department of Entomology and Plant Pathology, Muhammad Nawaz Shareef University of Agriculture, Multan on August 16, 2018. Dr. Sagheer Ahmad, Director Cotton Punjab was the chief guest on the occasion. The seminar was attended by scientists from MNSUAM, Cotton Research Institute, Multan; Central Cotton Research Institute, Multan and Bahauddin Zikariya University, Multan. Following talks were delivered by speakers in the seminar.

1. Prof. Dr. Shafqat Saeed and Dr. Hasan Riaz

“Whitefly and Cotton Leaf Curl Disease Complex in Pakistan”

2. Dr. Lihua Lyu

“Native cryptic species of *Bemisia tabaci* complex drive dramatic dissemination of CLCuMuV among host plants in China”

3. Dr. Shahid Mansoor

“Geminivirus complexes and whitefly in Pakistan and their control strategies”

The status of whitefly population dynamics was discussed by Prof. Dr. Shafqat Saeed, Dean, Faculty of Agriculture and Environmental Sciences, MNSUAM. He told the participants about the whitefly population surge in different months during the Cotton growing season. He told the audience about the whitefly cryptic species complex in Pakistan and their role in transmission of Cotton infecting begomoviruses. The second part of the presentation was delivered by Dr. Hasan Riaz, Assistant Professor, Department of Plant Pathology, MNSUAM. Dr. Riaz told the participants about the presence of different begomoviruses species and their associated betasatellites infecting Cotton in Pakistan. He further highlighted the likelihood of third epidemic of CLCuD. The next talk was



delivered by Dr. Lihua Lyu on “Native cryptic species of *Bemisia tabaci* complex drive



dramatic dissemination of CLCuMuV among host plants in China”. Dr. Lyu informed the audience about the presence of many whitefly cryptic species and their role in transmission of *Cotton leaf curl Multan virus* and *Cotton leaf curl Multan betasatellite*. The CLCuMV is infecting *Hibiscus* species in China and not

reported in Cotton till date whereas the Chinese scientists fear its a matter of time CLCuMV infects Cotton in China. The third speaker was the Director, NIBGE,

Faisalabad, Dr. Shahid Mansoor. Dr. Mansoor has a vast experience of studying begomoviruses disease complexes in Pakistan. He focused his talk on the *Tomato leaf curl New Dehli virus*, recently found to be infecting Cotton in Pakistan. He also shared the reports of ToLCuNDV trans-replicating the betasatellites associated with CLCuD. He informed the audience about the efforts being made by the plant virologists. The chief guest, Dr. Sagheer Ahmad, lauded the efforts of Pakistani and Chinese scientists and highlighted the need of more output-oriented research collaboration in future.



Whitefly and Cotton Leaf Curl Disease Complex in Pakistan



By

**Prof. Dr. Shafqat Saeed
and
Dr. Hasan Riaz**

Muhammad Nawaz Shareef University of Agriculture Multan

OUTLINE

- 🦟 Introduction
- 🦟 Current Status of Whitefly population
- 🦟 Whitefly Hosts
- 🦟 Whitefly species involved in CLCuD
- 🦟 Distribution of Whitefly species
- 🦟 Cotton leaf curl disease
- 🦟 CLCuD alternate hosts
- 🦟 Viral gene involved in symptoms induction
- 🦟 Current trend of CLCuD in Pakistan
- 🦟 Possible Solutions

INTRODUCTION

4th

production, 9.5% of global Cotton

3rd

consumption, 10% of global consumption

3rd

Yarn production, 9% of global yarn

2nd

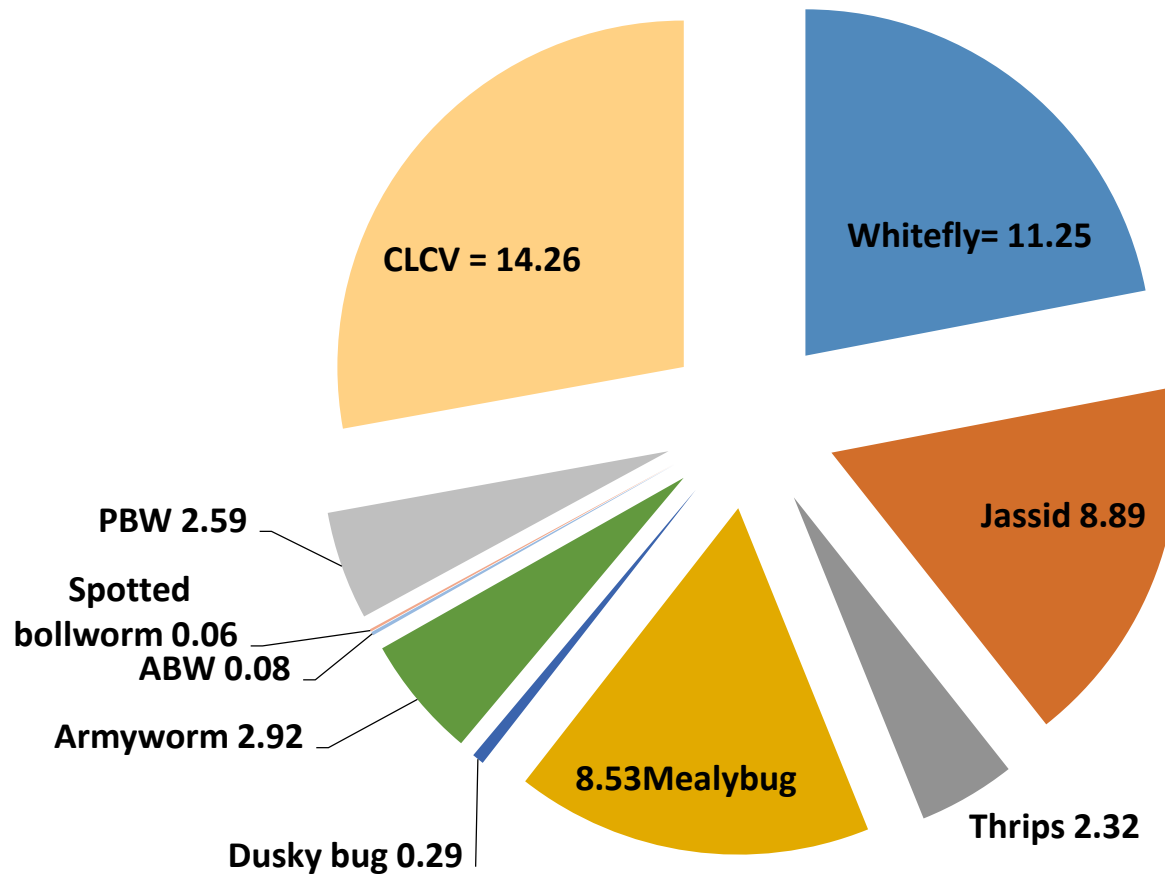
Yarn export, 25% of global produce

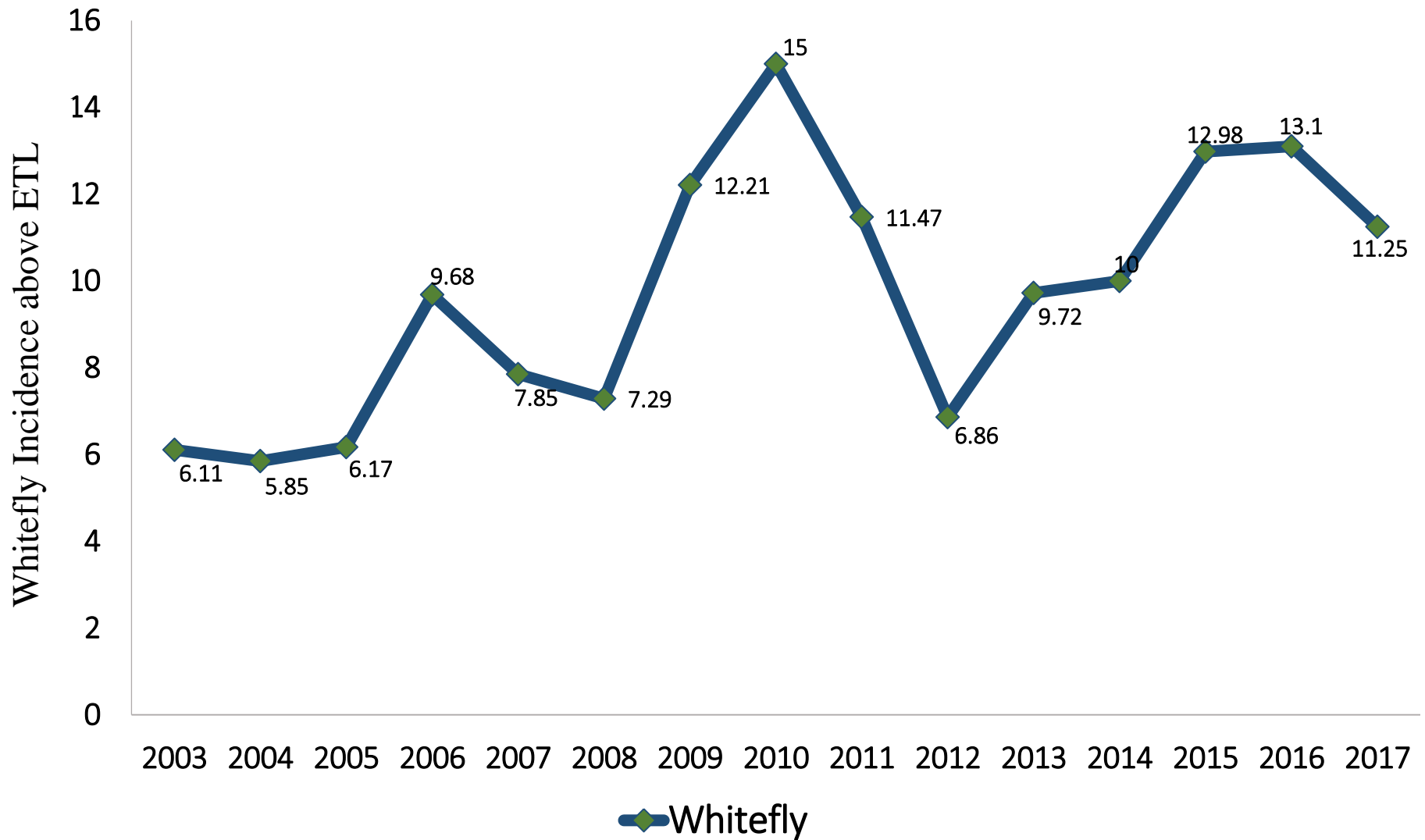
3rd

Cloth production, 7% of global gray cloth

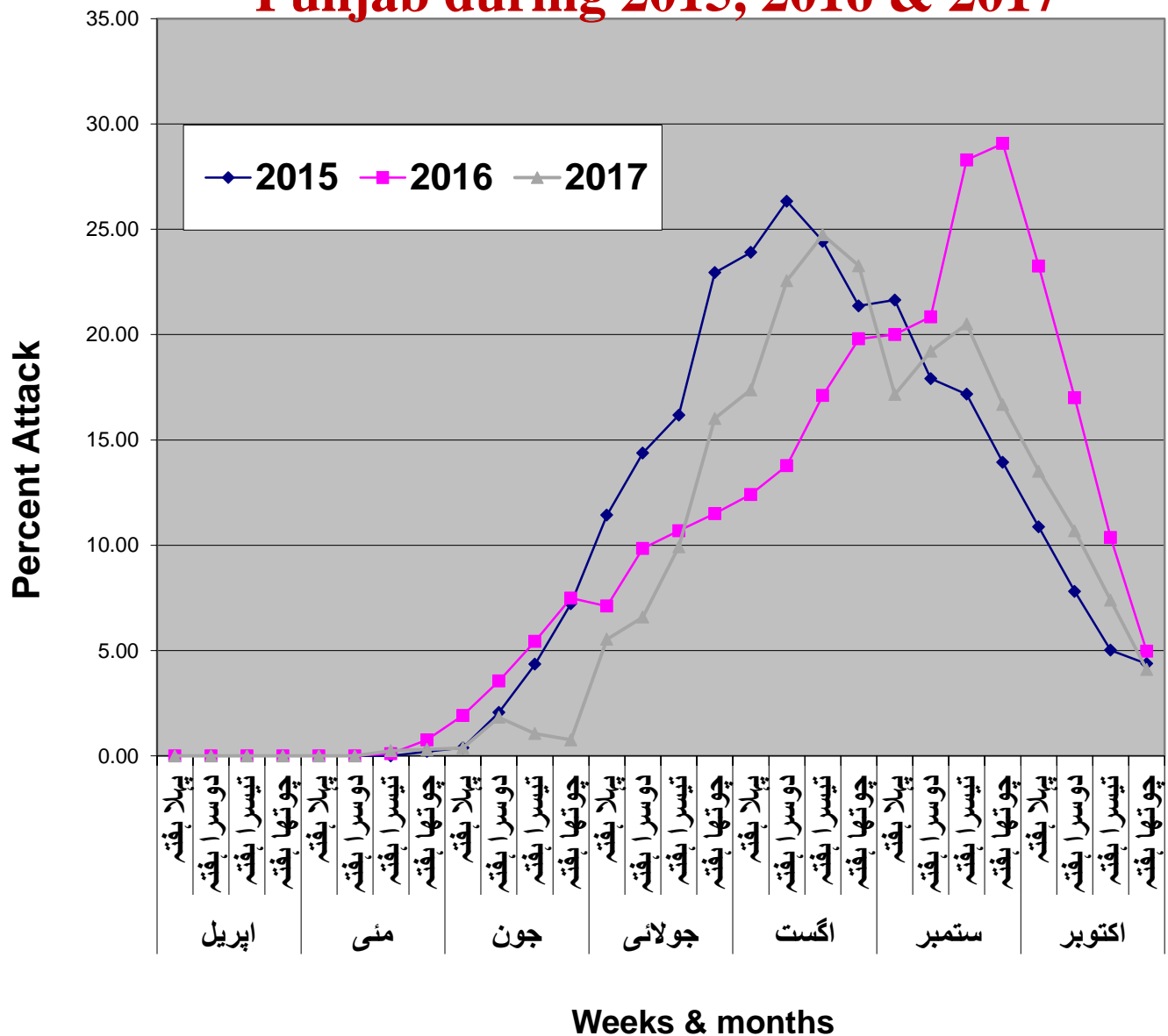
Situation of different insect pests on cotton above ETL during 2017

Total spots = 55429
Above ETL = 28372

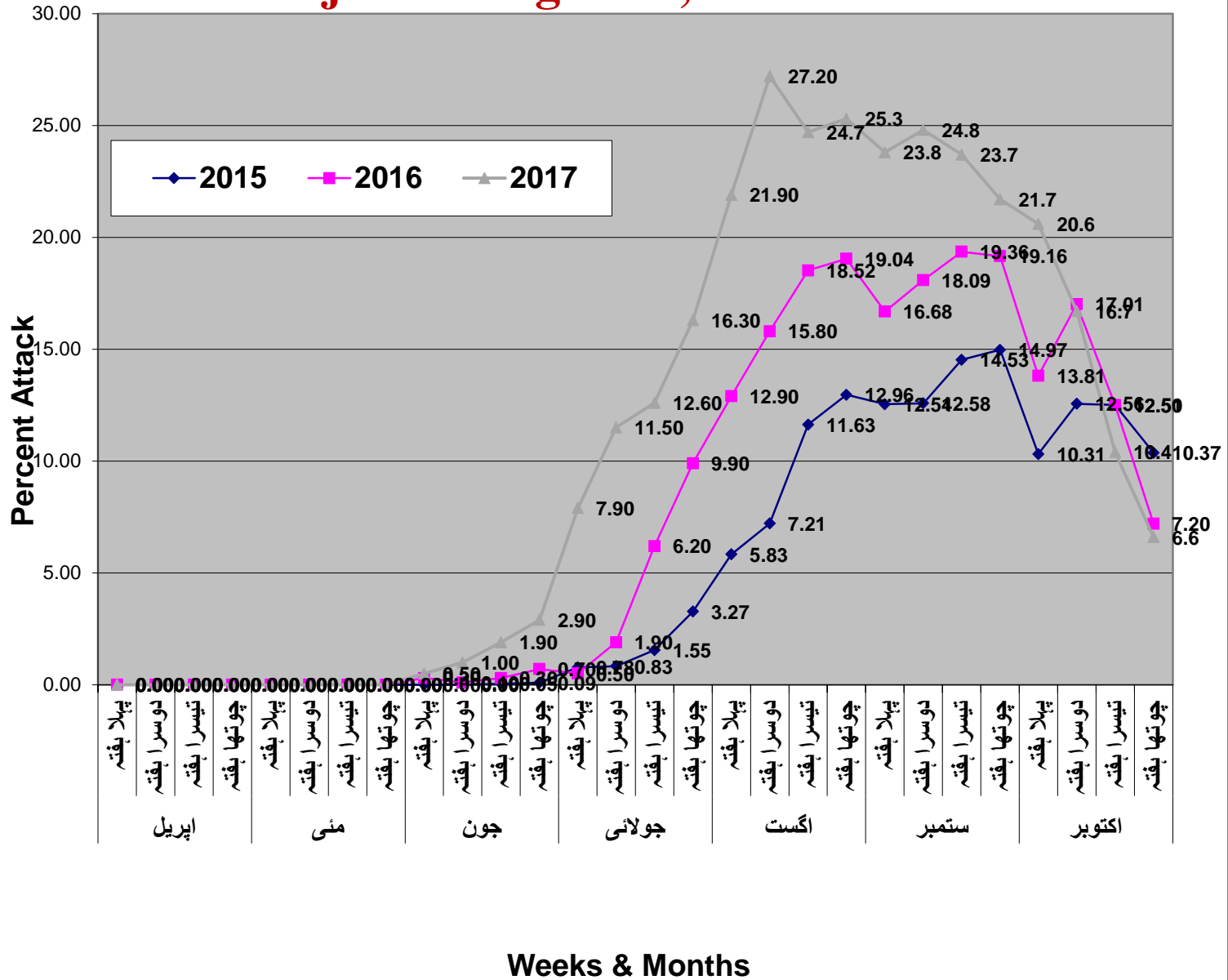




Week wise situation of Whitefly on cotton in Punjab during 2015, 2016 & 2017



Week wise situation of CLCV on cotton in Punjab during 2015, 2016 & 2017

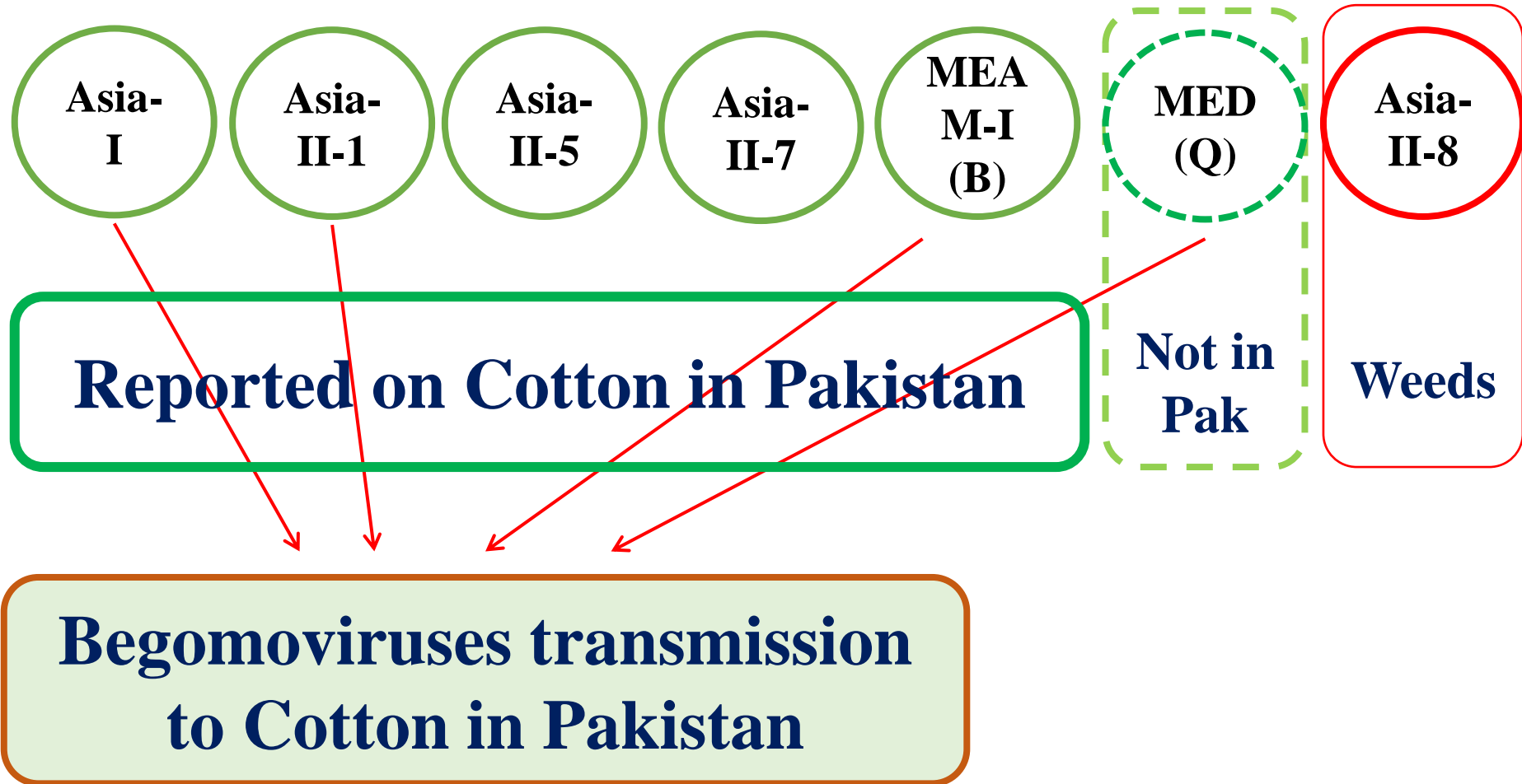


Whitefly Hosts in Pakistan

- 229 alternative hosts plants identified in Pakistan
- 361 alternative hosts plants recorded from 89 families in China
- *Compositae*, *Cruciferae*, *Cucurbitaceae*, *Solanaceae* and *Leguminosae* are favourable

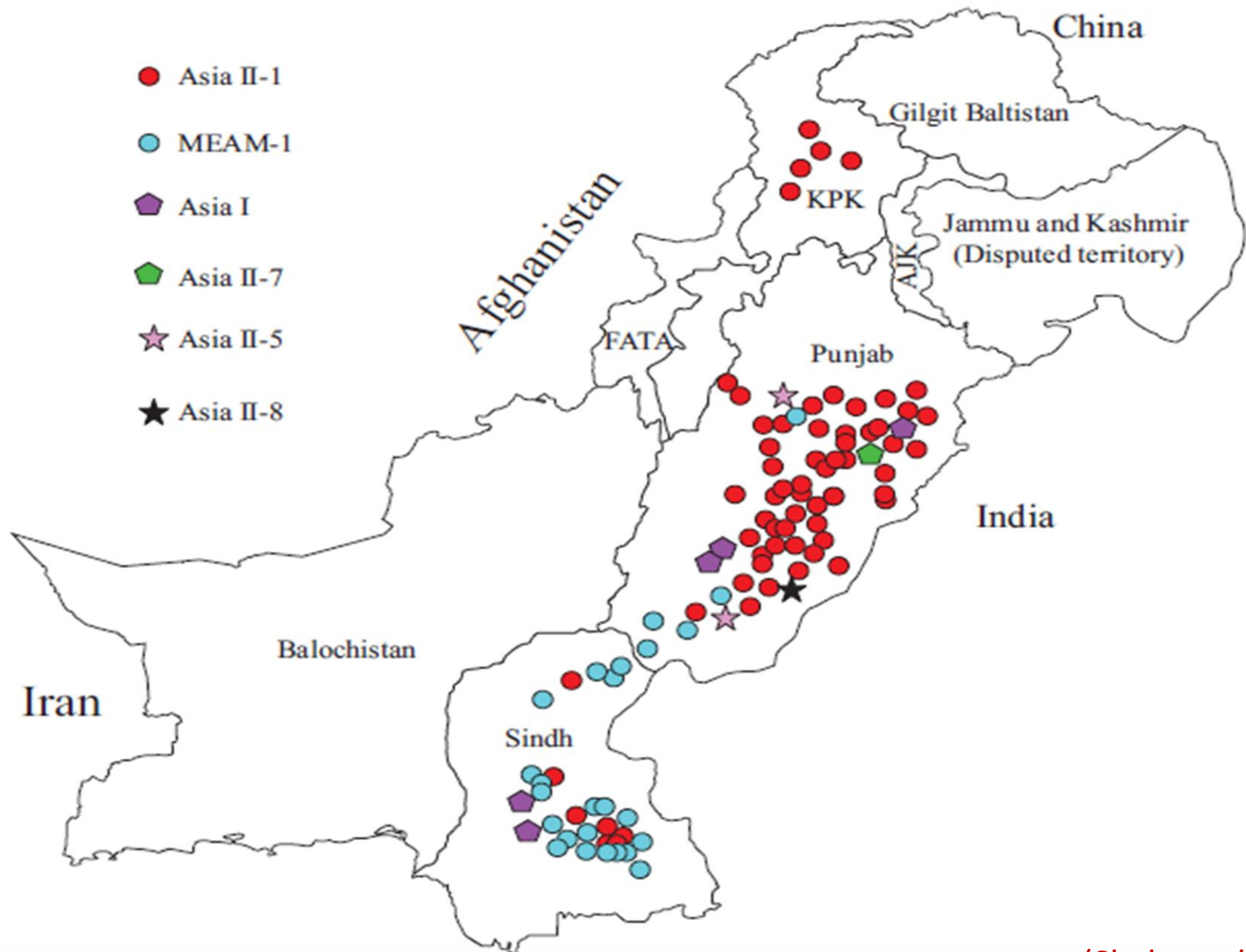
(Attique *et al.*, 2003; Jian Li *et al.*, 2011;
Pest warning department Punjab, 2017)

Whitefly Species in Pakistan



(Baoli *et al.*, 2008; Srinivasan *et al.*, 2008; Shah *et al.*, 2013; Masood *et al.*, 2017; Islam *et al.*, 2018, Li-long *et al.*, 2018)

