

Conservación de virus fitopatógenos

Enrique Moriones

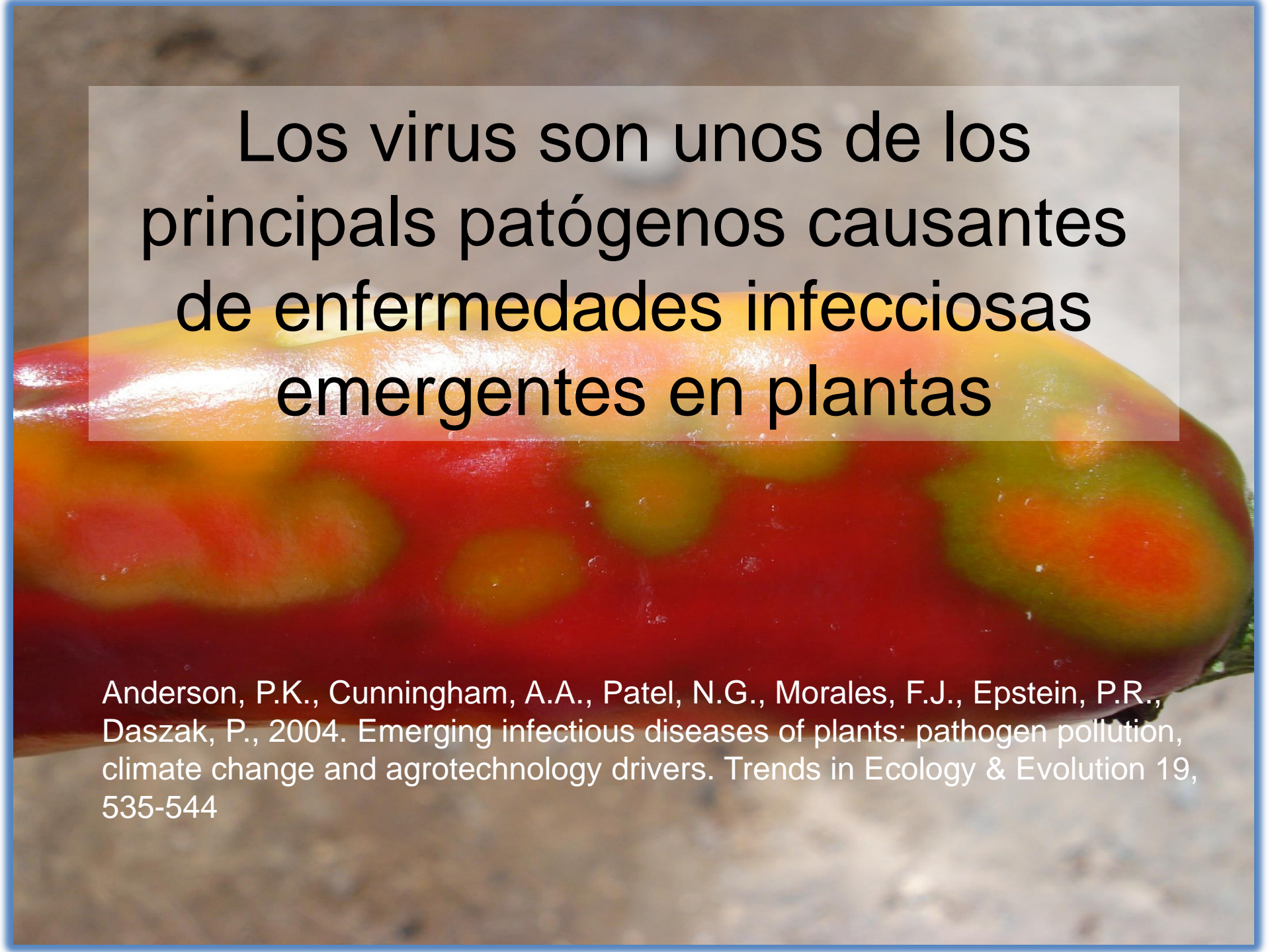
Laboratorio de Virus de Plantas

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Los virus son unos de los principales patógenos causantes de enfermedades infecciosas emergentes en plantas

