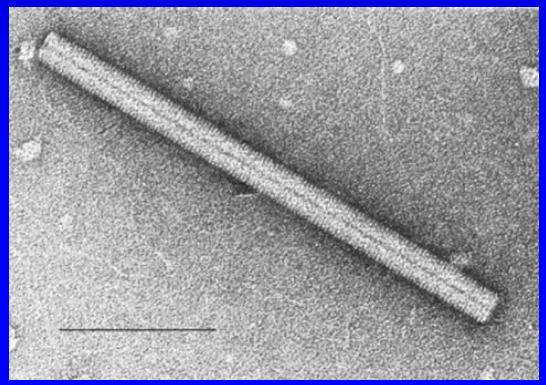
Emergence, detection and management of the new tobamovirus *Tomato brown rugose fruit virus* (ToBRFV)



Robert L. Gilbertson
Department of Plant Pathology
University of California Davis

Photos: N. Salem

There is a remarkable diversity of viruses that infect plants

- Most plant viruses have ssRNA genomes
- Diversity in virion shape and size and the nature of the genome
- Genetically flexible and respond to changes (selection pressure)
- Disease symptoms do not allow species ID
- A large number and diversity of viruses infect tomato
- IPM is the best management approach

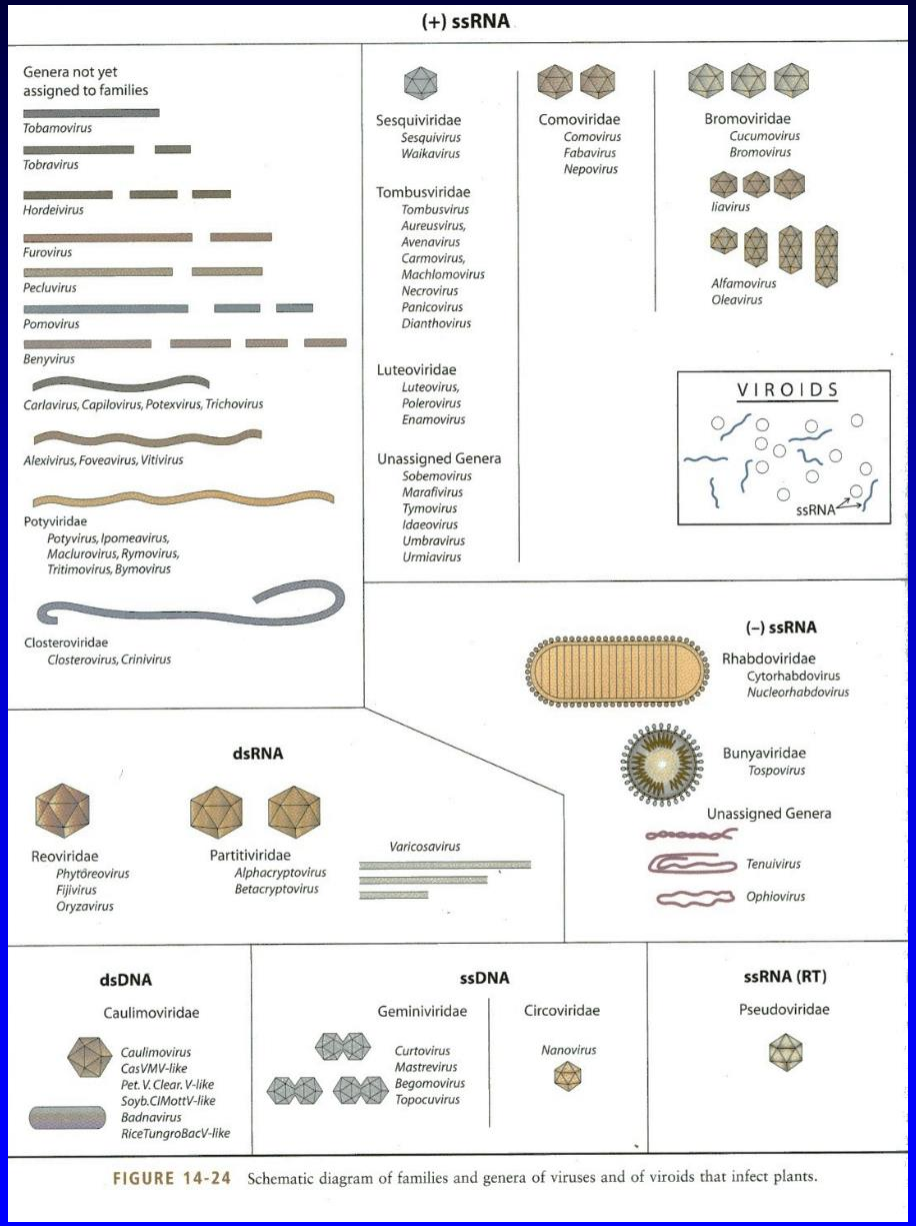
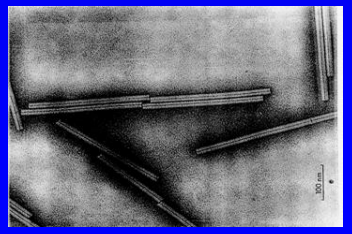