Distribution of Whitefly Biotypes in Pakistan



Cotton Leaf Curl Disease Complex in Pakistan

by

Dr. Hasan Riaz
Assistant Professor
(Plant Pathology)

INTRODUCTION

1967

Reported in Tiba Sultan Pur, Multan

1989

First epidemic, Multan Strain

12.8 to 8.04 million bales

Estimated losses US\$ 5 billion

2001

Second Epidemic, Burewala Strain

US\$ 87 million per annum

Annual 10% losses reported

(Briddon and Markham, 2000; Mahmood *et al.* 2003; Mansoor *et al.* 2006, Farooq *et al.* 2011)

CLCuD Complex

**Monopartite
Begomoviruses**

**Bipartite
Begomoviruses**

DNA-A

**DNA
 β**

**DNA
 α**

DNA-A

DNA-B

Monopartite Begomoviruses

1. *Cotton leaf curl Multan virus*
 2. *Cotton leaf curl Kokhran virus*
 3. *Cotton leaf curl Gezira virus*
 4. *Cotton leaf curl Bangalore virus*
 5. *Cotton leaf curl Alabad virus*
 6. *Okra leaf enation virus*
 7. *Papaya leaf curl virus*
 8. *Tomato leaf curl Bangalore virus*
 9. *Cotton leaf curl Barasat virus*
- *CLCu Multan Betasatellite*
 - *CLCu Gezira Betasatellite*
 - *PaLCu Betasatellite*



DNA-A



DNA
β

Bipartite Begomoviruses

1. *Tomato leaf curl New Dehli virus*
2. *Cotton leaf crumple virus*
3. *Cotton chlorotic spot virus*
4. *Cotton yellow mosaic virus*



DNA-A



DNA-B

Alternate Hosts of Begomoviruses

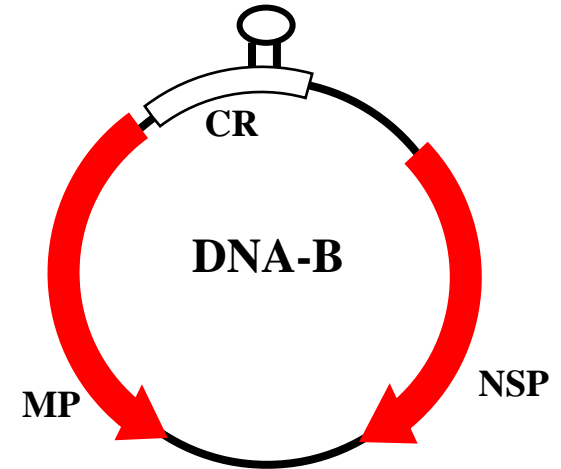
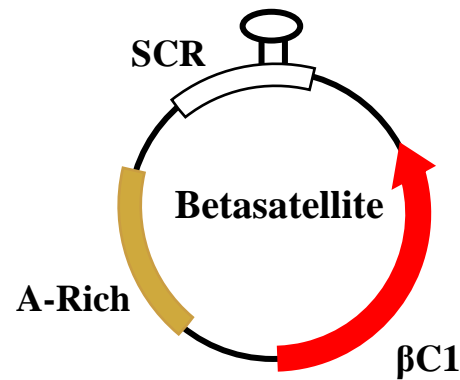
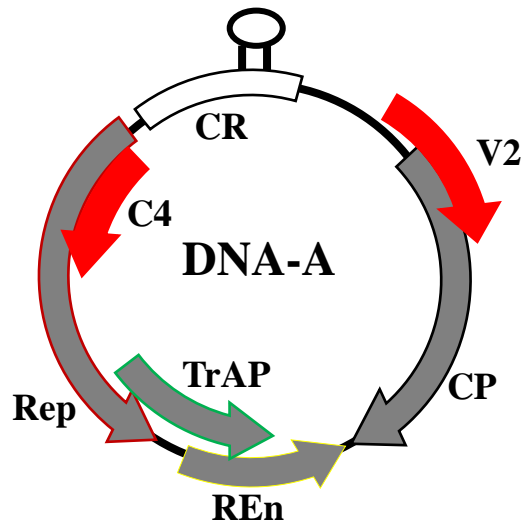
1. Okra
2. Hollyhock
3. *Hibiscus rosa-sinensis*
4. Tomato
5. Papaya

DNA +
DNA β

1. Chilli
2. *Malvastrum coromandelianum*
3. *Hibiscus rosa-sinensis*
4. Cucurbits

CLCuMB

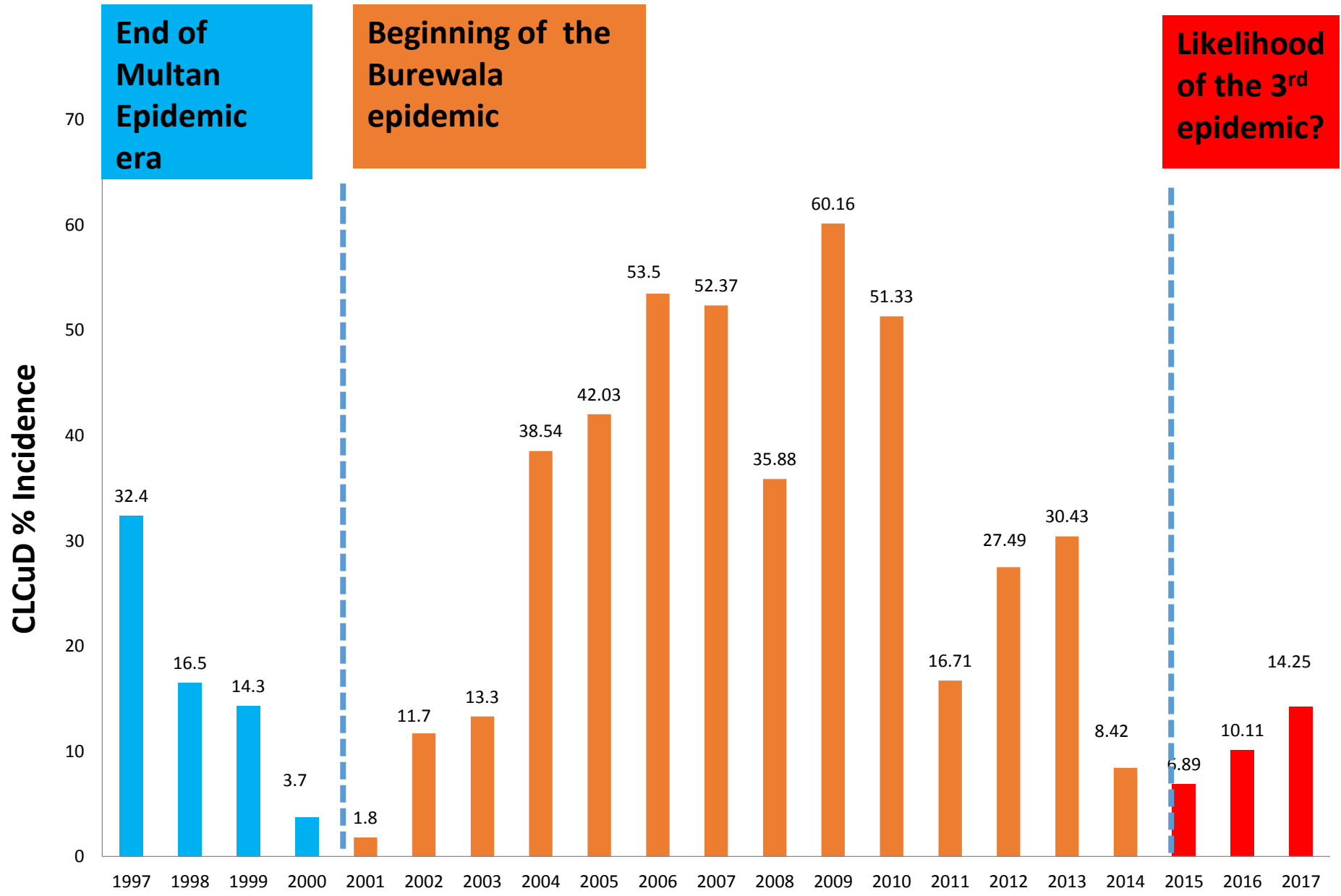
Viral Genes involved in Symptom Induction



**C4
&
V2**

β C1

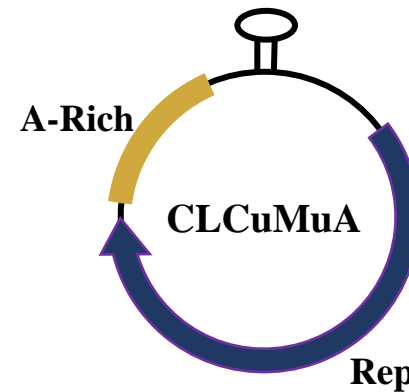
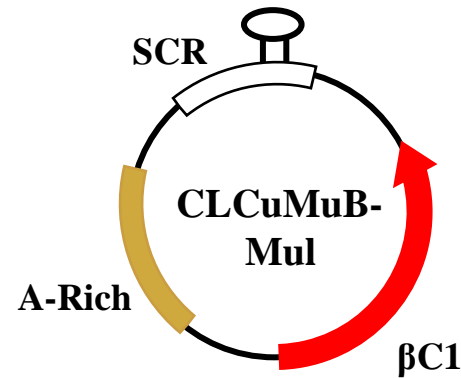
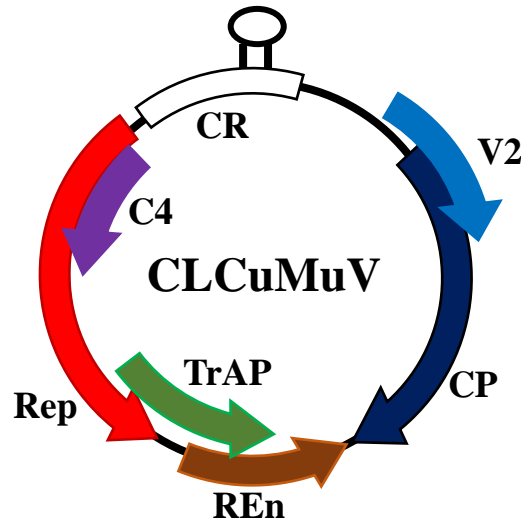
**MP
&
NSP**



(Sattar *et al.*, 2017)

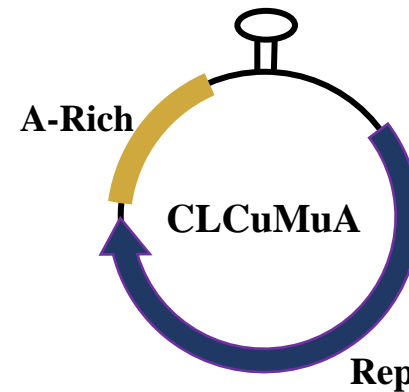
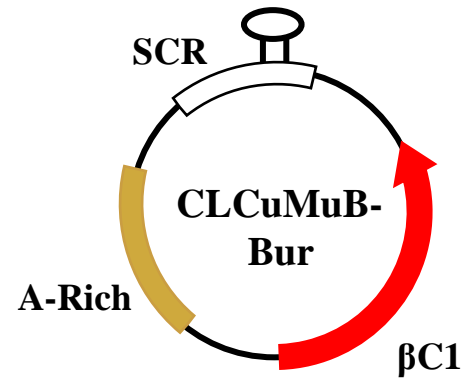
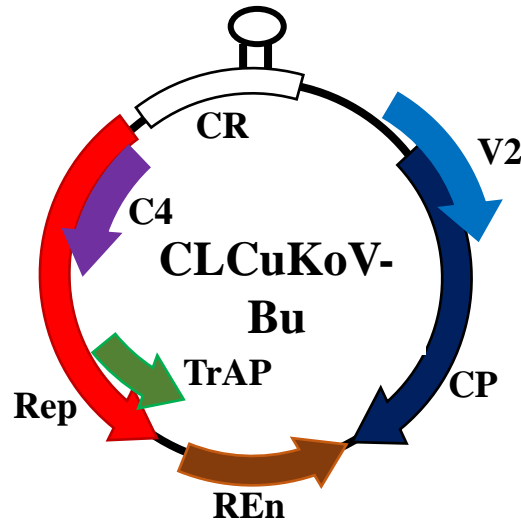
Components of Multan Epidemic

Monopartite Begomovirus Complex



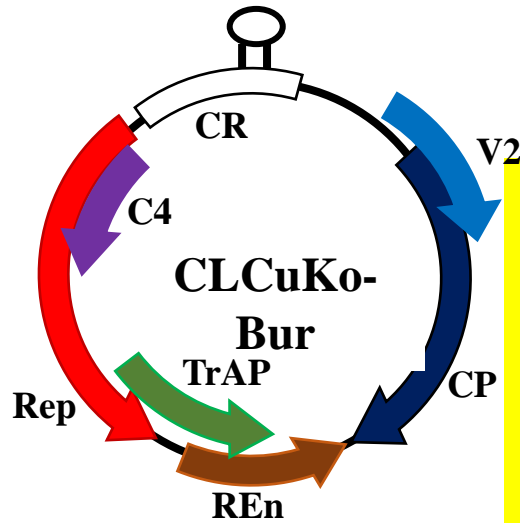
Components of Burewala Epidemic

Monopartite Begomovirus Complex

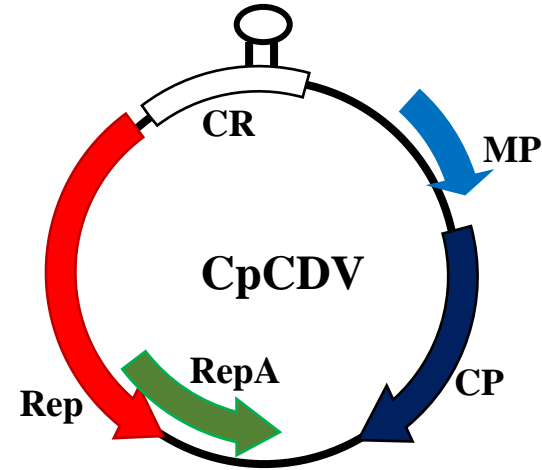
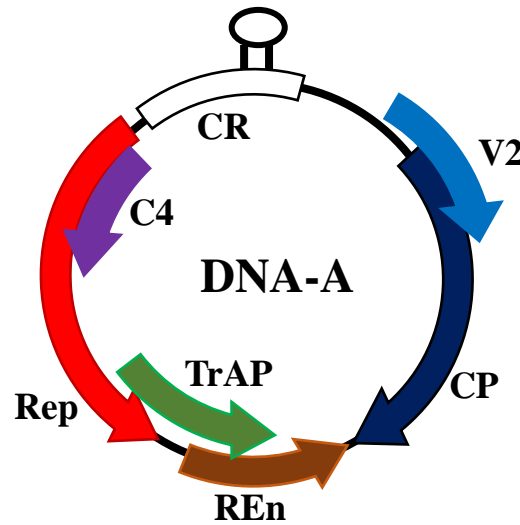


Conceivable Components of Third Epidemic

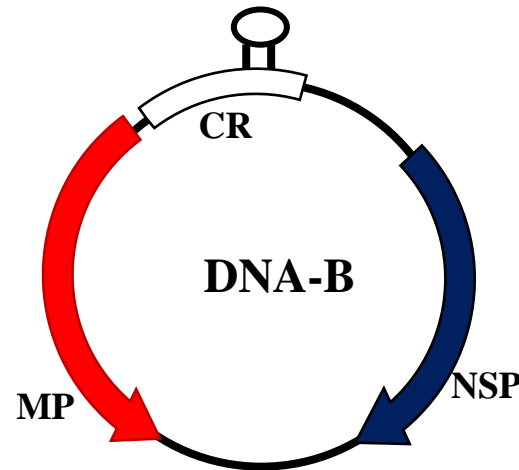
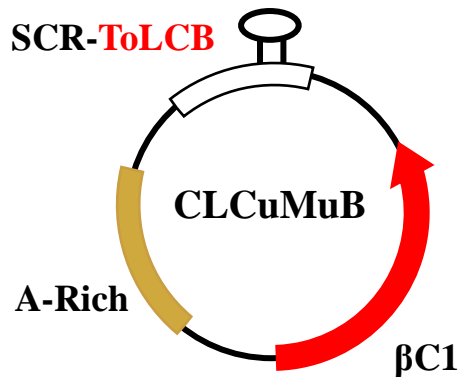
Monopartite Begomovirus



Bipartite Begomovirus



Mastrevirus



Possible Solutions

- 🦟 Development of Resistant Varieties
- 🦟 Effective management of Whitefly
- 🦟 Silencing and genome editing approaches to target viral symptom inducing genes

Thank you



广东省农业科学院
Guangdong Academy of Agricultural Sciences

Native cryptic species of *Bemisia tabaci* complex drive dramatic dissemination of CLCuMuV among host plants in China

Lihua, LYU PhD

Laboratory of Invasive Pests,
Plant Protection Research Institute, Guangdong
Academy of Agricultural Sciences



16 August, 2018 Multan, Pakistan

Cotton leaf curl disease and its threat on cotton in South Asia

Cotton Leaf Curl Disease

Symptoms: Curling of leaves, thickening and darkening of veins

Distribution: Pakistan, India, Sudan, Egypt, Nigeria, Malawi, South Africa and **China (2006)**.

Damage: Pakistan and India suffered the most, with losses of up to \$5 billion in Pakistan between 1992 and 1997.



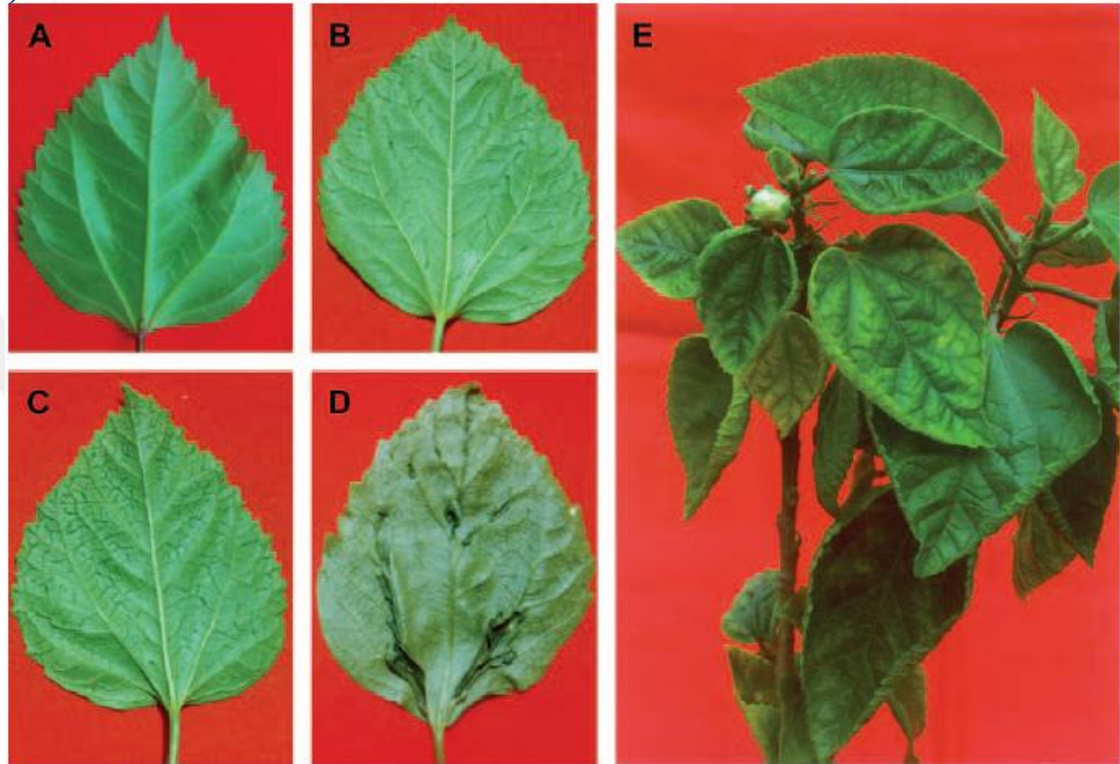
Infected Cottons showing regular symptom in Multan, Pakistan

Incidence of Begomovirus infecting *Hibiscus* recorded in India at first

Hibiscus Leaf Curl Disease (HLCuD)

Reports in India First reported in the northern states of India in the 1950s (Anon., 1950; Vasudeva et al., 1953), later in the central areas (Mali, 1980), and then in Southern areas (Ramanan, et al., 2005)

Typical symptoms vein thickening, upward curling of leaves and enations on the abaxial leaf surface, reduction in leaf size and stunting).



HLCuD incidence near Bhubaneswar, North India, and in **Bangalore** area, South India, in horticultural nurseries/gardens with disease incidences **2- 17%**.

(Ramanan, et al., 2005)

Progressive symptom types produced by cotton leaf curl Multan virus-Hibiscus [Bangalore] (CLCuMV-Hib[Ban]).

- (A) Abaxial surface of a virus-free hibiscus leaf
- (B, C) CLCuMV-Hib [Ban]-infected leaves showing gradual vein thickening and darkening of green veins
- (D) enations
- (E) twig from an infected plant with leaves exhibiting curling, yellowing and vein thickening at the upturned edges

CLCuMuV, a pathogen causing HLCuD in India of South Asia

Phylogenetic analyses of these DNA-A sequences clustered them with

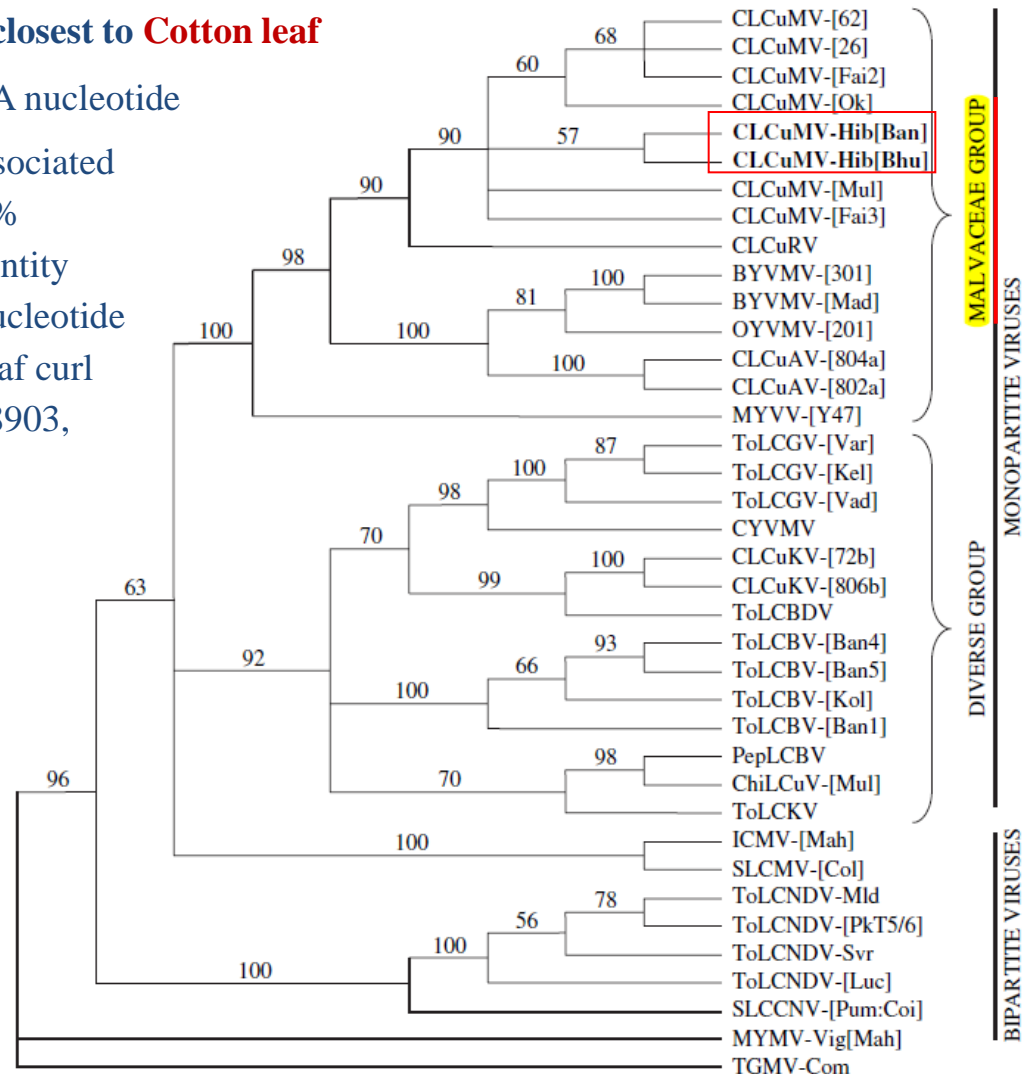
Old World cotton- infecting begomoviruses and closest to Cotton leaf

curl Multan virus (CLCuMuV) at 95–97% DNA-A nucleotide

identity. The 682-nucleotide satellite DNA molecules associated with the HLCuD samples Ban and Bhu shared 96.9% sequence identity with each other and maximum identity (93.1–93.9% over positions 158–682) with ;1350-nucleotide DNA-b satellite molecules associated with cotton leaf curl disease in Pakistan and India (accession Nos: AJ298903, AJ316038).

Phylogenetic tree showing predicted relationship of 1288-basesequences of cotton leaf curl Multan virus-Hibiscus [Bangalore] and CLCuMVHib [Bhu] to equivalent begomovirus DNA-A sequences in GenBank.

The sequences were generated by PCR using degenerate primers (Rojas et al., 1993), and the 1288 bases represent partial CP and AC1 genes and full AC2 and AC3 genes. Numbers at nodes represent the bootstrap percentage score out of 1000 replicates.



(Ramanan, et al., 2005)

HLCuV transmitted by *Bemisia tabaci* in India

CLCuMV transmission

CLCuMV-Hib [Ban] was transmitted by *B. tabaci* to *HIBISCUS* var. Geethanjali, and symptoms appeared about 2–4 weeks after virus inoculation.

Of the 16 plant species inoculated with CLCuMV-Hib [Ban] by *B. tabaci*, **10 species (+)** developed symptoms.

Symptoms in **COTTON** were similar to those in **Hibiscus** and included characteristic symptoms of dark green veins, vein thickening, curling and enations.

AN INDIGEOUS INDIAN BIOTYPE
of *Bemisia tabaci* complex, transmit CLCuMV.