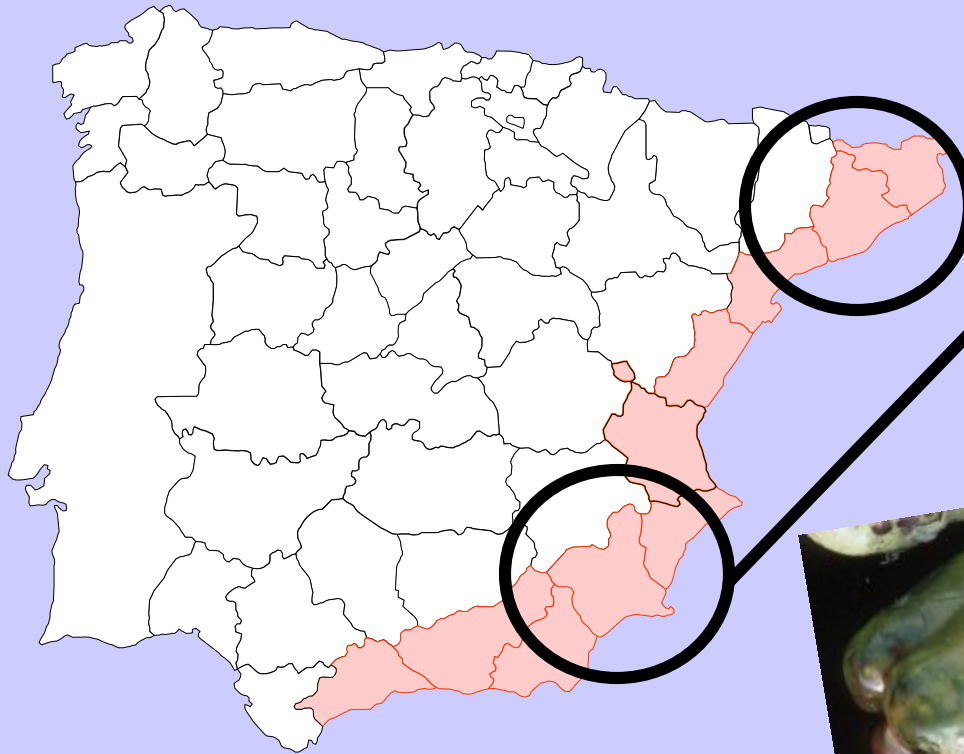
Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., Daszak, P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution* 19, 535-544

Virus "CLÁSICOS"

	TRANSMISIÓN	Tomate	Pimiento
PMMV	Semilla / contacto	-	+
TMGMV	Semilla / contacto	+	+
ToMV	Semilla / contacto	+	-
PVY	Pulgones	+	+
CMV	Pulgones	+	+

1980s -: V. bronceado del tomate

Tomato spotted wilt virus (*Tospovirus*, *Bunyaviridae*)



Tomate **Sw5...**

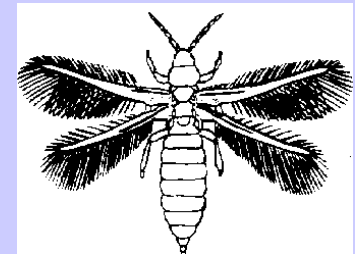
Pimiento **Tsw...**

Lechuga

Alcachofa

Habas

.....



Frankliniella occidentalis

1980s:

Trialeurodes vaporariorum

BPYV

Beet pseudoyellows virus
(*Crinivirus*, *Closteroviridae*)



1980s:

Bemisia tabaci

¡Amarillos de las cucurbitáceas!



1990s...: *Bemisia tabaci*

Virus transmitidos por Bemisia

VIRUS EMERGENTES

- Begomoviruses (Fam. *Geminiviridae*) DNA
- Criniviruses (Fam. *Criniviridae*) RNA
- Ipomoviruses (Fam. *Potyviridae*) RNA
- Torradoviruses (Picorna-like) RNA



CYSDV



ToCV



TToV



CVYV



TYLCV

¡Virus del mosaico del pepino dulce!