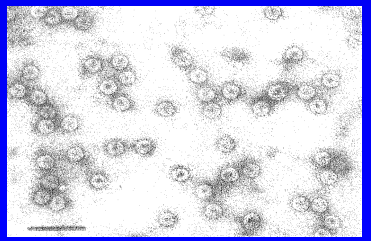
FIGURE 14-24 Schematic diagram of families and genera of viruses and of viroids that infect plants.

Different viruses can cause very similar symptoms

Tobacco mosaic virus
symptoms in tobacco

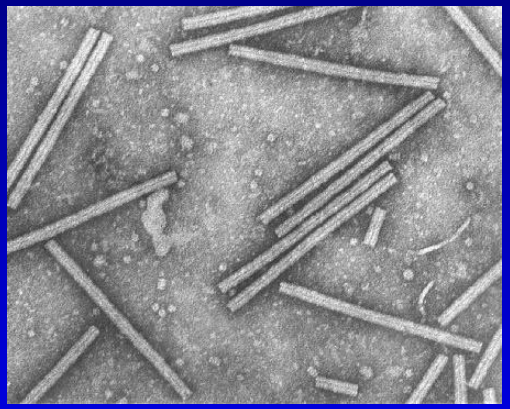


Cucumber mosaic virus
symptoms in tobacco



What is ToBRFV?

- A **new species** of a well-known group of viruses: **tobamoviruses**
- family *Virgaviridae*, genus *Tobamovirus*
- Genus name derived from **type species**: *Tobacco mosaic virus* (TMV)
- There are 37 **recognized species**
- All tobamoviruses possess: **rigid rod-shaped virions** and a **monopartite positive-sense RNA genome** (~6.4 kb)
- Virions are **extremely stable**
- **No insect vector**-transmitted by contact and touch facilitated by **activities of humans**
- TMV is one of the most **extensively characterized** viruses



Multiple tobamoviruses infect tomato

- **At least five tobamoviruses infect tomato and induce similar symptoms:**
  - ***Tobacco mosaic virus*** **(TMV)**
  - ***Tomato mosaic virus*** **(ToMV)**
  - ***Tobacco mild green mosaic virus*** **(TMGMV)**
  - ***Tomato mottle mosaic virus*** **(ToMMV)**
  - ***Tomato brown rugose fruit virus*** **(ToBRFV)**

		Symptoms	Symptoms			
Virus	Emergent	Leaves	Fruit	Tm-2^2	Distribution	Importance
TMV	No	Mo, Di, SS	Browning*	Resistant	WW	Low
ToMV	No	Mo. Di, SS	Browning*	Resistant	WW	High
TGMMV	No	Mo, Di, SS	Few or none	Resistant	WW	Medium
ToMMV	Yes (2013)	Mo, Di, SS	Few or none	Susceptible	MX, USA, ME, Spain	Medium
ToBRFV	Yes (2015)	Mild Mo	Necrotic lesion	Susceptible	ME, MX, USA, Europe	High

Emergence of ToBRFV: déjà vu all over again?