Which one?

NO ANSWER.

Table 1 Plant species inoculated with Cotton leaf curl Multan virus-Hibiscus[Bangalore] using *Bemisia tabaci*

Host	Family	No. of Plants Infected/ Inoculated (%) ^a	Days Post- inoculation ^b	Virus Detection ^c		
				PCR	ELISA	Symptoms
Cultivated plants						
<i>Abelmoschus esculentus</i> Moench	Malvaceae	0/15		–	–	NS
<i>Althea rosea</i> Cav.	Malvaceae	0/9		–	–	NS
<i>Gossypium barbadense</i> L. +	Malvaceae	6/13 (46.1)	18–20	+	+	LC, VT, En
<i>Hibiscus cannabinus</i> L.	Malvaceae	0/25		–	–	NS
<i>Hibiscus rosa-sinensis</i> L. +	Malvaceae					
var. Geethanjali		15/18 (83.3)	15–17	+	+	LC, VT, En
var. Viceroy +		6/15 (40.0)	24–28	+	+	LC, VT, En
var. White Daffodil		0/17		–	–	NS
var. Tall FB		0/13		–	–	NS
var. H600		0/10		–	–	NS
var. Cooperi		2/13 (15.3)	25–28	+	+	VT, En
var. Red Flake +		5/20 (25.0)	24–30	+	+	VT, En
<i>Hibiscus sabdariffa</i> L.	Malvaceae	0/20		–	–	NS
<i>Hibiscus schizopetalus</i> Hook. f.	Malvaceae	0/15		–	–	NS
<i>Lycopersicon esculentum</i> Mill. +	Solanaceae	5/20 (25.0)	20–25	+	+	ULC
<i>Nicotiana tabacum</i> L. +	Solanaceae					
var. Samsun		8/11 (72.7)	15–19	+	+	LC, VCT
var. Xanthi		12/18 (66.6)	14–15	+	+	LC, VC
Uncultivated plants						
<i>Ageratum conyzoides</i> L. +	Asteraceae	7/18 (38.8)	17–19	+	+	LC, VT
<i>Croton bonplandianum</i> Baill. +	Euphorbiaceae	6/15 (40.0)	18–21	+	+	LC, DGVT
<i>Euphorbia geniculata</i> Orteg.	Euphorbiaceae	2/15 (13.3)	21	+	+	LC, VT
<i>Malvastrum coromandelianum</i> (L.) Garcke	Malvaceae	0/15		–	–	NS
<i>Nicotiana benthamiana</i> Domin. +	Solanaceae	1/15 (6.6)	27	+	+	LC, VC
<i>Nicotiana glutinosa</i> L. +	Solanaceae	12/15 (80.0)	17–20	+	+	LC, VCT, En
<i>Parthenium hysterophorus</i> L. +	Asteraceae	10/14 (71.4)	14–20	+	+	LC, VT

NS, no symptoms; LC, leaf curl; VT, vein thickening; En, enations; ULC, upward leaf curling; VCT, vein clearing and thickening; VC, vein clearing; DGVT, dark green veins and thickening; ELISA, enzyme-linked immunosorbent assay.

^aGroups of 25–30 viruliferous *B. tabaci* inoculated to each plant and given a 24-h acquisition access period and 48-h inoculation access period.

^bDays from inoculation to the first and last plant expressing symptoms.

^c+ indicates hibiscus leaf curl disease diagnoses by PCR using Deng *et al.* (1994) primers and by double-antibody sandwich-ELISA and triple-antibody sandwich (TAS)-ELISA; – indicates no virus detection by TAS-ELISA.

Putative species groups & cryptic species across world

Putative species groups and cryptic species of the whitefly *B. tabaci* complex

Species group	Cryptic species	Biotypes (haplotypes) designations associated	Reference	
Africa/Middle East/Asia Minor	Mediterranean	Q, J, L, Sub-Saharan Silverleafing	Dinsdale et al. (2010)	
	Middle East-Asia Minor 1	B, B2		
	Middle East-Asia Minor 2			
	Indian Ocean	MS		
New World	New World	A, BR, C, D, F, Jatropha, N, R, Sida	Dinsdale et al. (2010)	
	New World 2		Dinsdale et al. (2012)	
Uganda	Uganda		Dinsdale et al. (2010)	
Sub-Saharan Africa	Sub-Saharan Africa 1		Dinsdale et al. (2010)	
	Sub-Saharan Africa 2	S		
	Sub-Saharan Africa 3			
	Sub-Saharan Africa 4			
Italy	Italy	T	Dinsdale et al. (2010)	
Ru	Ru		Parrella et al.(2012)	
Asia II -India	Asia II 8		Dinsdale et al. (2010)	
Asia II	Asia II 1	K, P, PCG-1, PK1, SY, ZHJ2	Dinsdale et al. (2010)	
	Asia II 2			
	Asia II 3	ZHJ1		
	Asia II 4			
	Asia II 5	G		
	Asia II 6			
	Asia II 7	Cv		
	Asia II 9			Hu et al. (2011)
	Asia II 10			
	Asia III	Asia III		
China	China1	ZHJ3	Dinsdale et al. (2010)	
	China2			
	China3			Hu et al. (2011)
Australia	Australia	AN	Dinsdale et al. (2010)	
Australia /Indonesia	Australia /Indonesia		Dinsdale et al. (2010)	
Asia I	Asia I	H, M, NA, PCG-2	Dinsdale et al. (2010)	
	Asia I -India		Chowda-Reddy et al. (2012)	

31 Cryptic species

34 Biotypes

(Liu YQ and
Liu SS , 2011)

Dominant cryptic species of *Bemisia tabaci* complex in South Asia's India

Distribution and composition

Invasive cryptic species (*LOC – location)
MEAM1 2 LOCs* (Bangalore and Kolar)

Indigenous cryptic species
Asia I 28 LOCs (the most wide)
Asia II-1 20 LOCs
 Asia II-8 6 LOCs
 Asia II-7 2 LOCs
 Asia II-5 5 LOCs
 China-3 1 LOC
 Asia I-India 1 LOC

Cryptic species in HLCuD occurring area

Bhubaneswar, North India
 No data

Bangalore, South India
 Asia I, **Asia II 1, Asia II 11, Asia II 7,**
Asia II 8

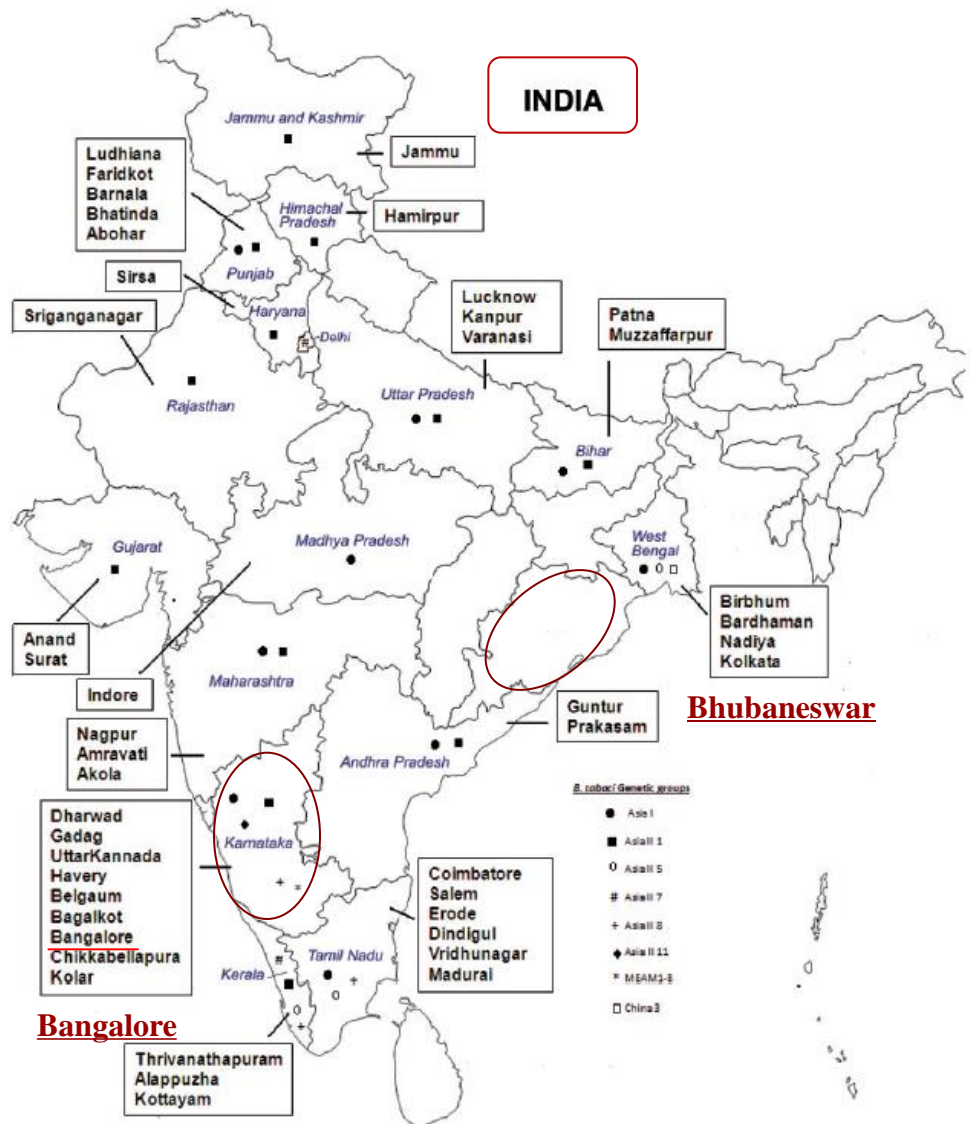


Fig. 1. Collection locations across India surveyed for *B. tabaci*.

Dominant cryptic species of *Bemisia tabaci* complex in South Asia's Pakistan

Distribution and composition of whitefly complex

- Invasive cryptic species** (*LOC – location)
MEAM1 (▲)
- Indigenous cryptic species**
Asia II 1 (the most wide) (●)
Asia II-5
Asia II-7 (⊕)
Asia II-8

Islam et al.

Acta Tropica 183 (2018) 119-

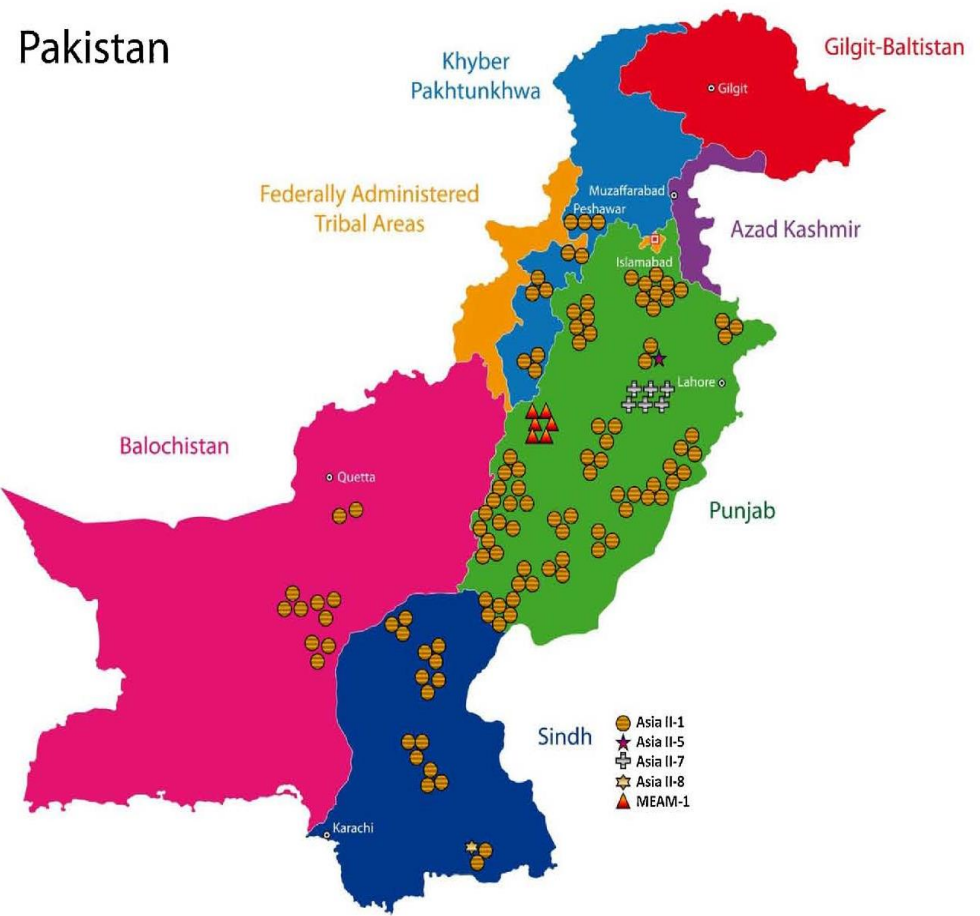


Fig. 2. Map of Pakistan showing distribution of various cryptic species.

(Islam et al., 2018)

Some facts and Question:

FACTS :

- **NO** evidence to show MEAM1 (Invasive) transmissible for CLCuMuV.
- **SOME INDIGENOUS** species are able to transmit CLCuMuV in **India**.

QUESTION:

– **WHICH ONE or MORE** cryptic species of *Bemisia tabaci* complex is /are capable to transmit CLCuMuV among Malvaviscus plants, especially cotton plants in **South Asia** ?

➤ **ANY** reports in Pakistan ?

Chronicle of Hibiscus leaf Curl Disease occurred naturally in China

- **2006** CLCuMuV infecting *Hibiscus rosa-sinensis* plants only in **Guangdong** (Mao, et al., 2008)
- **2008** Symptomatic *H. rosa-sinensis* in Nanning, **Guangxi** in 2008 (Cai, et al., 2010)
- **2008-2010** incidences often found in **GD and GX** (Tang, et al., 2013, 2015)
- **As of 2010** the disease on *H. rosa-sinensis* recorded in **Hainan, Fujian, Yunnan, and Jiangsu.**

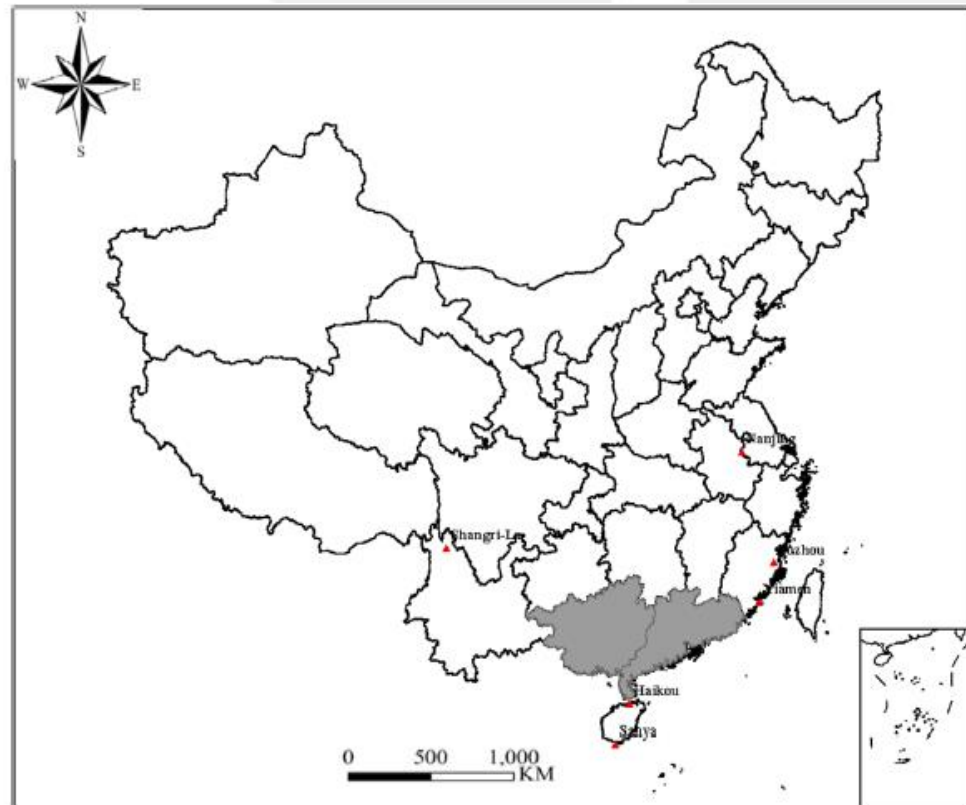


Fig. 1 Distribution of Cotton leaf curl Multan virus (CLCuMuV) and its associated betasatellite (CLCuMuB) in China. Guangdong and Guangxi, the two provinces in which CLCuMuV and CLCuMuB has been endemic are shaded. For other provinces, red triangles were used to indicate regions that have been surveyed and CLCuMuV and CLCuMuB have been detected

Natural plant host range of CLCuMD in South China

CLCuMuV invasion process in China



● **Upland cotton**
Gossypium hirsutum

● **Okra** *Abelmoschus esculentus*

● **Kenaf** *Hibiscus cannabinus*

● **Chinese rose**
Hibiscus rosa-sinensis

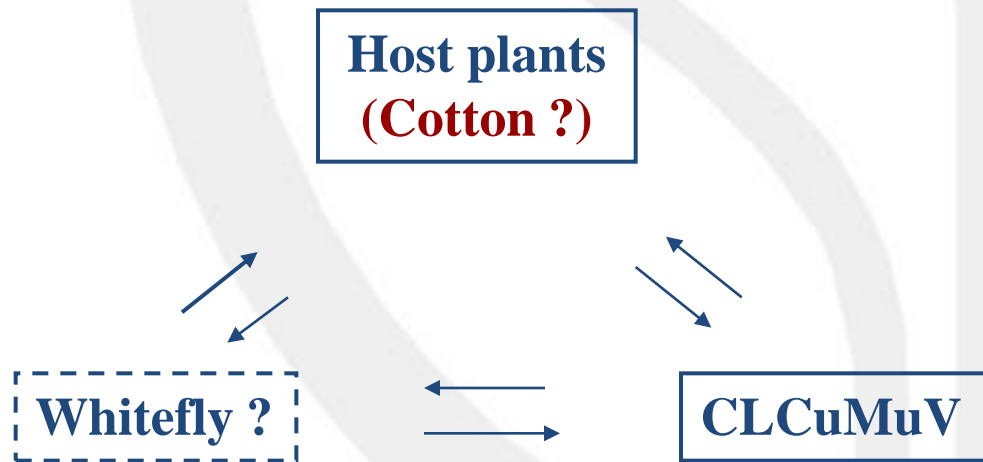


● **Pending Hibiscus**
Malvavirus arboreus



Question One:

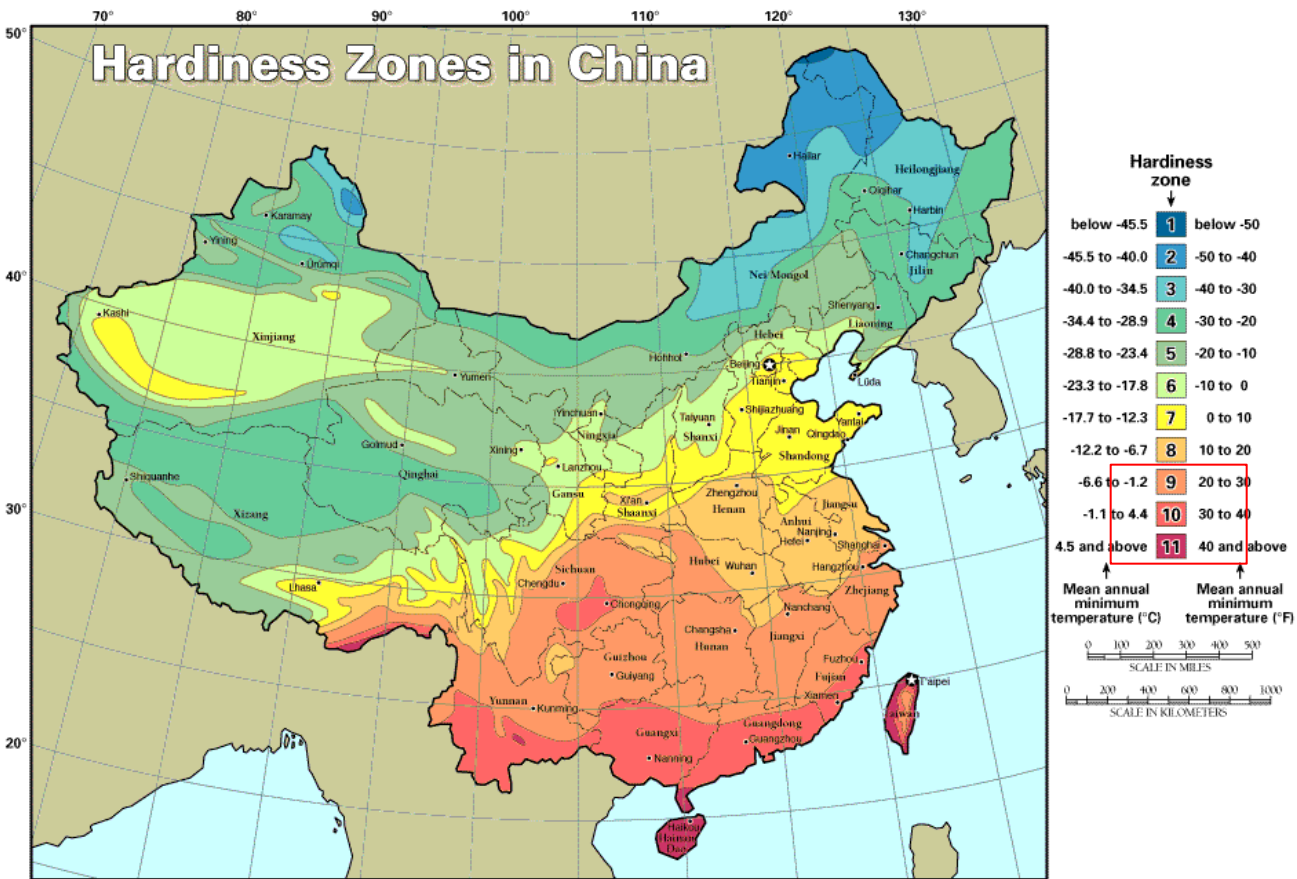
- **DOES CLCuMV pose any threat to COTTON production in China ?**
- Which cryptic species of *Bemisia tabaci* can transmit CLCuMuV to host plants ?



Distribution of *Hibiscus rosa-sinensis* L. in China

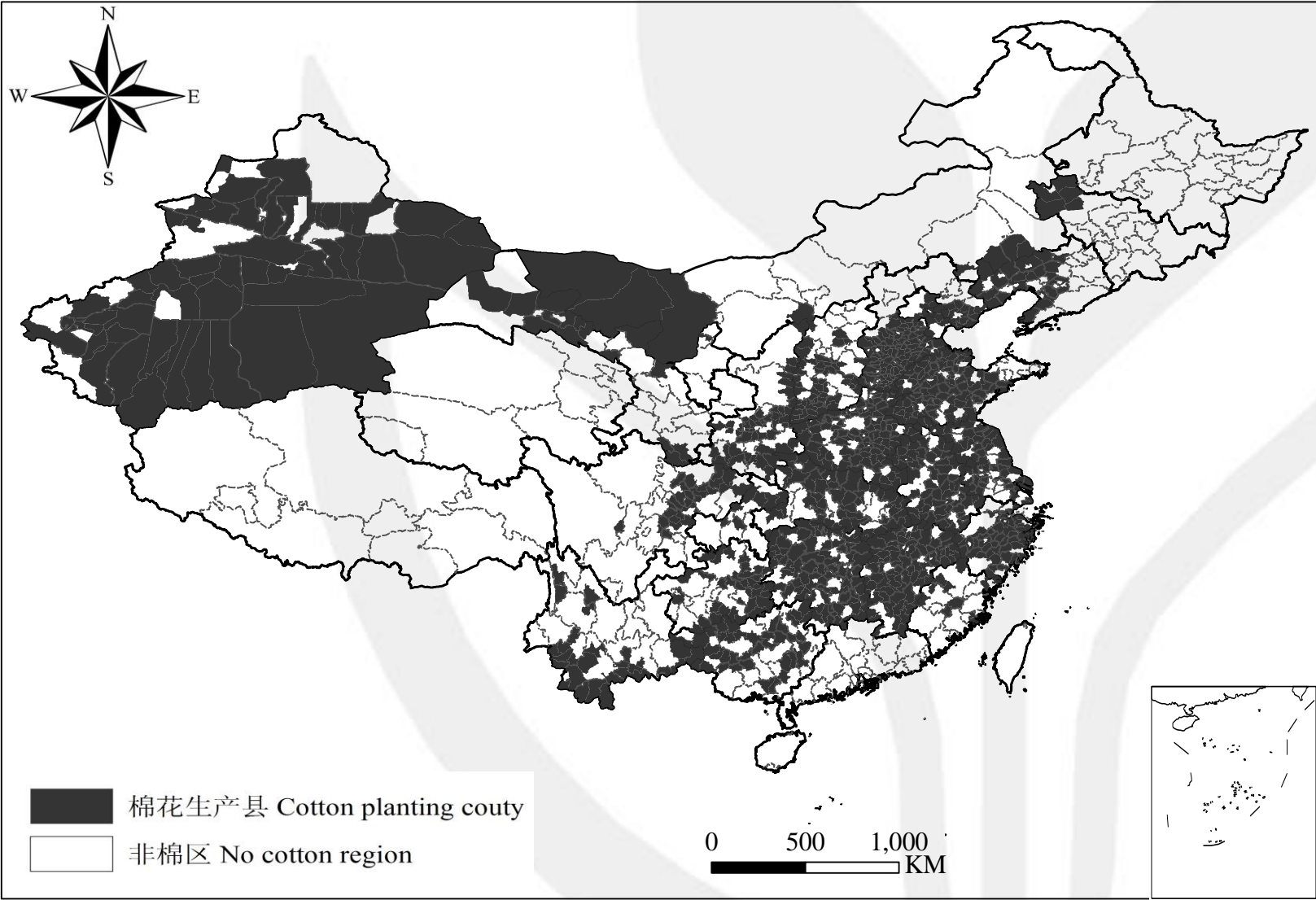
Hibiscus or China rose

- Cultivated widely as an ornamental flowering plant in tropical and subtropical regions (Taiwan, Fujian, Guangdong, Guangxi, Yunnan, and Sichuan).
- Popular particularly in Chinese landscape gardens from ancient times and along the roadside at present due to respect or its aesthetic value.