PepMV

Pepino mosaic virus (Potexvirus)

TRANSMISIÓN :

- . semilla
- . contacto



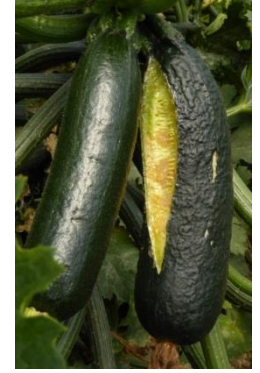
Torrado del tomate

genus *Torradovirus*, family *Secoviridae*



EMERGENCIAS RECIENTES:

- **Tomato leaf curl New Delhi virus en calabacín (Begomovirus)**



Pérdidas hasta 100%

- **Pepper vein yellowing virus** (*Polerovirus* / *Luteoviridae*)

- **Cucumber green mottle mosaic v.** (*Tobamovirus* / *Virgaviridae*)



Families and Genera of Viruses Infecting Plants

DNA

dsDNA (RT)
Caulimoviridae
 Caulimovirus
 Petuvirus
 Soymovirus
 Cavemovirus
 Badnavirus
 Tungrovirus

ssDNA

Geminiviridae
 Mastrevirus
 Curtovirus
 Topocovirus

Begomovirus

Nanoviridae

RNA

dsRNA

Reoviridae
 Fijivirus
 Phytoreovirus
 Oryzavirus

Partitiviridae
 Alphacryptovirus
 Betacryptovirus

100 nm

ssRNA (-)

Bunyaviridae
 Tospovirus

Rhabdoviridae
 Cytorhabdovirus
 Nucleorhabdovirus

Tenuivirus
 Ophiovirus

Varicosavirus

ssRNA (RT)

Pseudoviridae
 Pseudovirus
 Sirevirus

Metaviridae
 Metavirus

ssRNA (+)

Luteoviridae
 Sobemovirus
Sequiviridae
 Sadwavirus
 Cheravirus
Tombusviridae
 Tymoviridae

Bromoviridae
 Cucumovirus
 Bromovirus
 Ilarvirus
 Alfamovirus
 Ourmiavirus

Comoviridae
 Idaeovirus

Tobamovirus
 Tobravirus
 Hordeivirus
 Pecluvirus
 Furovirus
 Pomovirus
 Benyvirus
Flexiviridae
Potyviridae
Closteroviridae

CONSERVACIÓN DE VIRUS

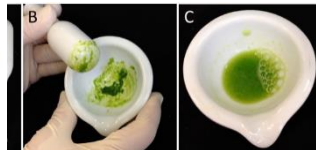
- Localización en planta
 (forma de inoculación / conservación)
- Estabilidad de la partícula

INOCULACIÓN MECÁNICA - CONSERVACIÓN LIOFILIZADO



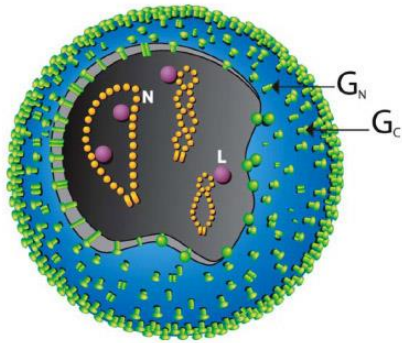
4 °C - En seco
(silica gel, CaCl₂)

- Cucumovirus (CMV, AIMV, PSV, ...)
- Tobamovirus (ToMV, TMV, PMMV, CGMMV, ...)
- Potyvirus (PVY, LMV, ZYMV, WMV, PRSV, ...)
- Potexvirus (PepMV, ...)
-

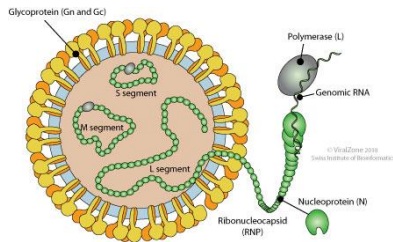


INOCULACIÓN MECÁNICA - CONSERVACIÓN TEJIDO CONGELADO

Tospovirus (TSWV)

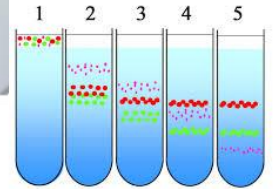
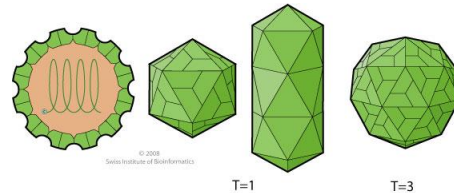
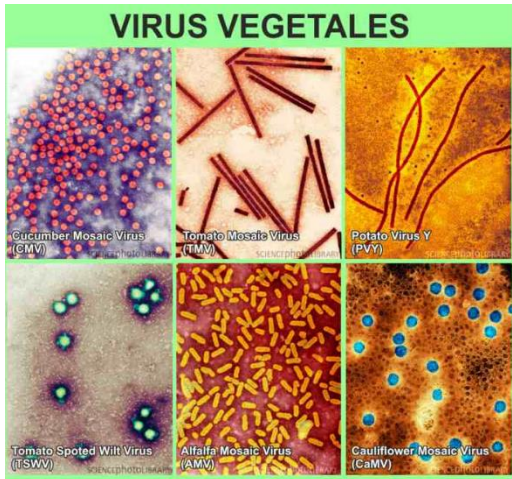