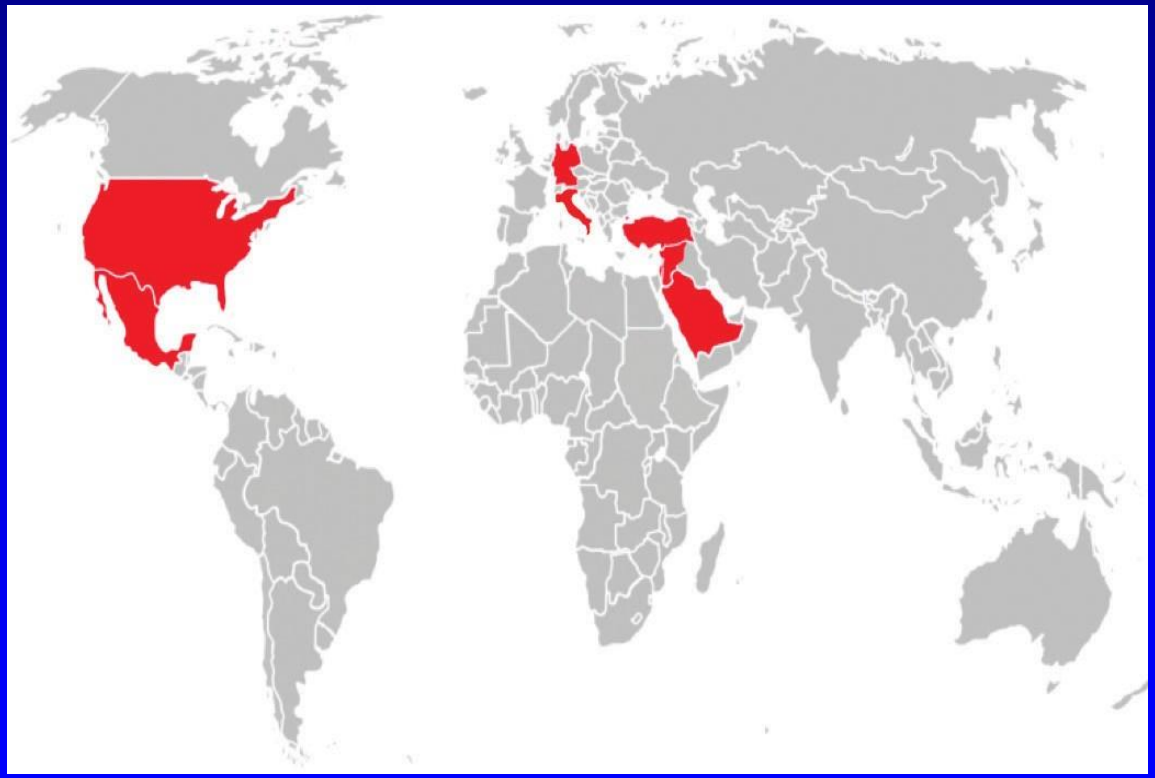
- First observed in Middle East (Jordan) in 2015
- Associated with tobamovirus symptoms on resistant tomato varieties grown in protected culture
- Virus was readily mechanically transmitted and samples tested positive for tobamovirus infection (ELISA, RT-PCR with general primers)

- Sequencing revealed it was a new tobamovirus species, most closely related to TMV (Ohio V) at 82.4% identity
- Spread to southern Israel and is causing substantial losses on resistant (Tm-2^2) varieties
- Another example of emergence of a resistance-breaking virus and the genetic flexibility of these pathogens