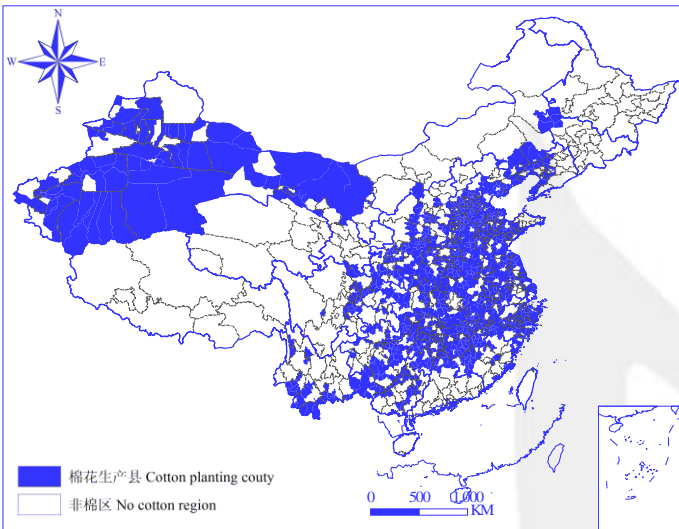


Based on *Hibiscus* plant hardiness rating, *Hibiscus rosa-sinensis* is grown outdoors in warm climates such as those found in zones 9 through 11, which cover a large swath of the Southern and Eastern China.

Distribution of cotton, a major cash crop, in China



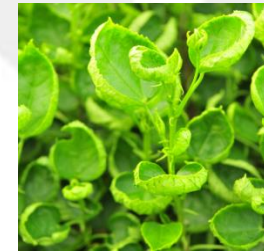
Any threat of CLCuMuV to cotton production in China ?



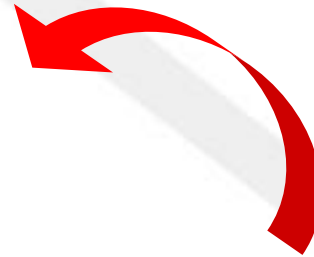
Cotton region :
potentially invaded area



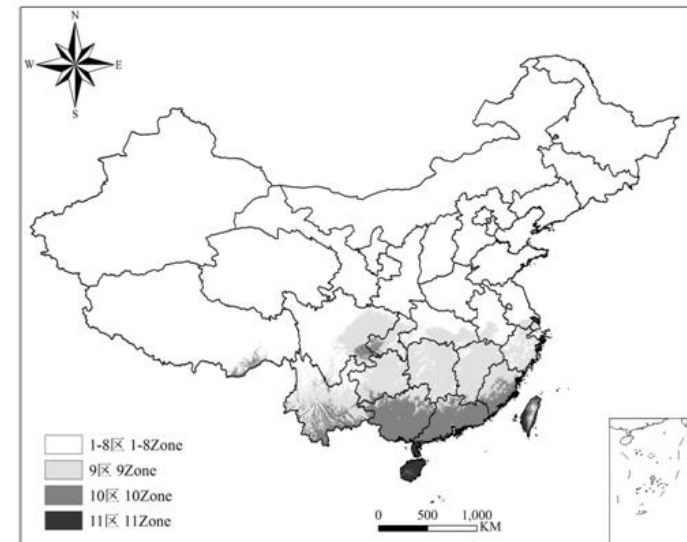
Whitefly complex ?



More CLCuMuV Host range?



CLCuMuV incidence region :
CLCuMuV invaded area + Hibiscus area



Potential geographic distribution of CLCuMuV in China using Maxent

Cotton leaf curl Multan virus already invaded **Southern China** poses a serious national-wide threat to cotton production, with the cotton-growing regions in the Yangtze River valley representing the highly suitable area for the prevention and control of CLCuMuV.

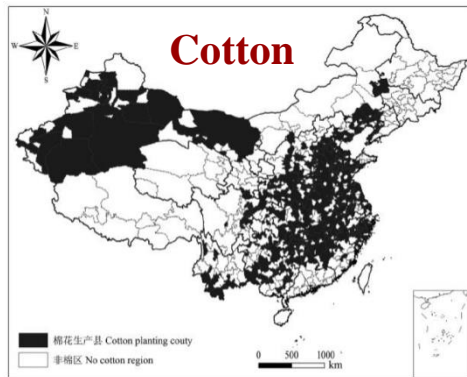


图1 中国棉花种植的空间分布图
Fig. 1 Spatial distribution of cotton planting in China

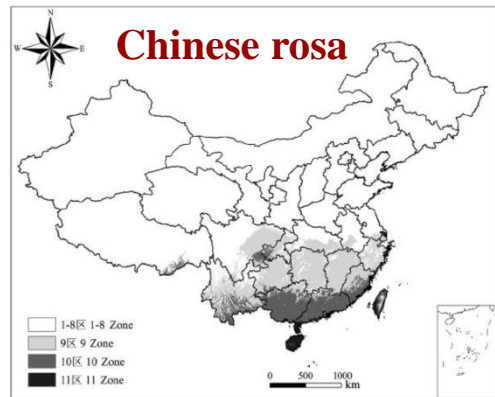
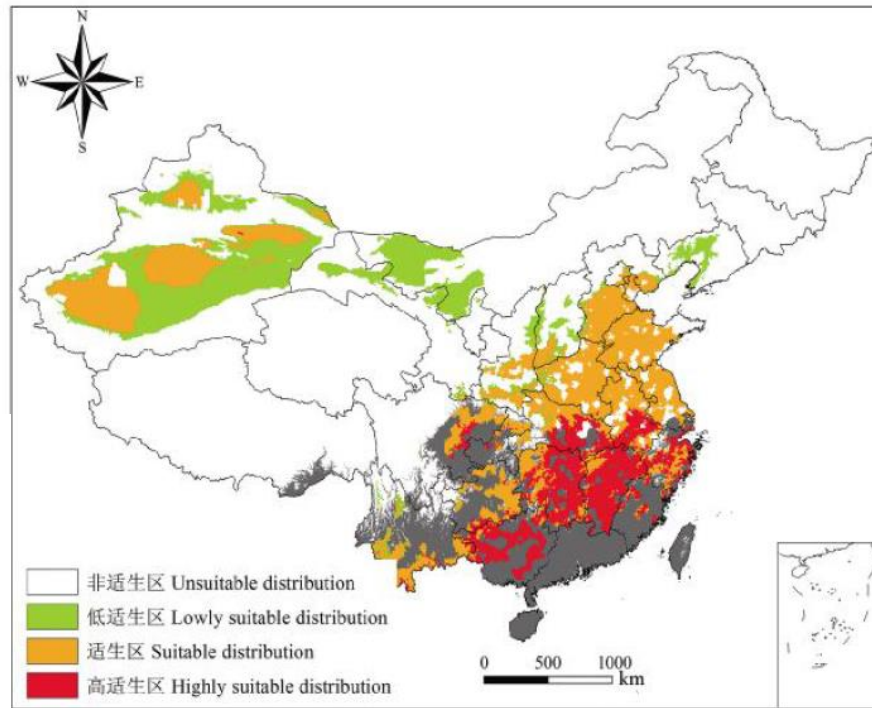


图2 中国玫瑰的潜在适生区
Fig. 2 Potential geographic distribution of *H. rosa-sinensis* in China



注:图中阴影部分为朱穆的潜在适生区。

Note: The shaded region represents potential geographic distribution of *H. rosa-sinensis* in China.

图5 基于 Maxent 模型、传毒介体和寄主植物的棉花曲叶病在中国的潜在适生区

Fig. 5 Potential distribution of cotton leaf curl disease in China by Maxent model, virus vector and host

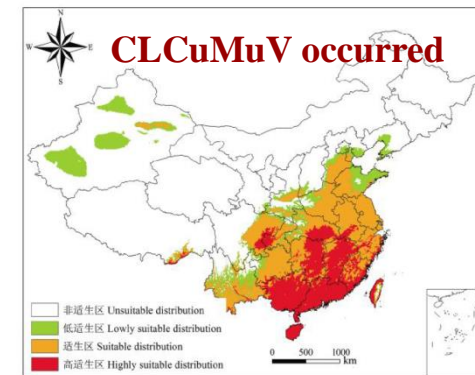


图3 基于 Maxent 模型的棉花曲叶病在中国的潜在适生区
Fig. 3 Potential distribution of cotton leaf curl disease in China by Maxent model

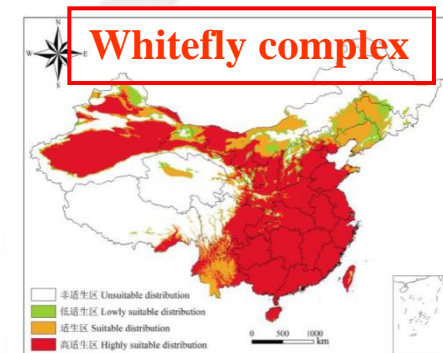
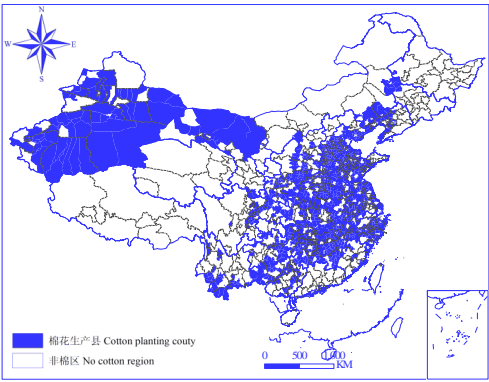


图4 基于 Maxent 模型的烟粉虱在中国的潜在适生区
Fig. 4 Potential distribution of *B. tabaci* complex in China by Maxent model

Occasional CLCuMD incidence in Guangdong & Guangxi



Cotton region :
potentially invaded area



Whitefly complex ?



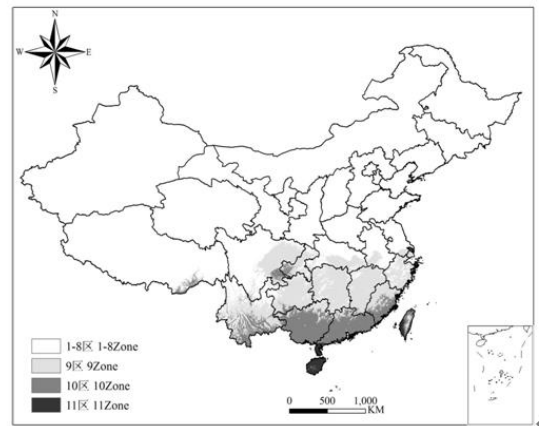
**More CLCuMuV
Host range?**

Occasional incidence

CASE I: Cotton infected by CLCuMuV via WHITEFLY, **Biotype B (MEAM1)**, in GX. (Lin et al. , 2011).

CASE II: Cotton plants infected CLCuMuV originated possibly from **diseased Hibiscus plants** via **WHITEFLY COMPLEX** in a trial plot in GZ (unpubl. data).

CLCuMuD incidence region :
CLCuMV invaded area + Hibiscus area



Investigation on Occurrence of CLCuMuV in China

Survey locations :

10 provinces, 31 cities or counties

Target host plants:

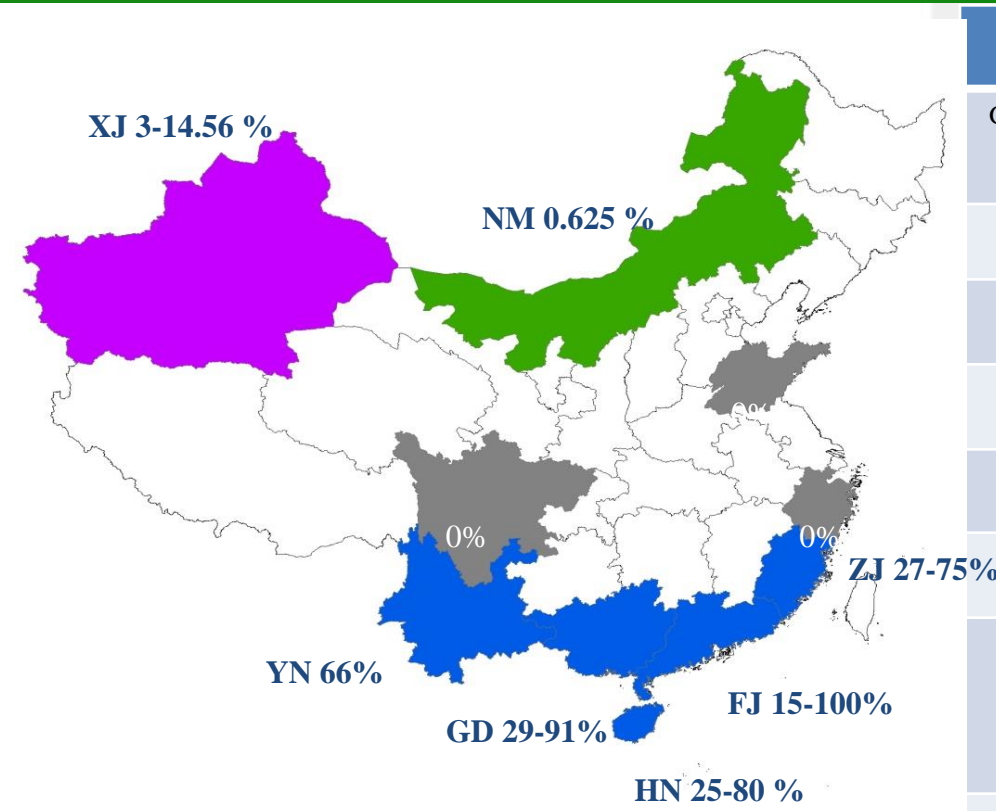
Cotton, Hibiscus, Okra,
Malvaisus arboreus

Survey habitats or sites:

Horticulture area in urban and rural
region, Flowers Farms or Markets



The incidents of CLCuMuV in China



Province	Habitat	Host plants (Virus +/-)	Incidence rate (%)
Guangdong	Horticultural belt	Hibiscus+, <i>Malvaisus arboreus</i> +, Okra+	15 -100
	Flowers Farms	Hibiscus+	87
Guangxi	Horticultural belt	Hibiscus+, Okra-, Cotton-	29 -91
Hainan	Horticultural belt	Hibiscus+, Cotton-, Okra-	25 - 80
Yunnan	Horticultural belt	Hibiscus+, <i>Malvaisus arboreus</i> -	66
Fujian	Horticultural belt	Hibiscus+, Okra-	27 -75
Xinjiang	Cotton Fields	Cotton-	0
	Flowers Market	Hibiscus+	3
	Flowers Farms	Hibiscus+	14.56
Inner Mongolia	Flowers Market	Hibiscus-	0.625
Shandong	Horticultural belt	Hibiscus-	0
Zhejiang	Flowers Market	Hibiscus-	0
Sichuan	Flowers Market	Hibiscus-	0

Horticultural areas

Hibiscus plants seriously damaged by CLCuMuV in **Guangdong, Guangxi, Hainan, Yunnan and Fujian** provinces

Other sichuang

A few of CLCuMuV-diseased Hibiscus plants in flowers farms and markets, usually in the greenhouses of North China's province during winter.

Distribution and homogeneity of CLCuMuV & CLCuMuB

CLCuMuV incidence

CLCuMuV spread rapidly in the last nine years and became established in Southern China.

High genetic homogeneity of CLCuMuV & CLCuMuB

suggests the establishment of them from a single founder event, first arriving in Guangzhou in 2006 or even earlier.

Supposed Possible original location

All isolates of CLCuMuV from China fell within a subclade that clustered with Faisalabad strain of CLCuMuV from Pakistan.

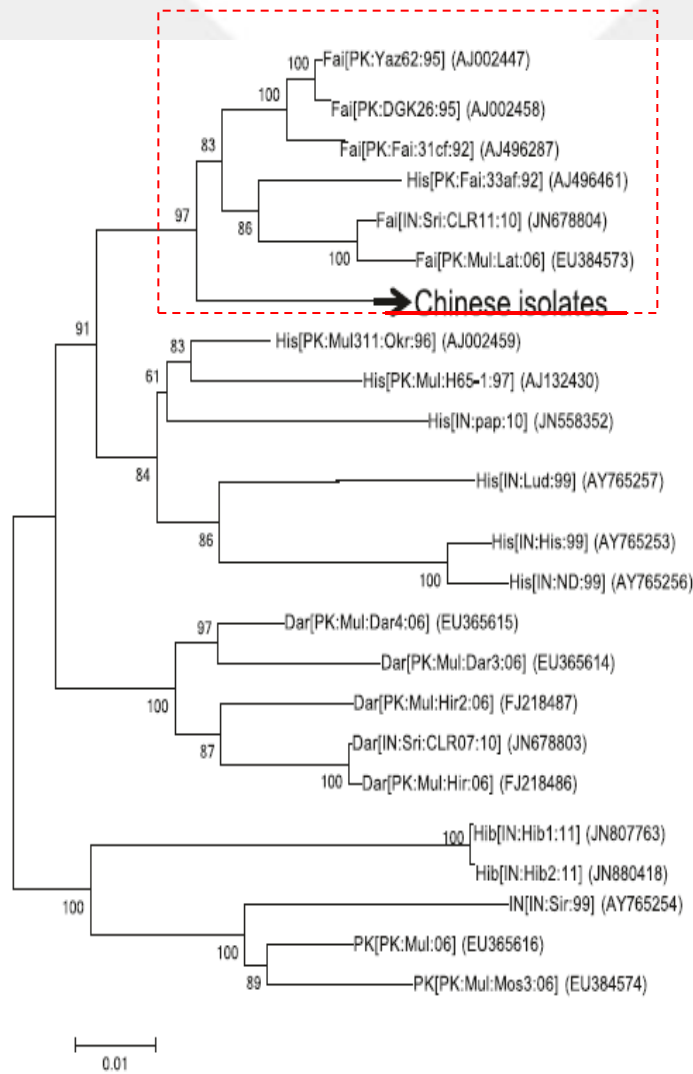
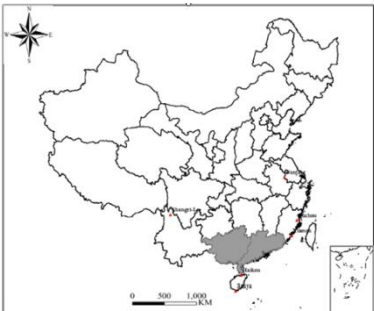
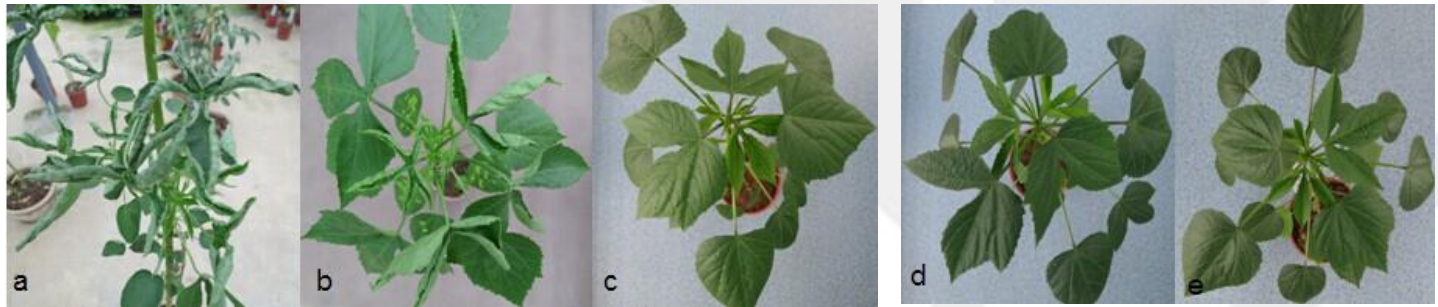


Fig. 2 Phylogenetic relationships between Chinese and South Asian CLCuMuVs. Neighbor-Joining analysis was performed with 1000 bootstrap replicates using MEGA6. Branches having branch support value smaller than 50 % were collapsed. For South Asian CLCuMuV isolates, the latest official names were used (talk.ictvonline.org/ictv_wikis/m/files_gemini/5120.aspx). Please see Additional file 1 for GenBank accessions and sequences of Chinese isolates of CLCuMuVs

The pathogenicity of CLCuMuV to host plants

Inoculation using agro-inoculation as follows:

- **Kenaf**: infectious clones DNA-A (KF444948) and its betasatellite
- **Cotton**: infectious clones pGreen II 049-1.6A of GD01 and pGreen II 049-2.0 β of its betasatellite



Conclusions : (1) Cotton leaf curl disease in Guangdong was caused by co-infection of CLCuMuV and its associated CLCuMuB isolate; (2) Successful infection cotton plants by CLCuMuV and its betasatellite using agro-inoculation technique according to Koch's Postulates

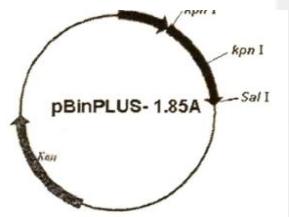
Tangyafei et al., Scientia Agricultura Sinica , 2015

Tangyafei et al., Acta Phytopathologica Sinica , 2013

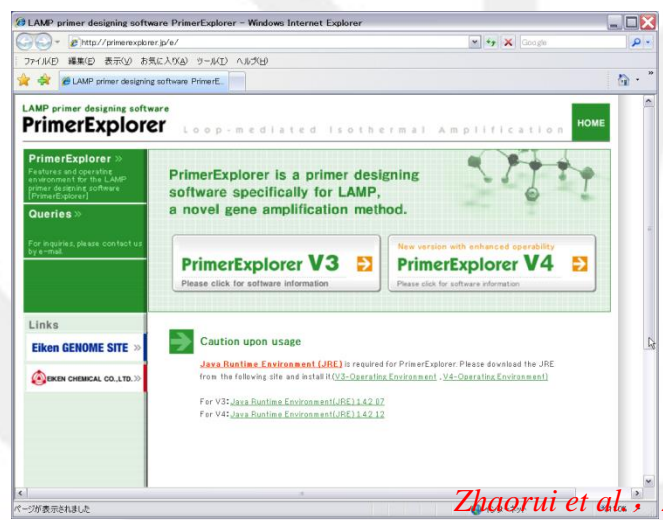
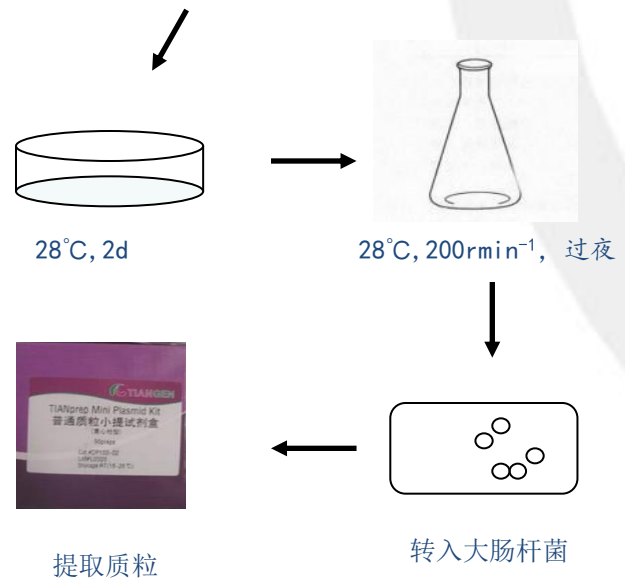
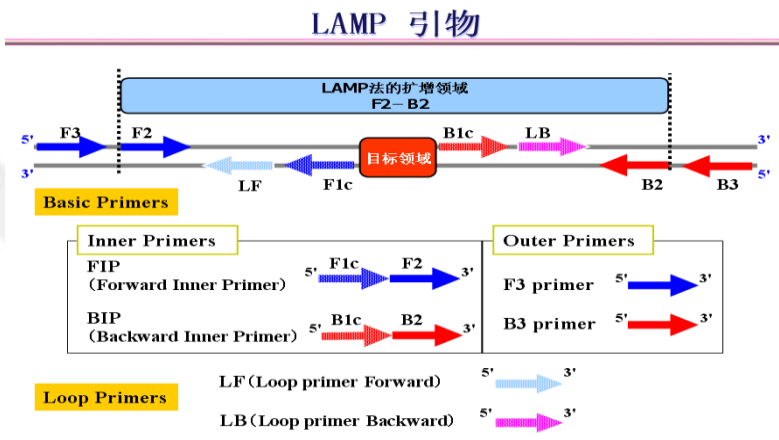
Establishment of CLCuMuV detection method

Step one: Set up a SYBR Green I real-time fluorescence quantitative PCR detection method for Cotton leaf curl virus

Step two: Detect of Cotton leaf curl Multan virus in the body of Bemisia tabaci adults using LAMP technique

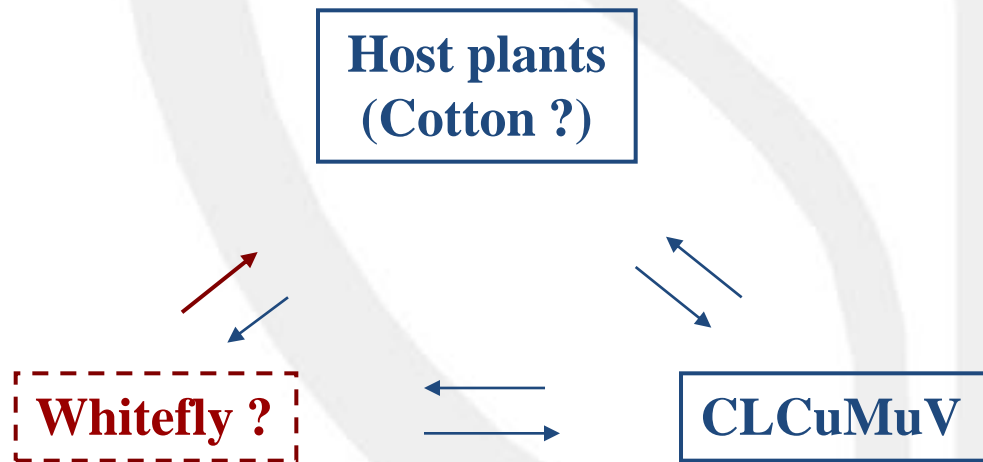


CLCuMV-pBinPLUS-1.85A侵染性克隆



Question THREE:

- Des CLCuMV pose threat to cotton production in China ?
- **WHICH** cryptic species of *Bemisia tabaci* can transmit CLCuMuV to host plants ?