N2 líquido



-80 °C

INOCULACIÓN MECÁNICA - CONSERVACIÓN PURIFICADO VIRIONES o ÁCIDOS NUCLEICOS



- Cucumovirus (CMV, AIMV, PSV, ...)
- Tobamovirus (ToMV, TMV, PMMV, CGMMV, ...)
- Potyvirus (PVY, LMV, ZYMV, WMV, PRSV, ...)
-

- VIRION (15% glycerol: -20 °C, -80 °C)
- RNA (-20 °C)



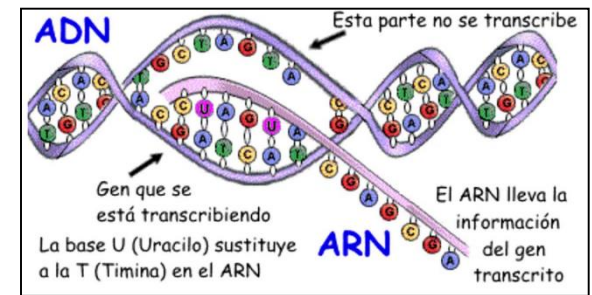
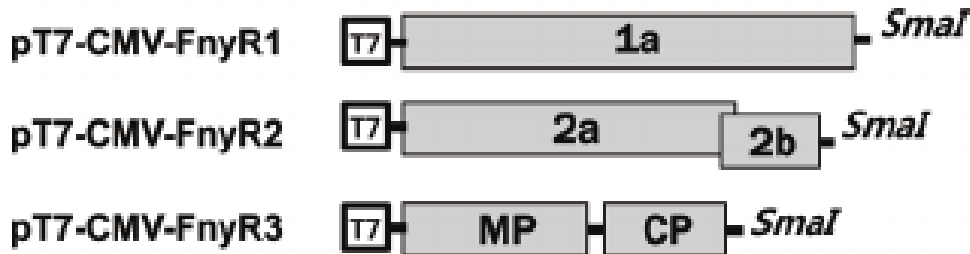
INOCULACIÓN MECÁNICA - CONSERVACIÓN CLONES MOLECULARES



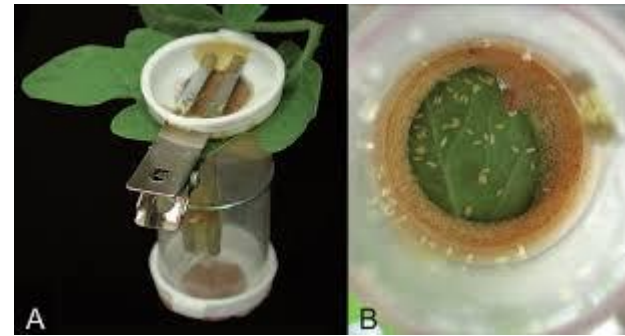
-20 °C

Cucumovirus, potyvirus,
potexvirus,

Transcripción genética
(polimerasa T7; transitoria ·35S)



RESTRINGIDOS A FLOEMA - CONSERVACIÓN IN VIVO (begomovirus -TYLCV, ToLCNDV-, crinivirus - ToCV, CYSDV, CTV - ...)