Photo: N. Salem

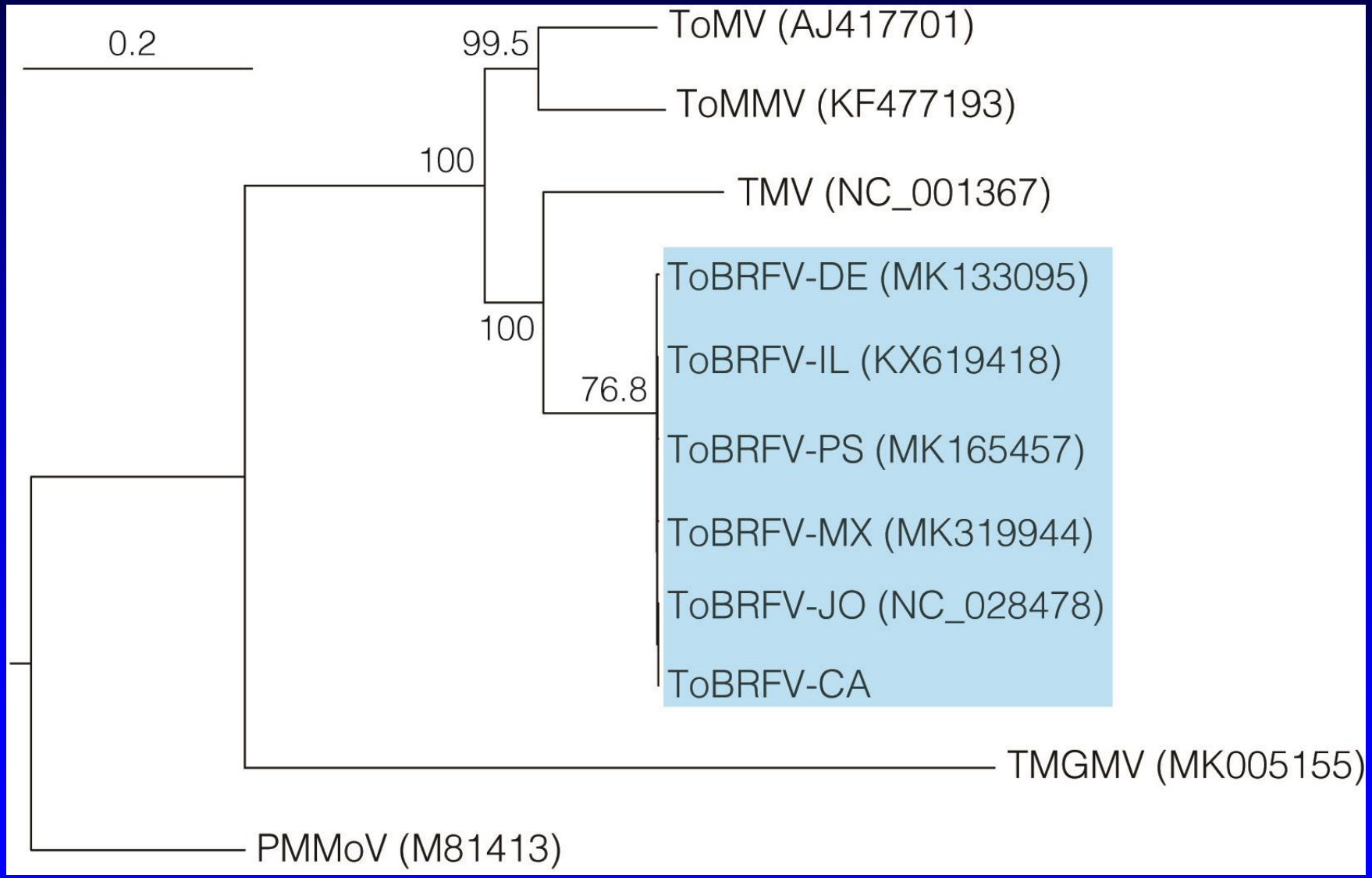
Emergence of ToBRFV: déjà vu all over again?