Cryptic species of *Bemisia tabaci* complex and their distribution in China

Whitefly composition : 13 indigenous and 2 invasive cryptic species.

Distribution traits: Indigenous species occur only in the Southern and Southeastern area of China and the diversity declined from the South to the North.

Interspecies competition: Two invasive cryptic species rapidly displaced the indigenous whiteflies in most of China. Later MED has been displacing MEAM 1 in many regions since 2005.

表 2 中国烟粉虱隐种及其分布区域

Table 2 Cryptic species of the whitefly *B. tabaci* and their distributions in China

隐种 Cryptic species	生物型 Biotype	在中国各省、市、自治区的分布 Distribution in China*
Mediterranean	Q	除西藏、宁夏、甘肃、吉林、辽宁外的其他省份 All provinces except Tibet, Ningxia, Gansu, Jilin, and Liaoning
Middle East-Asia Minor I	B	除西藏、宁夏、青海外的其他省份 All provinces except Tibet, Ningxia and Qinghai
Asia II 1	ZHJ2	海南、广东、广西、台湾、贵州、浙江 Hainan, Guangdong, Guangxi, Taiwan, Guizhou, and Zhejiang
Asia II 2		江苏 Jiangsu
Asia II 3	ZHJ1	广西、台湾、湖南、浙江、湖北 Guangxi, Taiwan, Hunan, Zhejiang, and Hubei
Asia II 4		广东 Guangdong
Asia II 6		云南、广东、广西、台湾、福建 Yunnan, Guangdong, Guangxi, Taiwan, and Fujian
Asia II 7	Cv	广东、广西、台湾、福建、江苏 Guangdong, Guangxi, Taiwan, Fujian, and Jiangsu
Asia II 9		湖南 Hunan
Asia II 10		广东 Guangdong
Asia III		台湾 Taiwan
China 1	ZHJ3	海南、广东、贵州、湖南、江西、浙江、四川、重庆、湖北、安徽 Hainan, Guangdong, Guizhou, Hunan, Jiangxi, Zhejiang, Sichuan, Chongqing, Hubei, and Anhui
China 2		广东 Guangdong
China 3		云南、湖南、江西、重庆、浙江、湖北、安徽 Yunnan, Hunan, Jiangxi, Chongqing, Zhejiang, Hubei, and Anhui
Asia I		海南、云南、广西、广东、台湾 Hainan, Yunnan, Guangxi, Guangdong, and Taiwan

Invasive (2) { MED around 2003
MEAM1 Mid-1990s

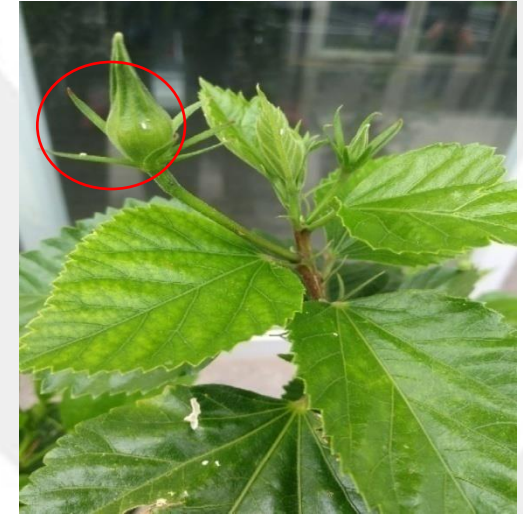
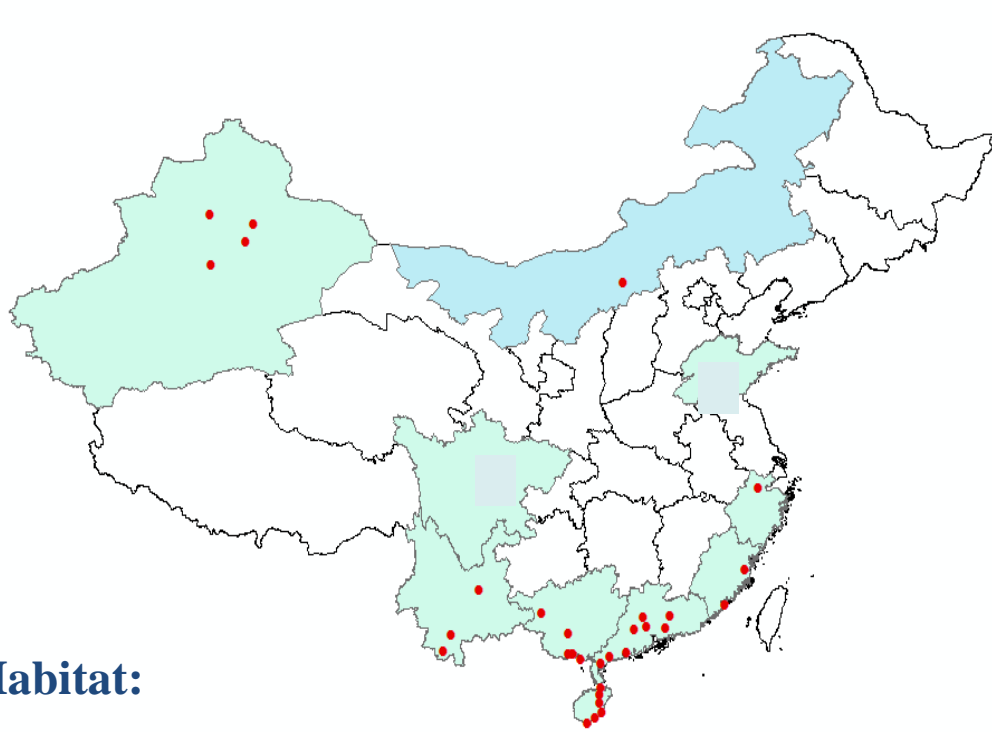
Indigenous (13) {

* 主要依据文献 Guo *et al.* (2012)、Hu *et al.* (2011)、Pan *et al.* (2011)、Qiu *et al.* (2007) 汇总。

* This table is mainly based on data in above references.

Composition and dominant CS of whitefly in China

Survey locations : 8 provinces, 21 cities or counties)



Survey Habitat:

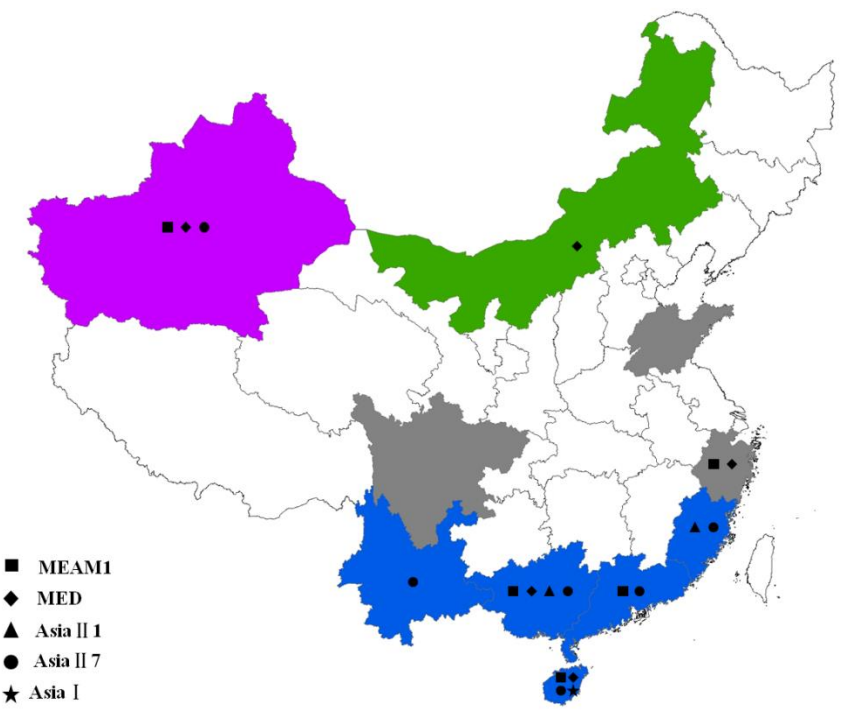
Horticultural belts, Flowers Market, Flowers farms

Target host plants:

Cotton, Hibiscus, Okra, *Malvaisus arboreus*, Tomato, Golden Potato, **Magenta**

Sample total: 399 individuals

Distribution and Composition of whitefly in China

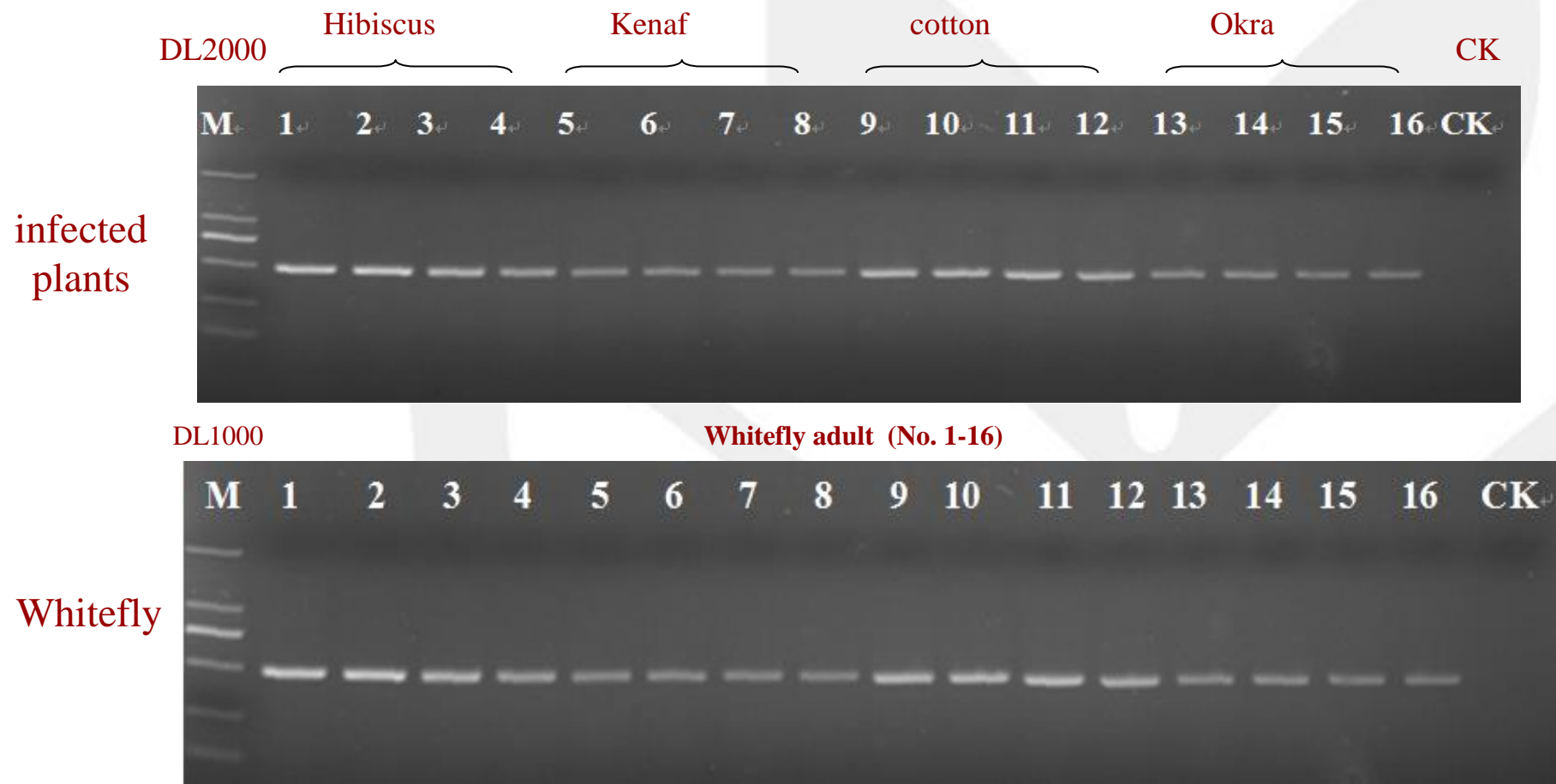


Province	Habitat	Host plants	Number	Cryptic species	
				MEAM1/ MED	Asia II 7/ Asia II 1
Guangdong	Horticultural belts	Hibiscus	192	100	92
	Flowers farms	Hibiscus	7	5	2
Guangxi	Horticultural belts	Hibiscus cotton	12 5	1/4	10/2
	Horticultural belts	Hibiscus\ Okra\ Furong	23 10 7	4/2 10/0	7/10 7/0
Yunnan	Horticultural belts	Hibiscus	15		15/0
Fujian	Horticultural belts	Hibiscus\ Okra	10 4		10/0 0/4
	Flowers Markets	Hibiscus	20	0/20	
Xinjiang	Cotton fields	Cotton	18	6/12	
	Flowers Markets	Hibiscus	42	0/42	
	Flowers Markets	Hibiscus\ Tomato	6 6 6		6/0 6/0 6/0
Zhejiang	Flowers Markets	Hibiscus\ Gold potato\ Magenta	0 10 10	- 1/9 1/9	

Conclusions :

- Two invasive species: **MEAM1**, **MED**
- Three indigenous species: **Asia II 7**、**Asia II 1** and **Asia I**
- A mixed population of MEAM1 & Asia II 7 on Hibiscus plants of Guangdong Province.
- Asia II 7 , a dominant cryptic species.

Detection for CLCuMV in four infected plants & whitefly



PCR detection of CLCuMV in infected plants of four hosts and whitefly

- The sampled plants of Kenaf, cotton, and Okra showing symptom were detected with CLCuMuV.
- The whitefly adults collected from diseased plants detected as positive CLCuMuV.

Which cryptic species of the complex can transmit CLCuMV ?



Virus reservoir
(Naturally infected plants)

Transmission Method

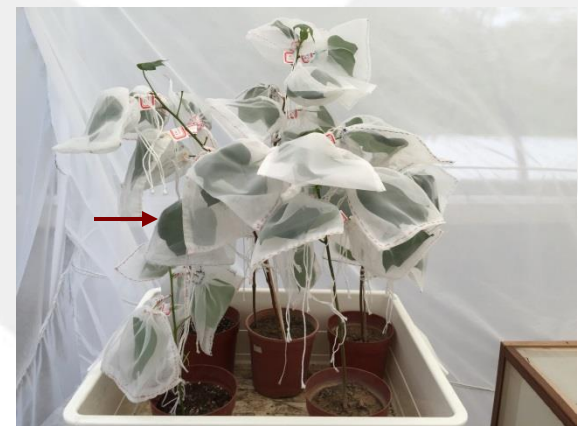
Host plants

Establishment of lab colony and pure strain of a specific cryptic species

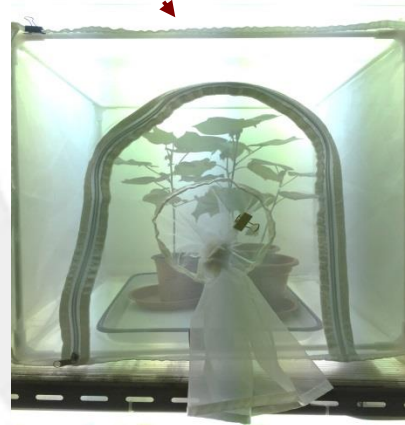
Field collection



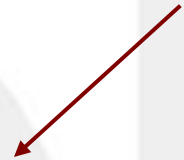
Strain from one pair



mt CO I-aided
Identification



Purification



MEAM 1(B)

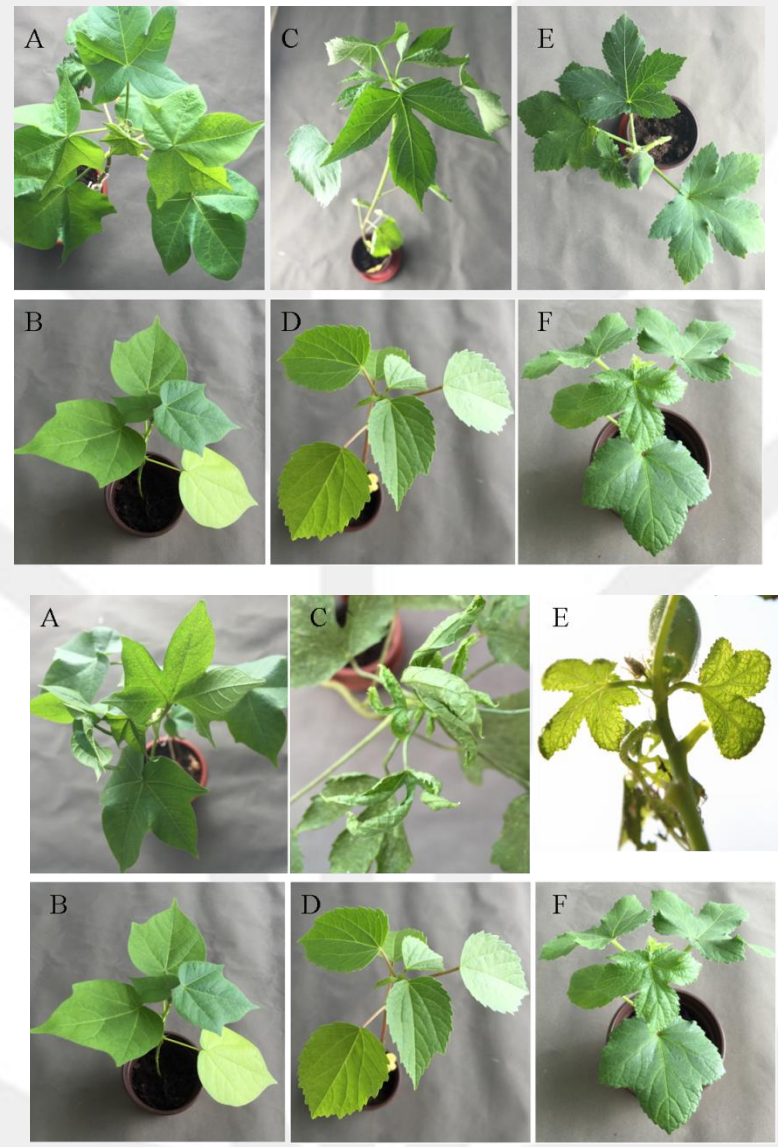
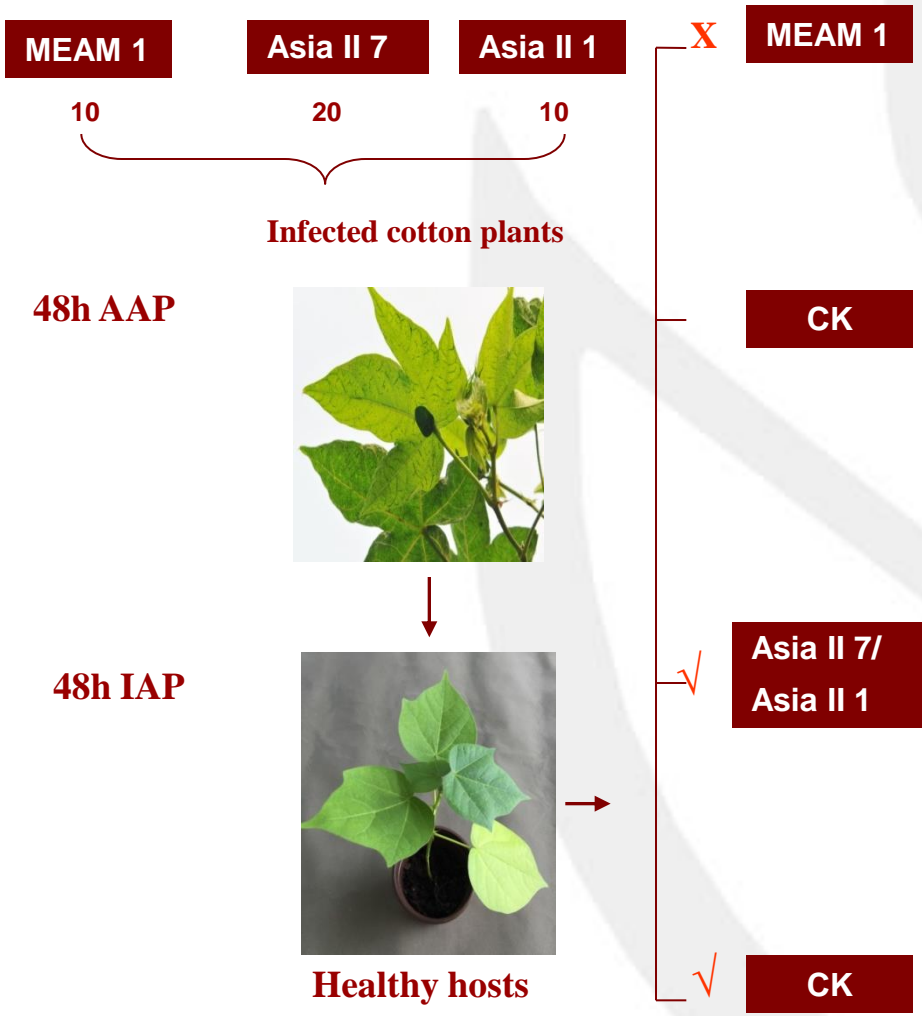
Invasive cryptic species

Asia II 7(CV)

Asia II 1(ZHJ-2)

Indigenous cryptic species

Identification of the cryptic species of whitefly transmitting CLCuMuV



Cotton Kenaf Okra

Two indigenous cryptic species of **Asia II 7** and **Asia II 1** are able to transmit CLCuMuV to cotton, Kenaf, and Okra plants.

Transmission difference of CLCuMuV by Asia II 7, MEAM1, and MED.



Symptoms of *G. hirsutum* (112-2) inoculated with Asia II 7 whitefly transmitting CLCuMuV

Transmission efficiency of CLCuMuV to *G. hirsutum* plants by *Bemisia tabaci* cryptic species of Asia II 7, MEAM1, and MED

Cryptic species	Variety	Tested plants	Whitefly no	Diseased plants	Positive with PCR	Transmission rate (%)
MEAM 1	112-2	30	10	0	0	0
	Zhongmian-40	30	10	0	0	0
MED	112-2	30	10	0	0	0
	Zhongmian-40	30	10	0	0	0
Asia II 7	112-2	30	10	12	12	40.0
	Zhongmian-40	30	10	0	0	0

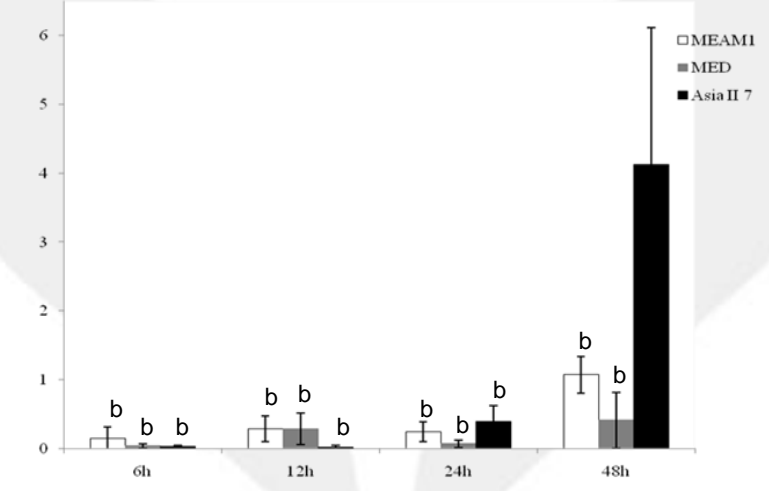
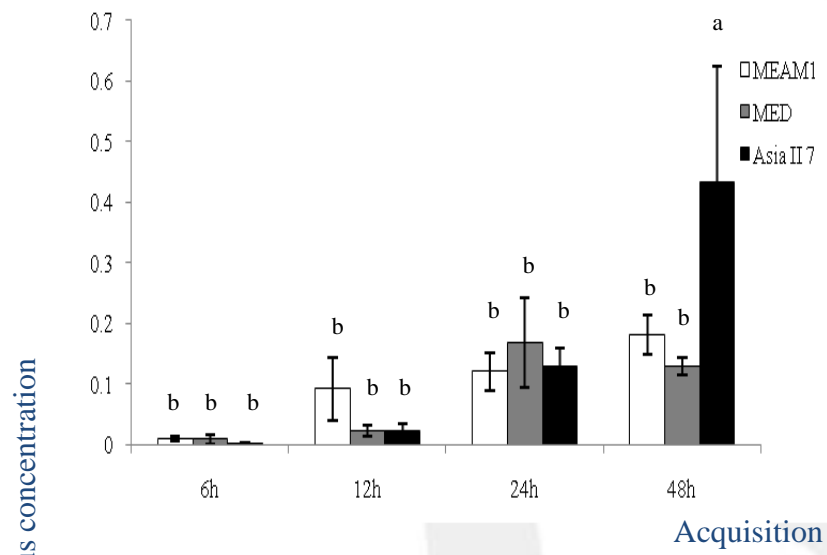
Impact of inoculation density Asia II 7 on transmission efficiency of CLCuMuV to *G. hirsutum* plants

Variety	No. of inoculated plants	No. of whiteflies per plant	No. of diseased plants	No. of plants PCR-detected with positive	Transmission efficiency (%)
112-2	30	1	1	1	3.33
	30	5	13	13	43.33
	30	10	12	12	40.00
	30	15	15	15	50
	20	1	0	0	0
Xinhai 21	20	5	6	6	30
	20	10	6	6	30
	20	15	5	5	25

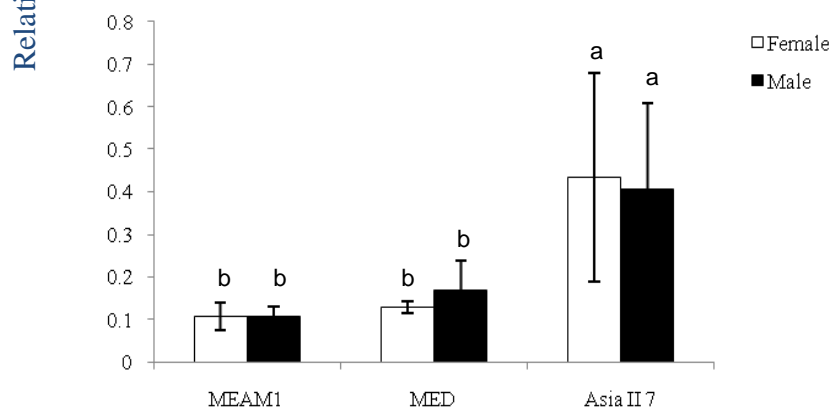
- The native cryptic species Asia II 7 were able to transmit CLCuMuV to infect 112-2 and Xinhai-21
- The invasive cryptic species MEAM1 and MED unable to transmit CLCuMuV to infect cotton

(Chen ting et al., Unpublished)

Differential retention of CLCuMuV in three cryptic species



Dosage of CLCuMuV and betasatellite in three cryptic species by qPCR



Conclusions:

- The viral amount in Asia II 7 which acquired the virus for 48 h was significantly higher than other cryptic species MEAM1, MED
- There are no significant difference of viral amount between females and males which acquired the virus for 48h in the three species of whitefly.