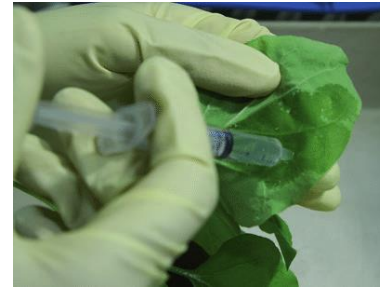
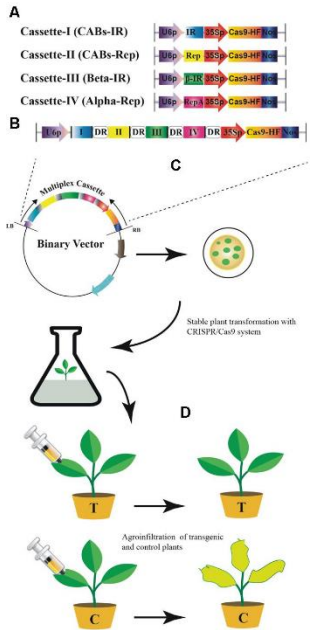
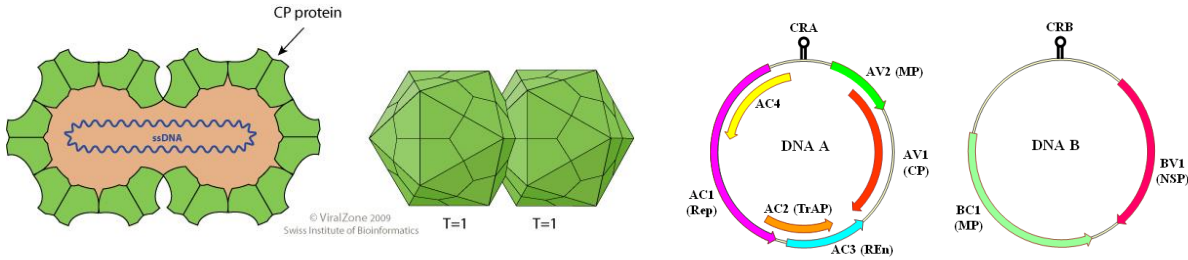


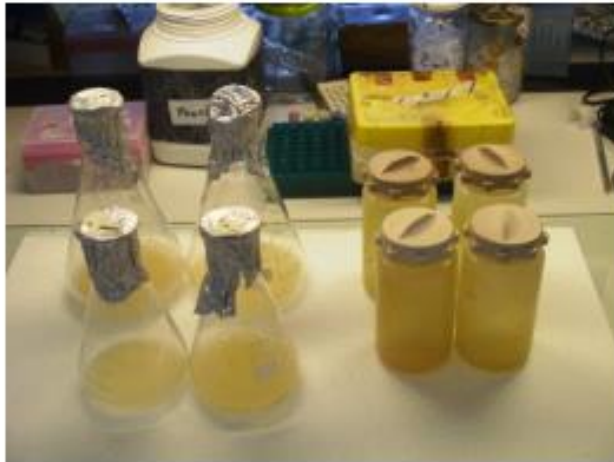
RESTRINGIDOS A FLOEMA - CONSERVACIÓN CLONES MOLECULARES - Agroinoculación (*Agrobacterium tumefaciens*)

crinivirus - ToCV, CYSDV, CTV- ...

Transcripción transitoria promotor 35S

BEGOMOVIRUS





Agrobacterium tumefaciens culture
harboring the infectious viral clone

Inoculation of plants with infectious virus clone using *Agrobacterium tumefaciens* culture (agroinoculation).



In nature

In the laboratory

Healthy plant



Agroinoculation using *A. tumefaciens* infectious culture

Introduction of the Ti-plasmid into *Agrobacterium tumefaciens*

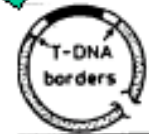
Bemisia tabaci insect vector carries viruses after feeding on infected plant



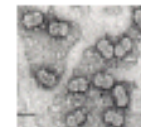
Geminivirus Infected plant



Subcloning of infectious virus genome copy into *A. tumefaciens* Ti-plasmid



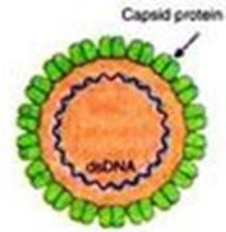
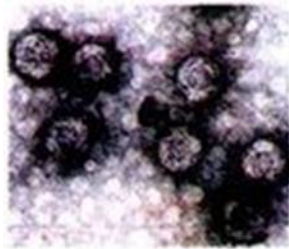
Cloning of infectious virus genome copy into *Escherichia coli* plasmid



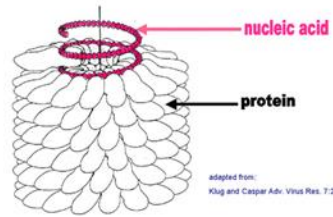
Extraction of virus nucleic acids



¡¡GRACIAS!!



TOBACCO MOSAIC VIRUS



adapted from:
Klug and Caspar Adv. Virus Res. 7:225