- Spread to **Europe** in 2018
- Major outbreak(s) in **Mexico**
- **Detected in USA** (California) in 2018

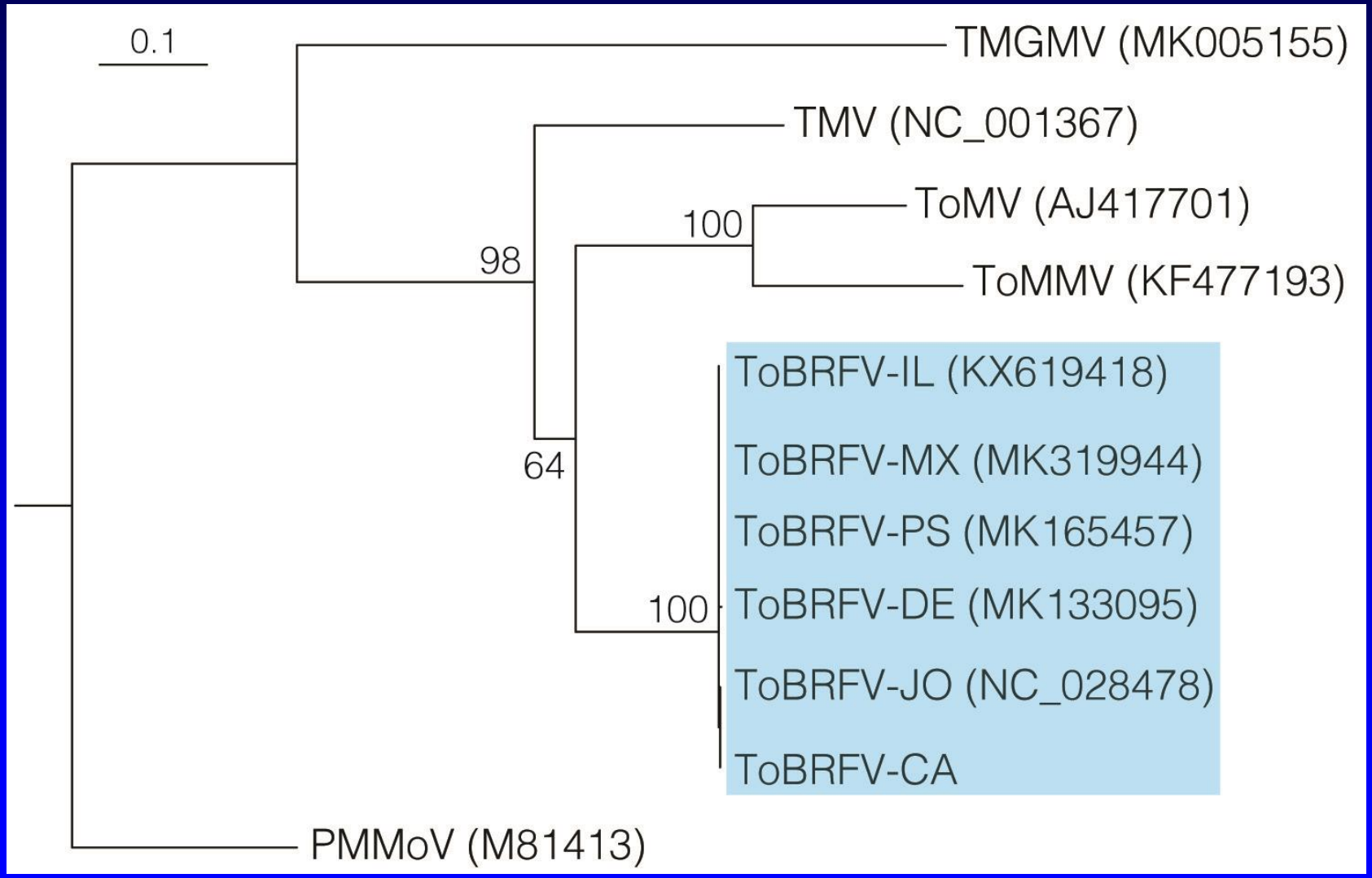


Long-distance spread of ToBRFV

Rooted phylogenetic tree derived from the nucleotide sequences of the complete viral genome