CLCuMuV dosage in three cryptic species feeding on diseased cotton plants at 48h AAP

(Chenting et al., Unpublished)

Summary or Conclusions

- CLCuMuV poses severe threat to cotton production, due to presence of CLCuMuV-infected Hibiscus, transmittable indigenous cryptic species in China.
- Until now, **Asia II7** and **Asia II 1** have been proven to be more efficient CLCuMuV-Vector and also to transmit to **cotton, kenaf, Okra, and pending Hibiscus**.
- The indigenous cryptic species play more important role on CLCuMuV-dissemination among Malvaviscus plants and its approach to cotton region in China.

Future work

- **Keep identifying more cryptic species and testing what kind of CS (invasive and native)is able to transmit CLCuMuV in both China and Pakistan.**
- **Further test if Asia II 7 & Asia II1 or other native CS can survive in Northern & Western regions for reevaluation impact of CLCuMuV on cotton production.**
- **Detect CLCuMuV potential hosts of field weeds off- season in Pakistan.**
- **Set-up lab colonies of cryptic species for transmission test in Pakistan.**

Acknowledgements:

- **Prof. Shafqat Saeed, seminar organizer**
 - **Muhammad Nawaz Shareef University of Agriculture**

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 - **Guangdong Academy of Agricultural Sciences**
 - **Laboratory of Vegetable Diseases**
 - **My colleagues: Ting CHEN, Guojun QI, Rui ZHAO et al..**
- 



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Geminivirus complexes and whitefly in Pakistan and their control strategies



Dr. Shahid Mansoor, *Sitara-e-Imtiaz*

HEC Distinguished National Professor
Fellow Pakistan Academy of Sciences

DIRECTOR

National Institute for Biotechnology and Genetic Engineering (NIBGE),
Faisalabad, Pakistan

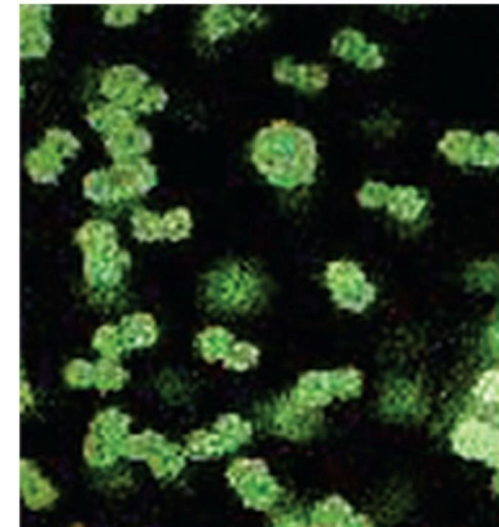
Geminiviruses

- Circular, single-stranded DNA viruses,
- Genome ~2.5–5.4 kb
- Derive their name from their twinned icosahedral particles,
- Currently 360 species in the family,
- **Are divided among 9 genera,**

Epidemics of geminiviruses are due to;

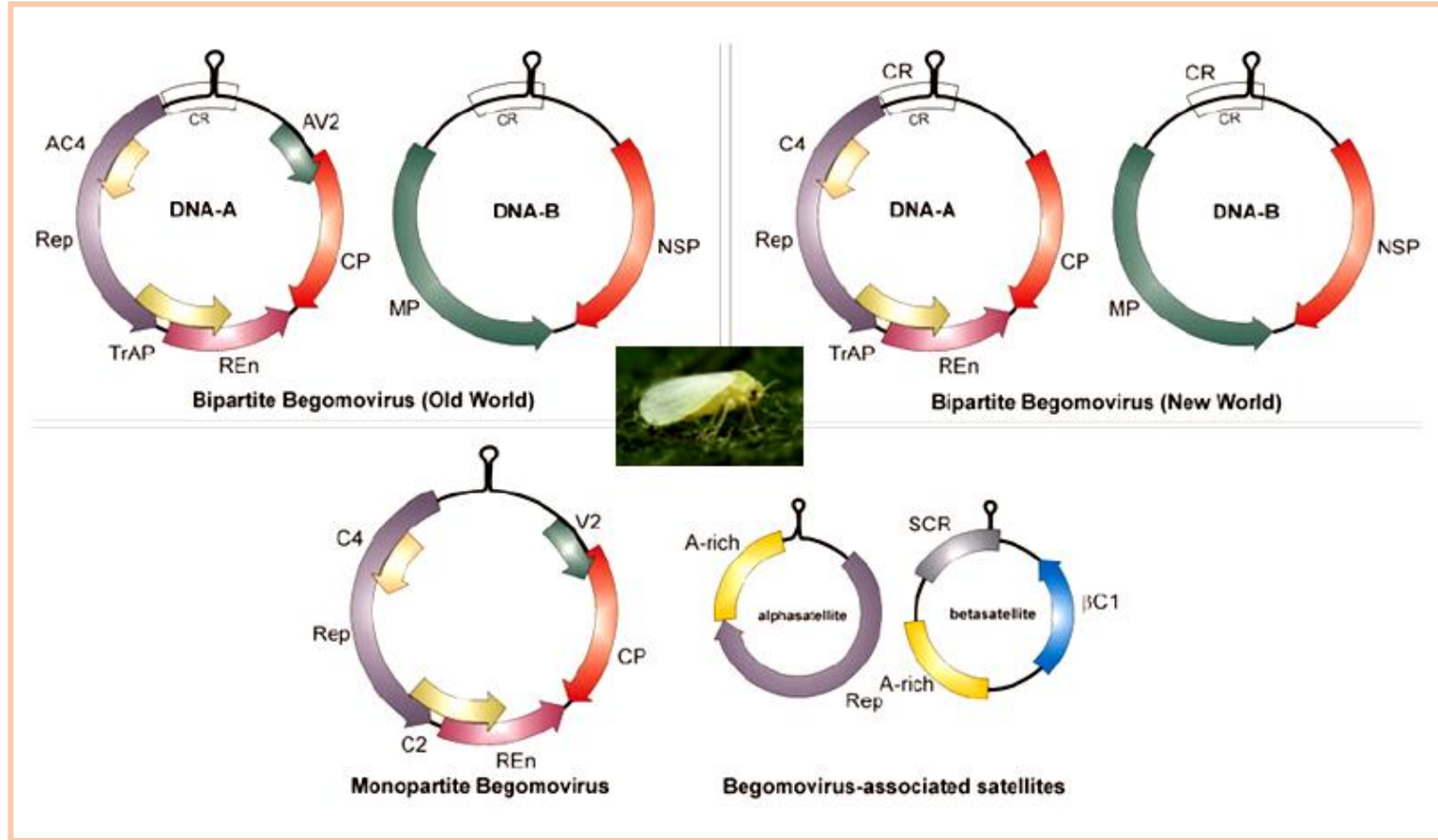
- Recombination
- Expansion and migration of vectors
- Transport of infected plant material

Geminivirus

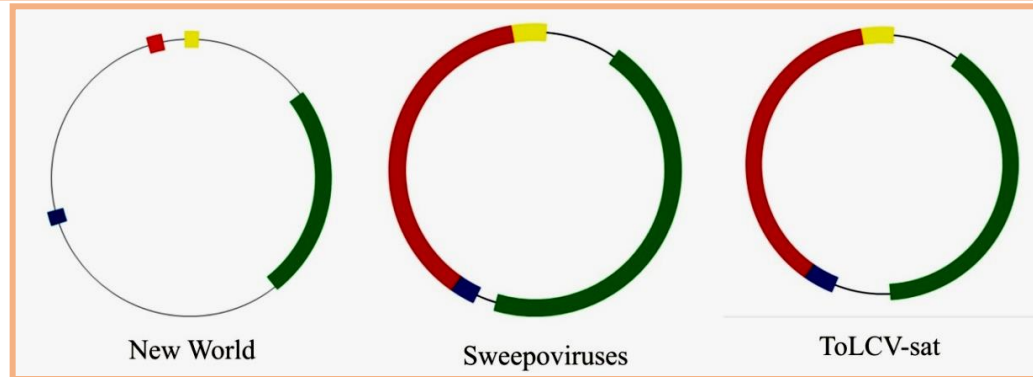


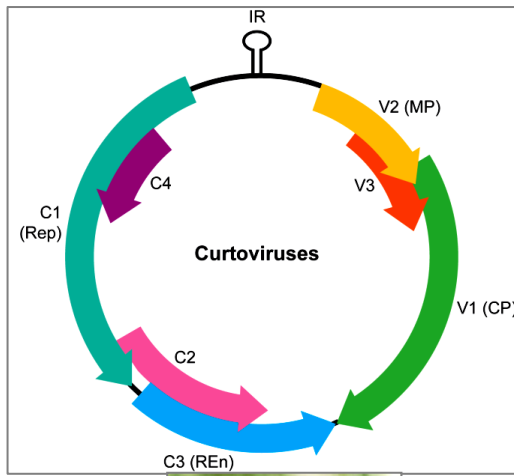
Geminiviridae

Begomoviruses

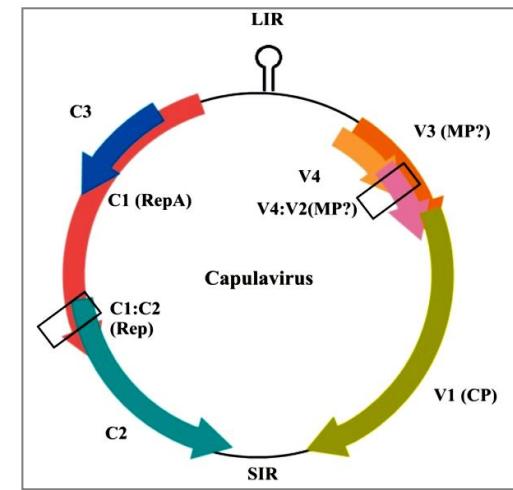
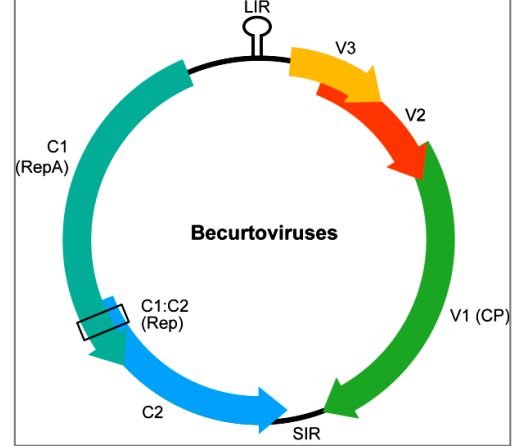


Deltasatellites

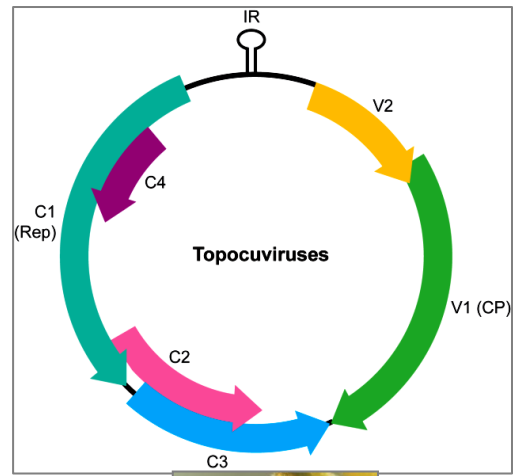




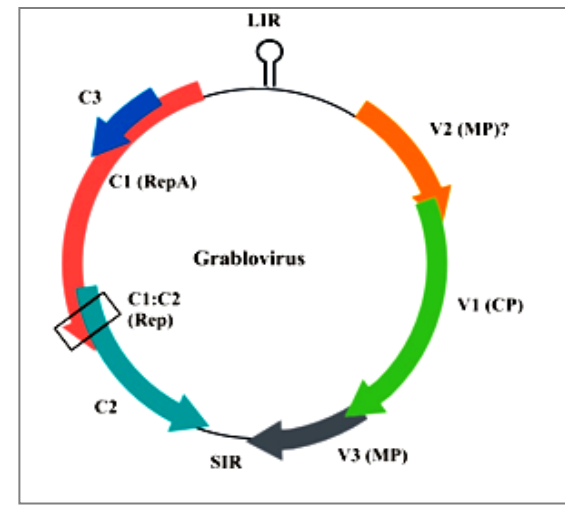
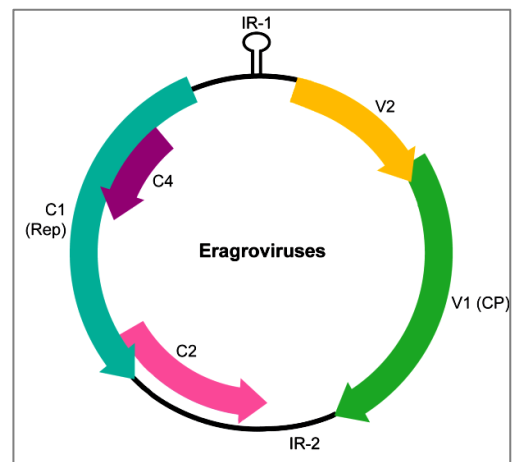
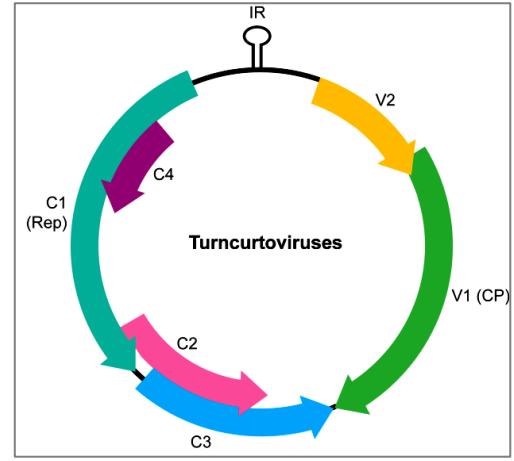
Circulifer tenellus



Aphis craccivora



Micrutalis malleifera



Spissistilus festinus

The most important begomoviruses in Pakistan

- **Tomato** is one of the most important crop affected by tomato leaf curl disease in Oman
- **Cotton Leaf Curl Disease** (CLCuD) first reported in Pakistan and India in 1960's and became major problem in 1990's
- **Whitefly** (*Bemisia tabaci*) is the vector of begomoviruses but whitefly species are distinct
- The begomovirus disease complexes found in Middle East are distinct from those found in the Indian subcontinent



The presence of *Tomato yellow leaf curl virus* (TYLCV) in Pakistan

- *Tomato yellow leaf curl virus* (TYLCV) is a monopartite *Begomovirus* which belongs to the family Geminiviridae
- It is transmitted by white fly (*Bemisia tabaci*) and causes tomato yellow leaf curl disease (TYLCD)
- It is the most damaging and threatening virus for tomato production worldwide
- The ongoing global spread of TYLCV from the Mediterranean basin and Middle-East poses a serious threat to tomato production
- First time a novel strain of TYLCV (named TYLCV-PK) was reported in Pakistan on cluster bean

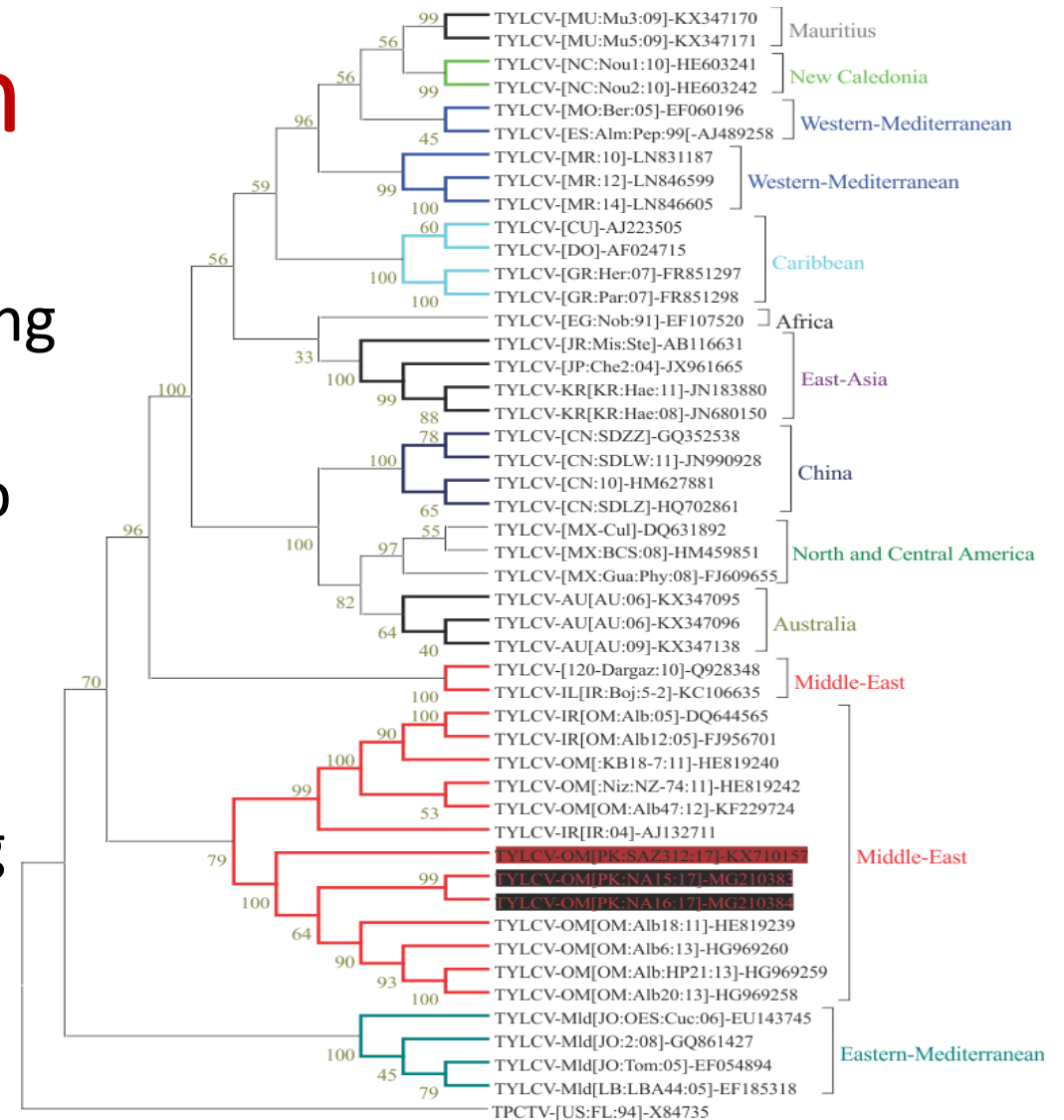


Figure 1. Cluster bean plant showing typical symptoms of begomovirus infection like leaf curling and leaf yellowing.

(Zaidi et al. 2017)

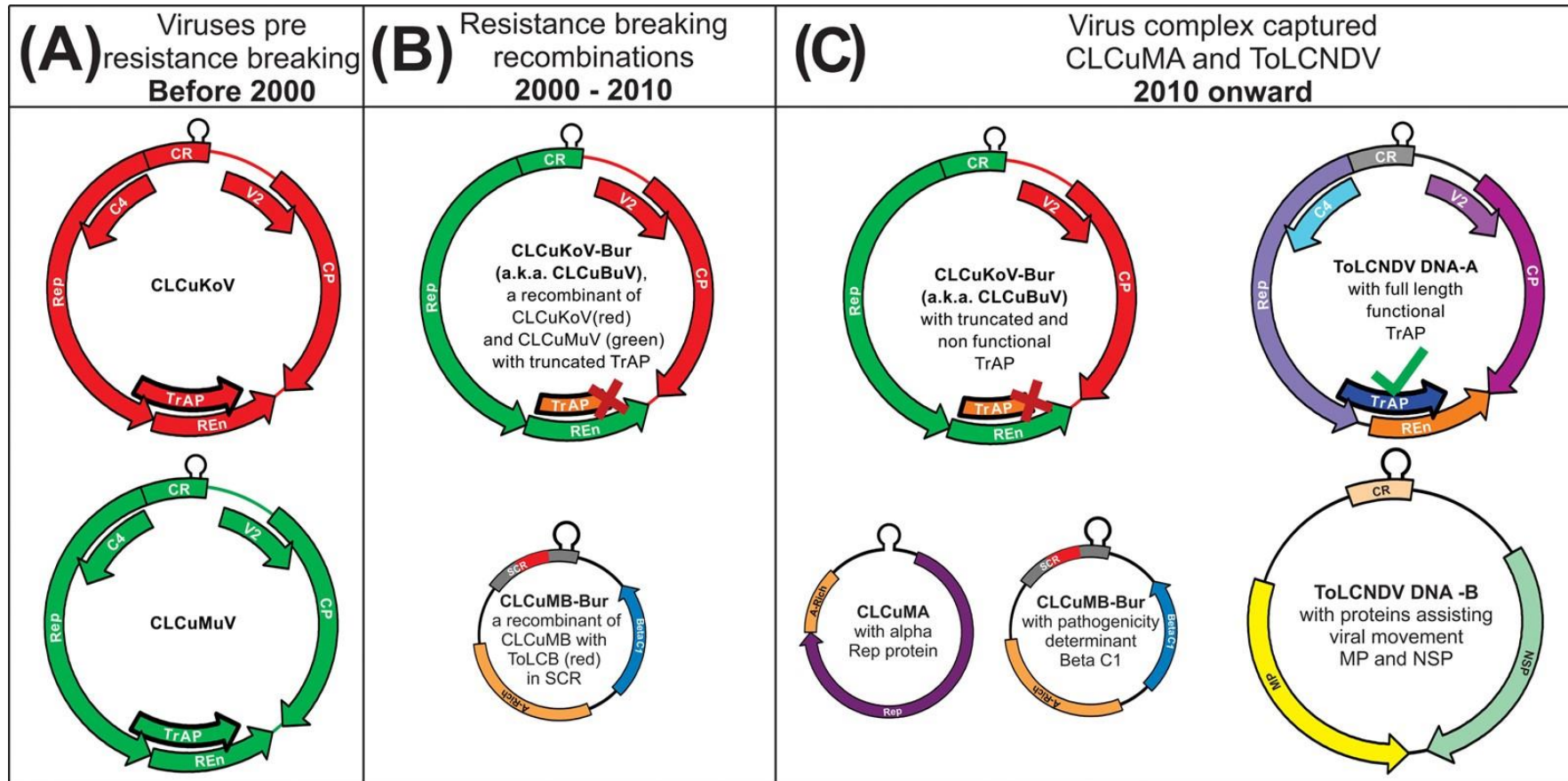
Diversity of TYLCV in Pakistan

- Recently we have also found TYLCV infecting its major host “tomato” in Sindh, Pakistan
- This novel strain showed close relationship with TYLCV identified in Iran
- Many recombination events were also identified in these novel strains
- These findings show that TYLCV is evolving and spreading in Pakistan
- It poses a looming threat to tomato and other crop production in Pakistan



Maximum likelihood phylogenetic tree to show the relatedness of TYLCV identified on tomato in Pakistan

Evolution of CLCuD complex during three decades; the presence of Tomato leaf curl New Delhi virus in cotton

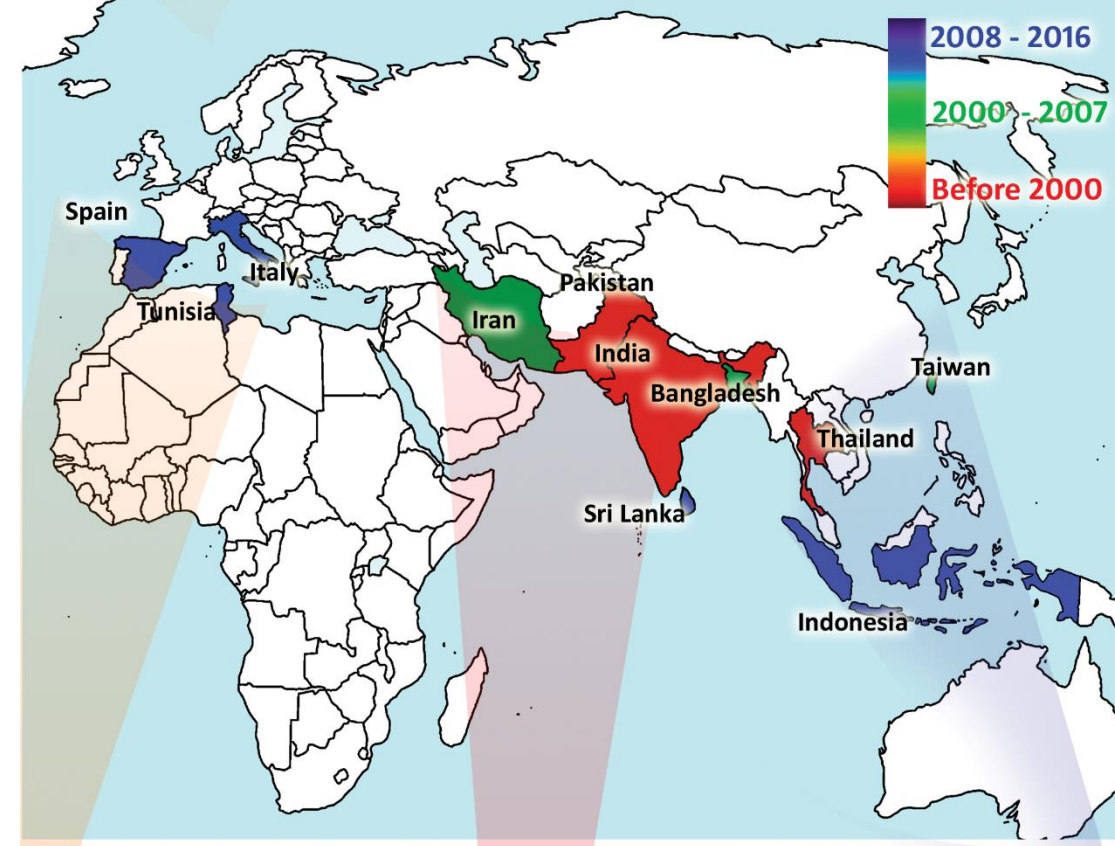


How successful is it
globally ?

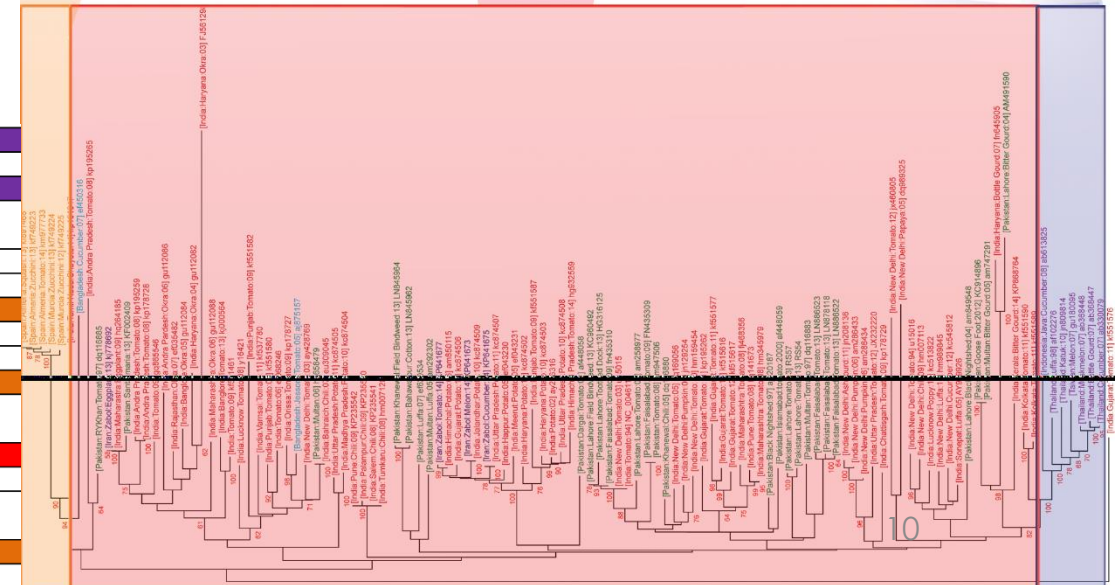
ToLCNDV is a globally widespread begomovirus infecting several crops

ToLCNDV infects

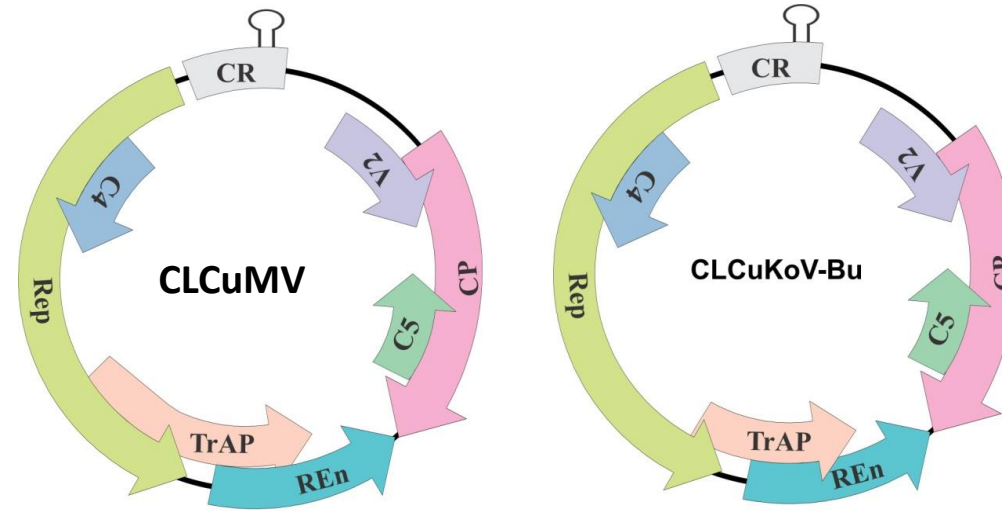
- 44 different plant species
- 11 different countries



India		Pakistan		Indonesia	
<i>Ageratum</i> spp.	<i>Datura stramonium</i>	<i>Momordica charantia</i>	<i>Chenopodium album</i>	<i>Capsicum annuum</i>	<i>Cucumis sativus</i>
<i>Benincasa hispida</i>	<i>Luffa cylindrica</i>	<i>Convolvulus arvensis</i>	<i>Luffa cylindrica</i>	Thailand	
<i>Momordica charantia</i>	<i>Aleyrodes brassicae</i> on <i>Catharanthus roseus</i>	<i>Solanum nigrum</i>	<i>Cestrum nocturnum</i>	<i>Sauropus androgynus</i>	<i>Luffa cylindrica</i>
<i>Lagenaria siceraria</i>	<i>Carica papaya</i>	<i>Capsicum annuum</i>	<i>Parthenium hysterophorus</i>	<i>Lagenaria siceraria</i>	<i>Cucumis melo</i>
<i>Daucus carota</i>	<i>Catharanthus roseus</i>	<i>Gossypium hirsutum</i>	<i>Solanum lycopersicum</i>	<i>Cucumis sativus</i>	
<i>Saccharum edule</i>	<i>Papaver somniferum</i>	<i>Eclipta prostrata</i>	<i>Rumex dentatus</i>	Spain	
<i>Capsicum annuum</i>	<i>Solanum tuberosum</i>			<i>Cucurbita</i> spp.	<i>Cucurbita pepo</i>
<i>Cucumis sativus</i>	<i>Cucurbita pepo</i>	Bangladesh		<i>Solanum lycopersicum</i>	
<i>Solanum melongena</i>	<i>Luffa cylindrica</i>	<i>Cucumis sativus</i>	<i>Solanum lycopersicum</i>	Tunisia	
<i>Crossandra infundibuliformis</i>	<i>Trichosanthes cucumerina</i>	Iran		<i>Cucumis melo</i>	<i>Cucurbitaceae</i> family
<i>Cyamopsis tetragonoloba</i>	<i>Luffa aegyptiaca</i>	<i>Cucumis sativus</i>	<i>Capsicum annuum</i>	Sri Lanka	
<i>Coccinia grandis</i>	<i>Nicotiana tabacum</i>	<i>Solanum melongena</i>	<i>Solanum lycopersicum</i>	<i>Momordica charantia</i>	<i>Luffa</i> spp.
<i>Jasminum multiflorum</i>	<i>Solanum lycopersicum</i>	<i>Cucumis melo</i>		<i>Trichosanthes cucumerina</i>	<i>Cucurbita pepo</i>
<i>Jatropha</i> spp.	<i>Citrullus lanatus</i>	Taiwan		Italy	
<i>Hibiscus cannabinus</i>	<i>Abelmoschus esculentus</i>	<i>Cucumis melo</i>		<i>Luffa</i> spp.	<i>Cucumis sativus</i>

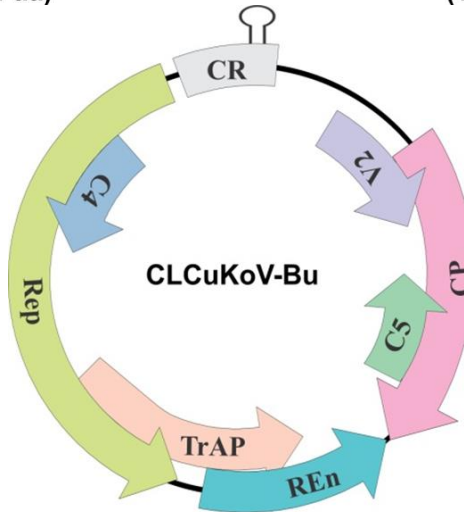


Further changes in the cotton leaf curl disease complex – an indication of things to come?



Before resistance breakdown
(TrAP: 134 aa)

Resistance breaking strain
(TrAP: 35 aa)



2013
(TrAP: 127 aa)

Conclusions

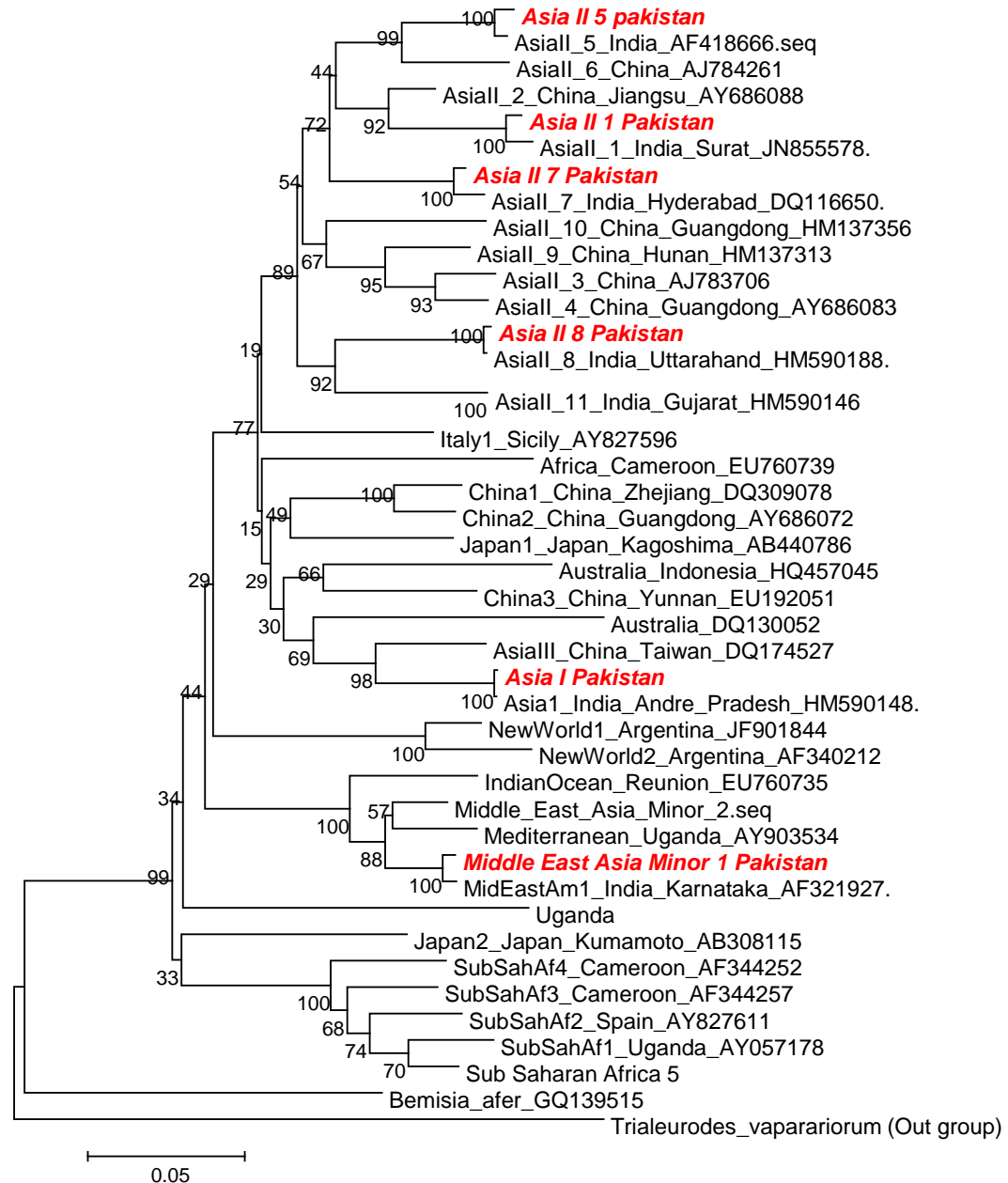
- Geminivirus disease complexes are continuously evolving by recombination, component capture and mutations
- New viruses are introduced through global trade
- Oman has invasion of begomoviruses both from Africa/Middle East as well as the Indian subcontinent
- Several new geminiviruses are expected to be found in the region
- New sources of resistance are required to control these diseases

Whitefly biotypes and endosymbionts diversity

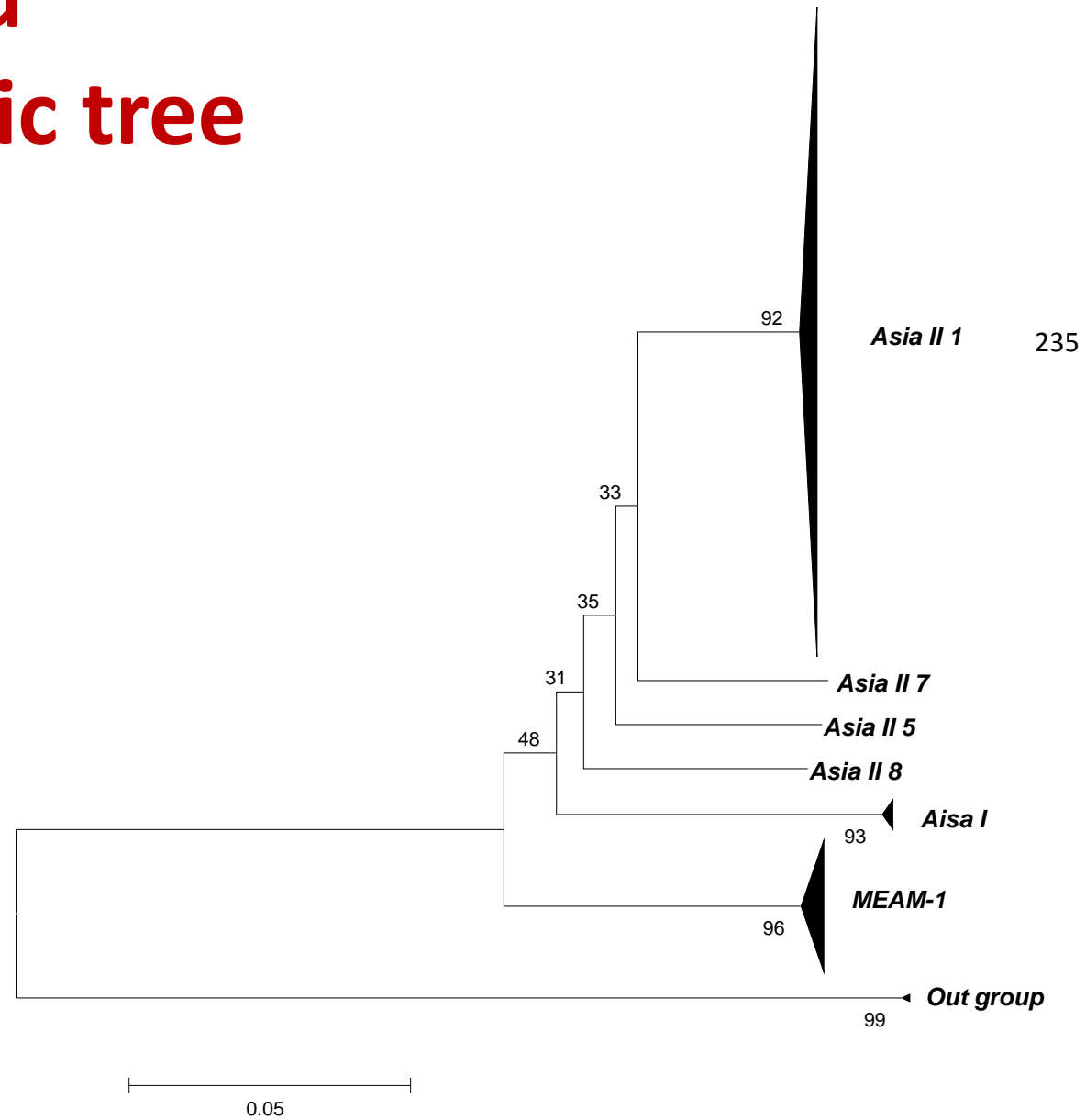
Map of Pakistan: Samples collection sites



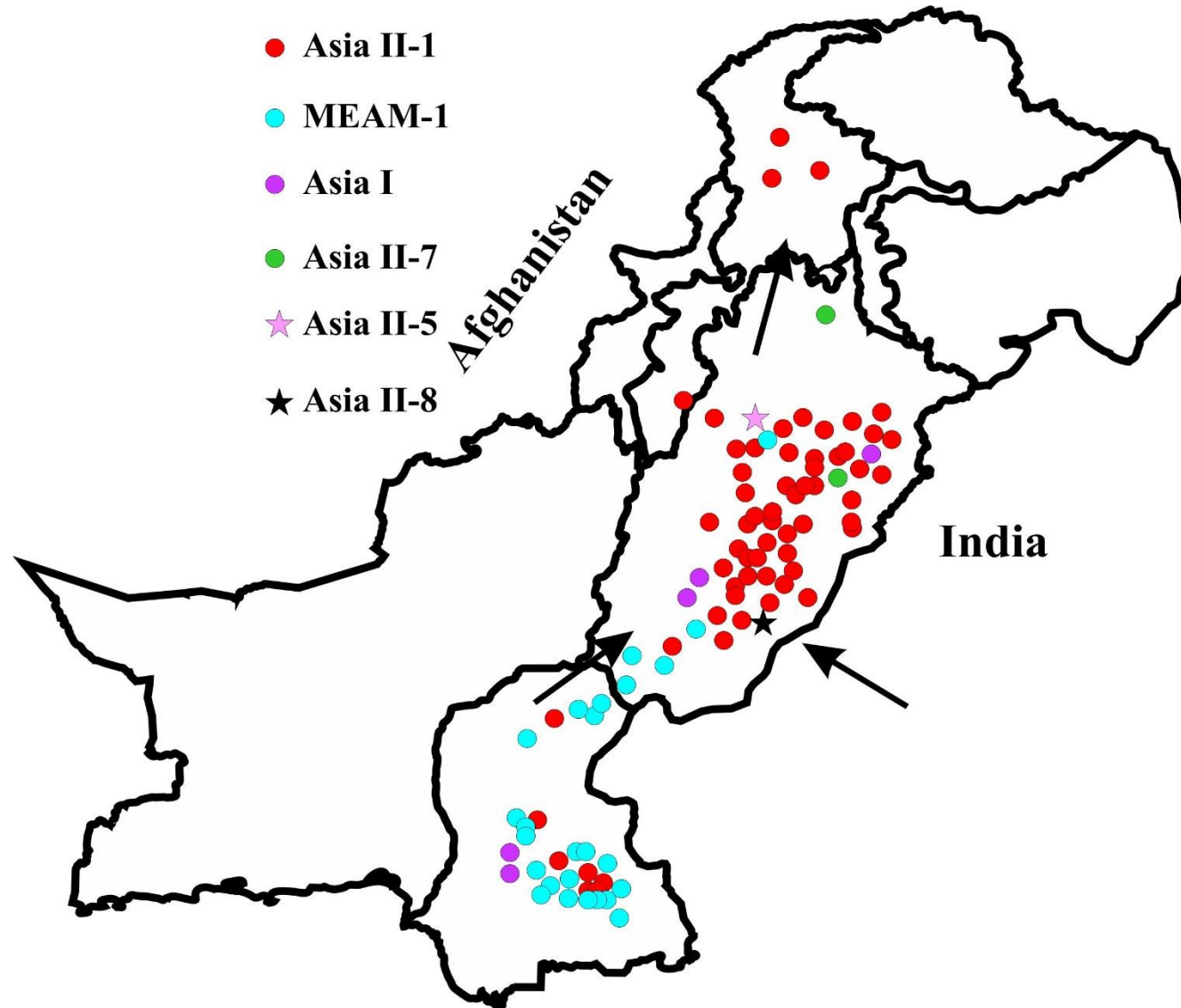
Comparison with known (database) Biotypes/species



COI-3 based phylogenetic tree



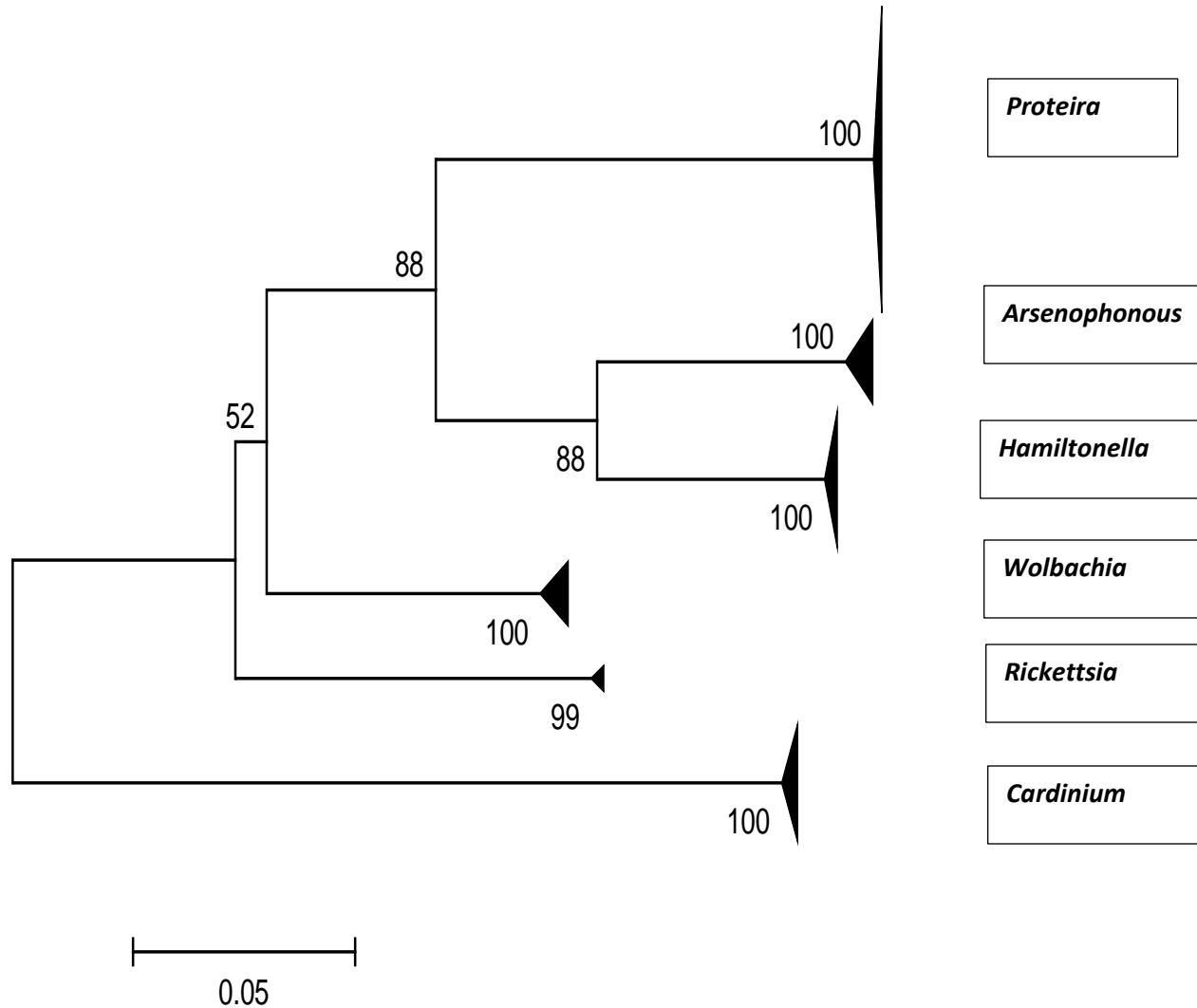
Distribution pattern of *Bemisia tabaci*



Diversity of Endosymbionts

- Whiteflies which were already identified were selected for screening symbionts
- Six primer sets were used against six types of endosymbionts
- Total number of 227 insect were screened, 101 from Punjab region, 95 from Sindh and 31 from KPK.
- Total number of 409 clones were produced during this study
- Proteira was found positive in all the samples

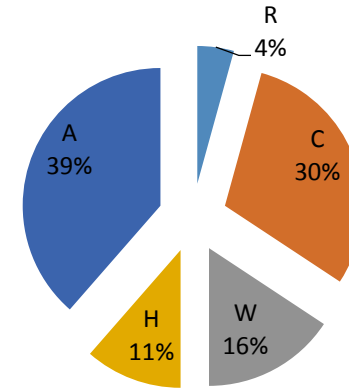
Phylogenetic tree showed the presence of six endosymbionts



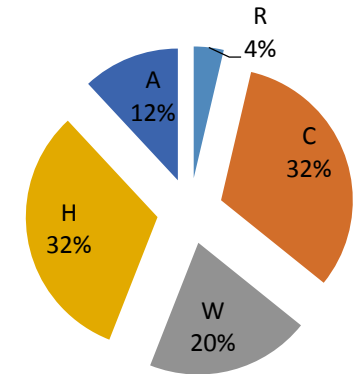
Fritschea false positive results

Presence of different endosymbionts in different regions

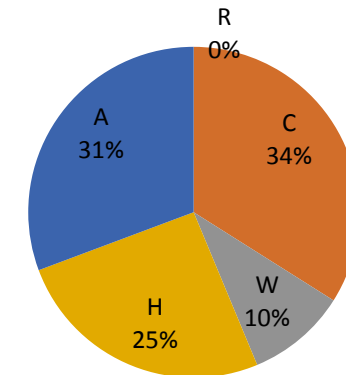
	No of Biotypes found	Proteia	Arsenophonous	Hamiltonella	Cardinium	Wolbachia	Fritschia	Rickettsia
Punjab (101)	06	92	66	55	73	22	15	0
Sindh (95)	03	87	13	35	35	22	09	04
KPK (31)	01	29	27	08	21	11	13	03



KPK



Sindh



Punjab

Distribution of different endosymbionts in different regions

- The whiteflies collected from Sindh contain *Hamiltonella* and *Wolbachia* in most of their samples while *Wolbachia* was least in case of whiteflies collected from Punjab
- *Arsenophonus*, *Hamiltonella* and *Cardinium* were found in combination in most of the samples in all regions
- In Punjab *Cardinium* was found in most of the samples than Sindh and KPK
- While *Wolbachia* was found in most of the samples in Sindh region