Rooted phylogenetic tree derived from the nucleotide sequences of the viral gene that encodes the movement protein (MP)



ToBRFV isolates are closely related and genetically divergent from other tomato-infecting tobamoviruses

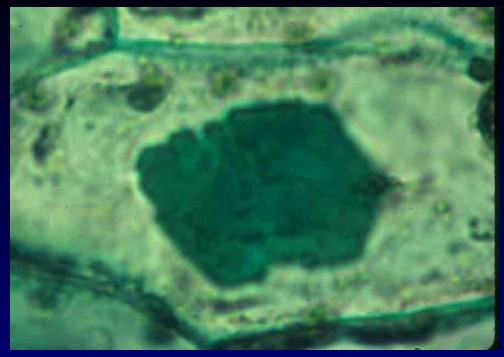
TABLE 1. Relative level of sequence diversity of the *Tomato brown rugose fruit virus* (ToBRFV) isolate of California and other closely related tobamovirus species

ToBRFV and tobamovirus species	Year	Location[a]	Genome and open reading frames (ORFs)							
			Total	Replication Proteins				Movement protein		Capsid protein	
				126-kDa		183-kDa					
				nt	aa	nt	aa	nt	aa	nt	aa
ToBRFV [tomato]	2015	JO	99.9	99.9	100 (100)	100	100 (100)	100	100 (100)	99.4	100 (100)
ToBRFV [BC]	2018	MX	96.7	99.8	99.8 (99.9)	99.9	99.9 (99.9)	99.9	99.6 (99.6)	99.4	100 (100)
ToBRFV [tomato:Ps]	2018	PS	99.7	99.8	81.5 (84.2)	99.8	87.2 (89.1)	99.8	89.5 (91.7)	100	100 (100)
ToBRFV [tomato]	2014	IL	99.8	99.9	99.9 (100)	99.9	99.9 (100)	99.9	99.6 (99.6)	99.4	100 (100)
ToBRFV[tomato]	2018	DE	99.7	99.7	99.7 (99.8)	99.7	99.6 (99.8)	99.6	99.2 (99.2)	99.4	100 (100)
TMV [TMVgp1]	ND	ND	81.8	81.6	92.8 (97.0)	82.4	93.6 (97.4)	75.1	78 (84.3)	82.7	89.3 (95.6)
ToMV [camellia]	ND	CH	81.0	80.8	92.6 (96.5)	81.7	92.7 (96.4)	76.6	78.3 (84.6)	78.5	86.2 (91.8)
ToMMV [tomato:mx5]	2009	MX	80.9	80.5	91.5 (96.0)	81.9	92.1 (96.1)	74.1	70.9 (81.3)	80.0	86.2 (91.2)
TMGMV [*N. glauca*]	2018	BR	65.3	63.7	64.7 (77.6)	66.0	68.2 (80.1)	62.2	53.6 (67)	66.0	70.4 (84.9)

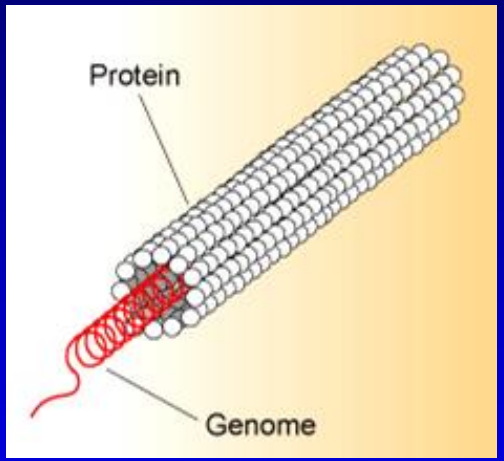
The tobamoviruses used for comparison and their corresponding GenBank accession numbers are: ToBRFV (NC_028478, MK319944, MK165457, KX619418 and MK133095), *Tobacco mosaic virus* (TMV) (NC_001367), *Tomato mosaic virus* (ToMV) (AJ417701), *Tomato mottle mosaic virus* (ToMMV) (KF477193) and Tobacco mild green mosaic virus (TMGMV) (MK005155). ND, not determined.