Virus Control Strategies

Conventional Strategies

Vector management by

- Pesticides
- Insect traps
- Protective shields

Natural resistance

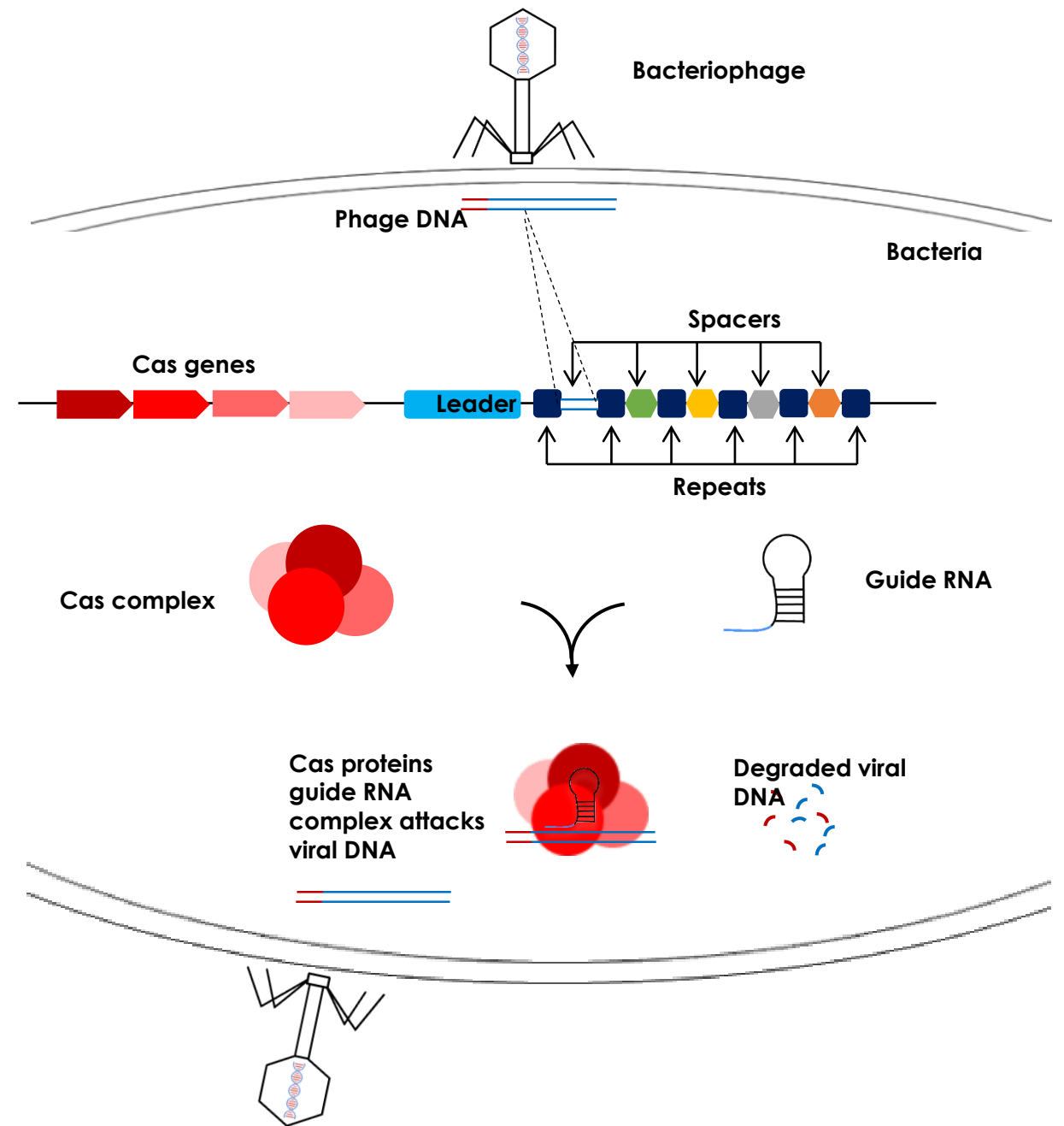
Identification and introgression of novel sources of natural resistance

Transgenic Strategies

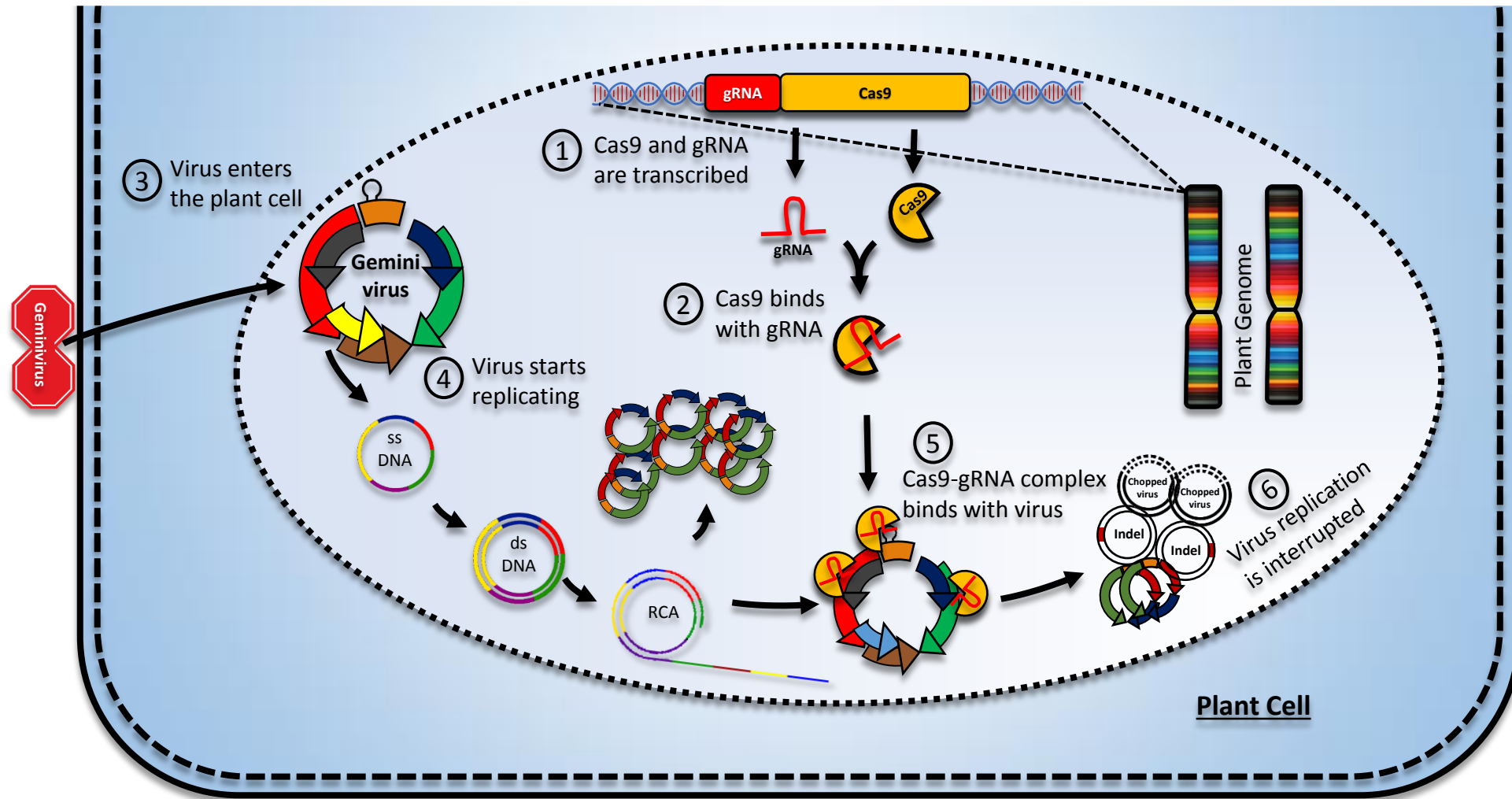
- RNAi mediated resistance
- Tma12 mediated resistance
- **G5** mediated resistance
- **GroEL** mediated resistance
- Genome engineering to target and cleave geminiviruses
 1. **AZP**
 2. TALEN
 3. **CRISPR/Cas9**

CRISPR/Cas9 – A bacterial immunity system

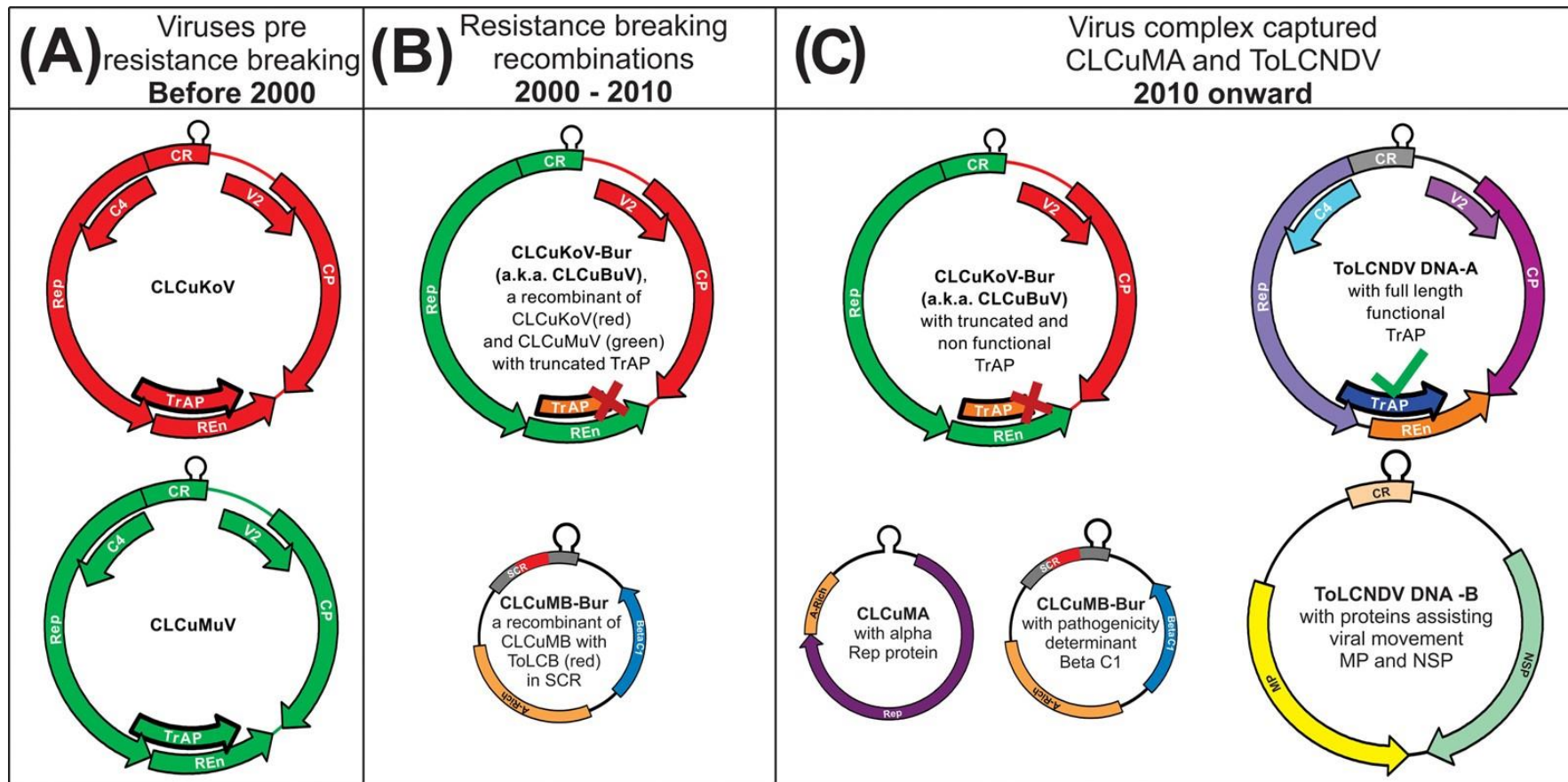
1. Phage infection
2. Spacer acquisition
3. Biogenesis
4. Target degradation



Stepwise depiction of the CRISPR/Cas9-mediated virus interference in the plant cell



Evolution of CLCuD complex during three decades



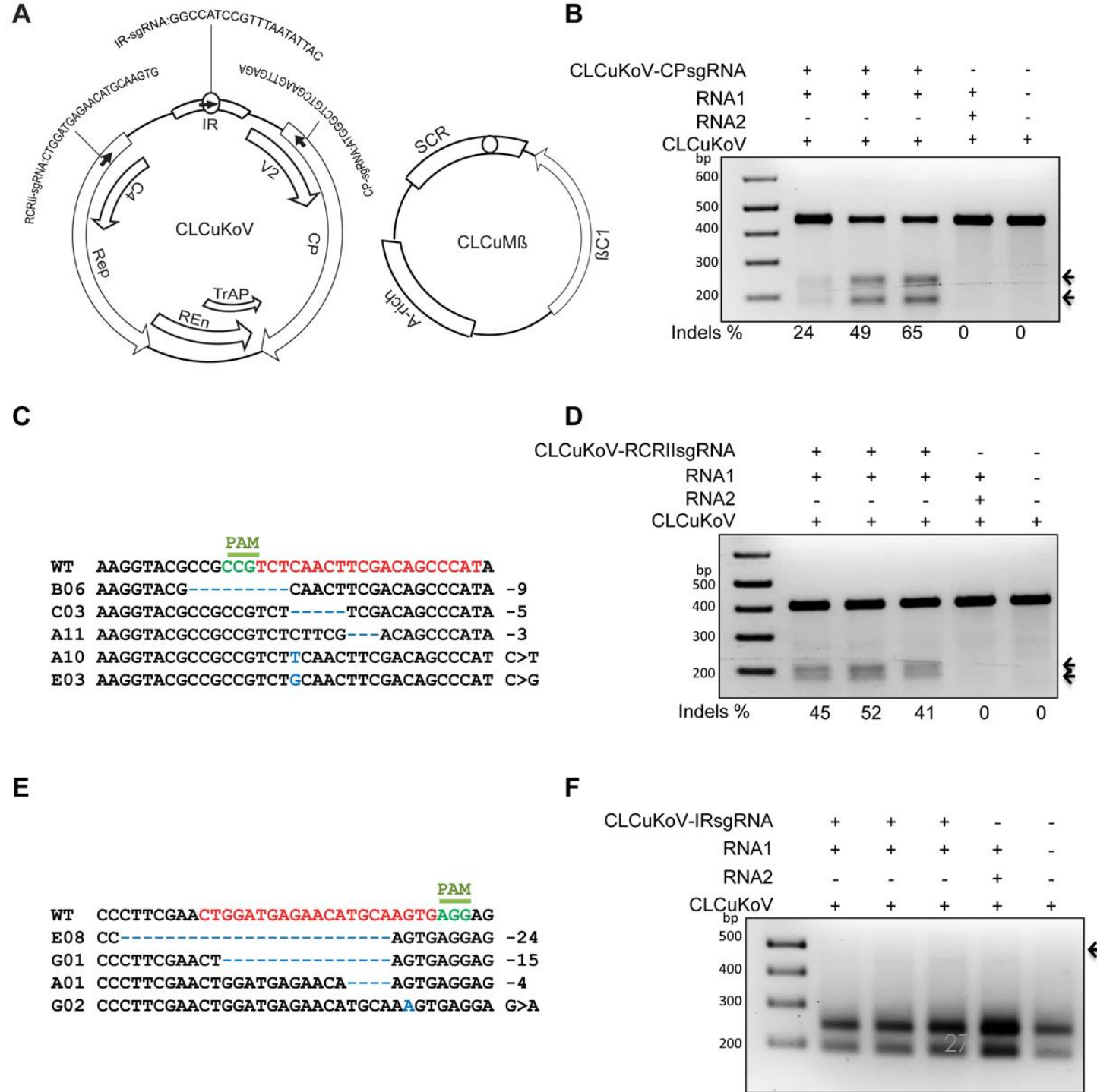
CRISPR/Cas9 mediated viral interference

Target selection

- Nonanucleotide sequence **TAATATTAC** was selected to target through CRISPR/Cas9
- It is conserved among all begomoviruses and betasatellites - expected to give **broad spectrum resistance**
- Two other targets were also selected to target **CP** and **Rep** proteins of virus

CRISPR/Cas9 can target and cleave CLCuKoV

- All three targets (Nonanucleotide, CP and Rep) were targeted and cleaved with the CRISPR/Cas9 machinery
- Indels were detected with T7E1 assay and confirmed with restriction site loss and Sanger sequencing



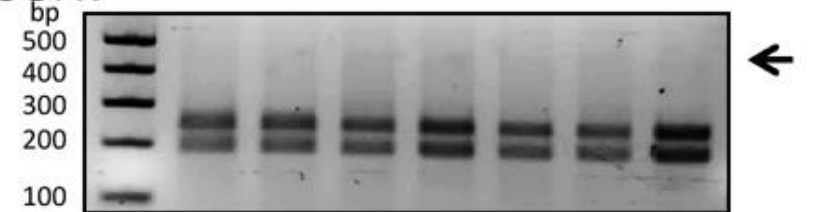
Can CRISPR/Cas9 engineer
one plant resistant to
multiple begomoviruses ?

Targeting conserved region of virus via CRISPR/cas9 can develop broad spectrum resistance

Plants developed to target nonanucleotide via CRISPR/Cas9 could target 6 viruses simultaneously

1. CLCuKoV
2. TYLCV
3. CHF
4. LOGAN
5. MeMV
6. WORLAND

CLCUKV -IRsgRNA	-	-	-	-	-	-	+
TYLCV2.3 -IRsgRNA	-	-	-	-	-	+	-
CFH- IRsgRNA	-	-	-	-	+	-	-
LOGON-IRsgRNA	-	-	-	+	-	-	-
MeMV- IRsgRNA	-	-	+	-	-	-	-
WORLAND - IRsgRNA	-	+	-	-	-	-	-
RNA1	+	+	+	+	+	+	+
RNA2	+	-	-	-	-	-	-
CLCUKV	+	+	+	+	+	+	+

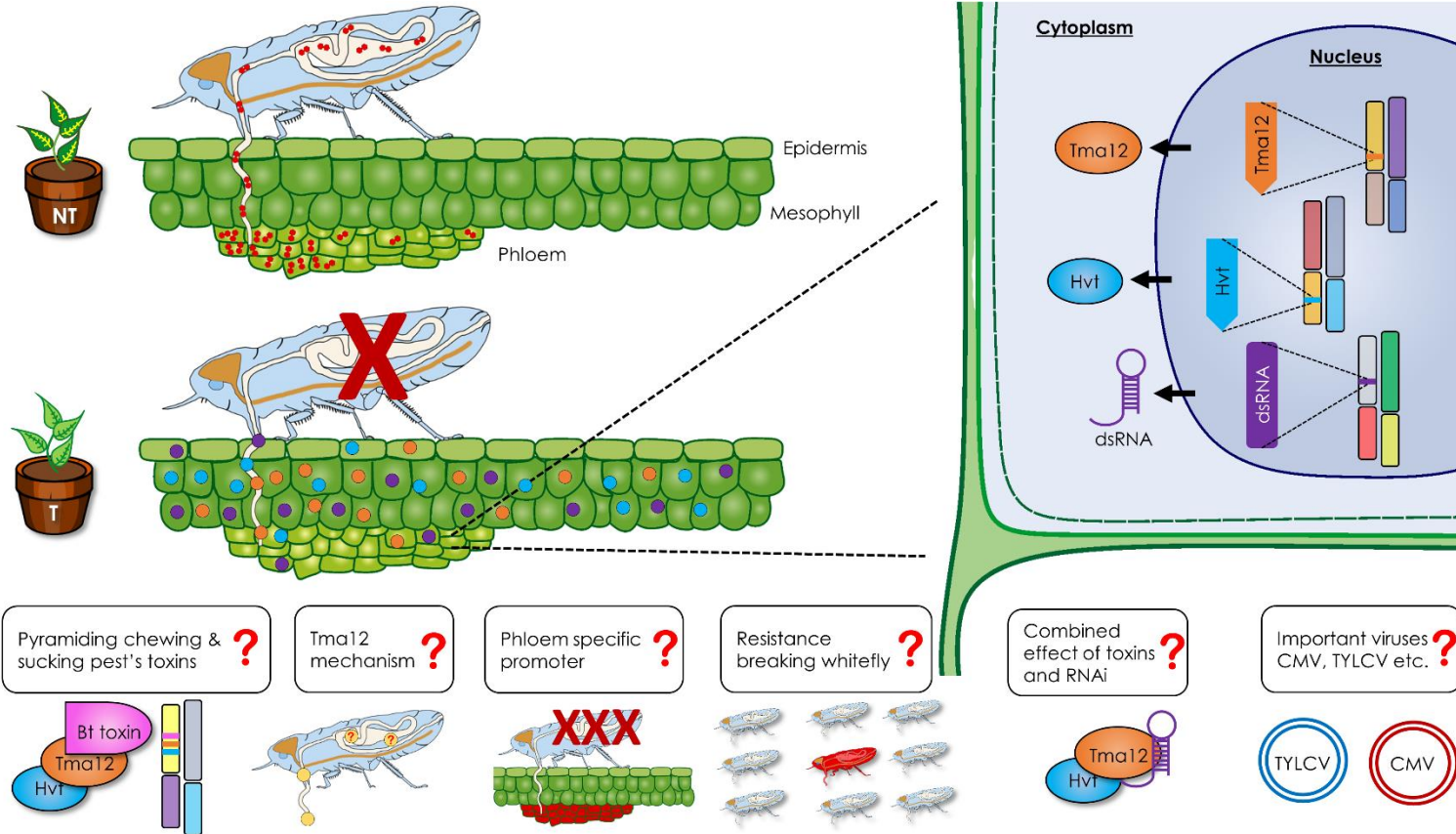


Conclusions

- CRISPR/Cas9 is an efficient strategy to target geminiviruses
- Targeting conserved regions of viruses via CRISPR/Cas9 can provide broad spectrum resistance
- Other broad spectrum resistance strategies like stacking G5-GroEL-AZP, are also promising
- Confirmed transgenic plants developed in this study are valuable resources for future experimentation

Is it possible to create dual virus and whitefly resistance?

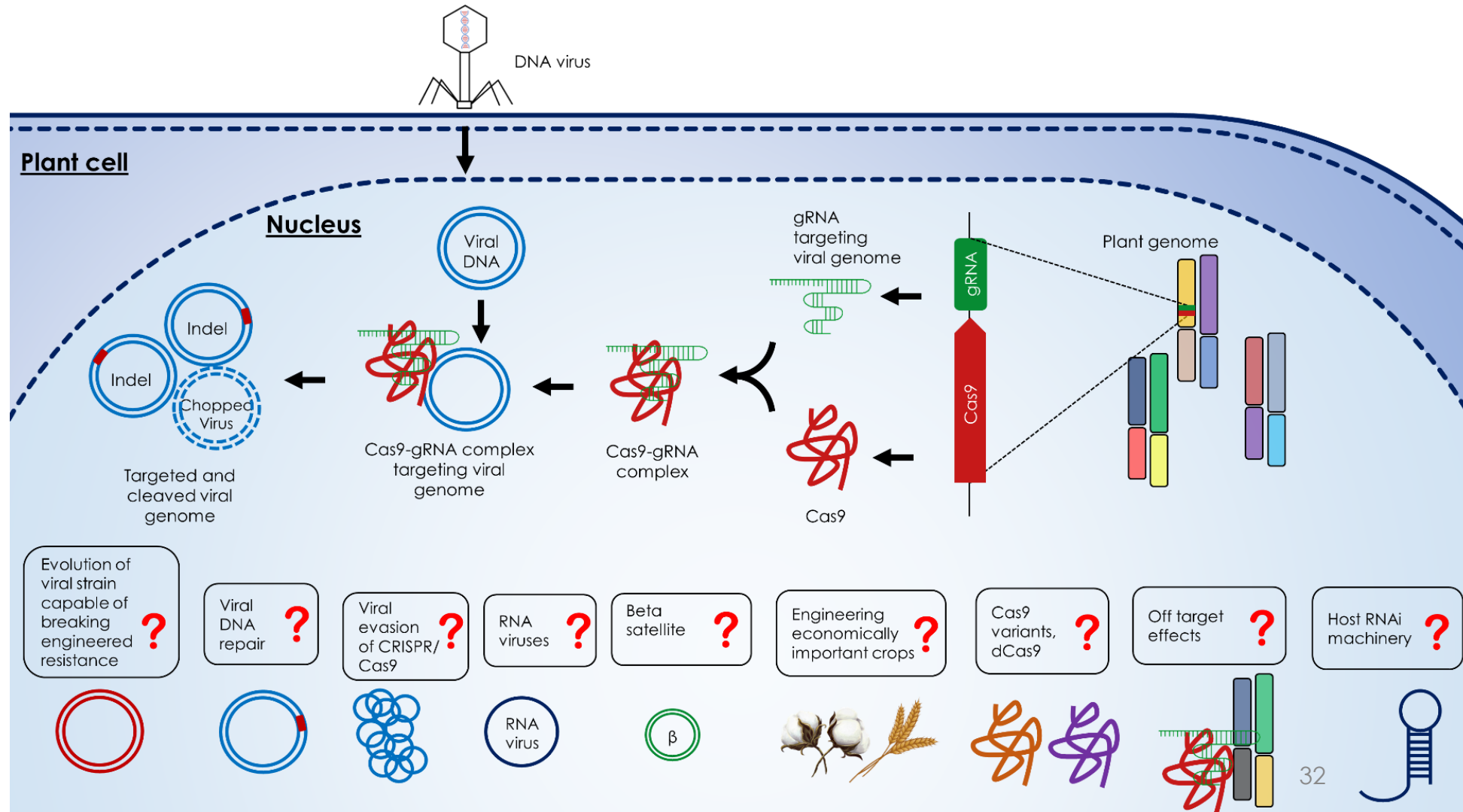
- RNAi
- Protein-mediated resistance



Zaidi SS, Briddon RW, **Mansoor S**.2017. [Engineering dual begomovirus-Bemisia tabaci resistance in plants.](#) Trends in Plant Science. 22(1):6-8. (impact factor 10.899)

Future Prospects

Several issues should be addressed before taking CRISPR/Cas9 strategy to field



Thank you