[a] BR, Brazil; CH, China; DE, Germany; IL, Israel; JO, Jordania; MX, Mexico; PS, Palestine.

Tobamoviruses share many biological properties



- **Stable virions** allow efficient transmission by contact and persistence in production systems, including in soil
- **Humans are the main vector** of tobamoviruses!
- **Seed transmission**-mostly via contamination of the seed coat-seed can be a primary source of inoculum
- **Symptoms vary** among tobamovirus strains and species
- **Methods for diagnosis** are available (indicator plants, serology [immunostrips and ELISA], RT-PCR and general/specific primers], and RT-qPCR) but for ToBRFV, **sequencing is still required**

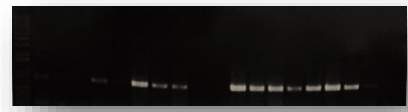


Identification of ToBRFV in resistant tomato cultivars with tobamovirus symptoms

Typical tobamovirus symptoms in Tm-2^2 varieties

Positive test with TMV immunostrips

Confirm tobamovirus by RT-PCR



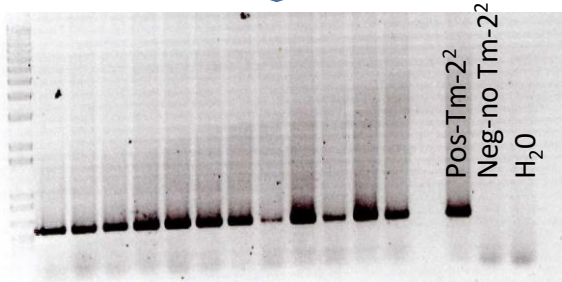
Confirm ToBRFV by sequencing RT-PCR fragments and comparing with database

If sequence is >90% identical to ToBRFV → **Isolate of ToBRFV**

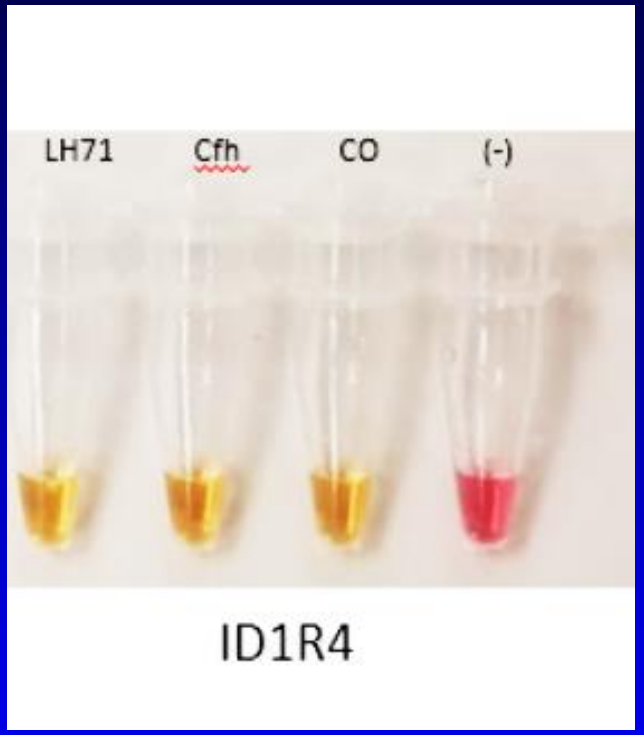
If sequence is >90% identical to other tobamovirus → **Isolate of this tobamovirus**

If sequence is <90% identical to tobamovirus sequences → **Isolate may be a new tobamovirus**

Confirm tomato is a resistant variety by PCR for Tm-2^2 gene



A LAMP assay for detection of ToBRFV could allow for rapid identification in leaf tissue



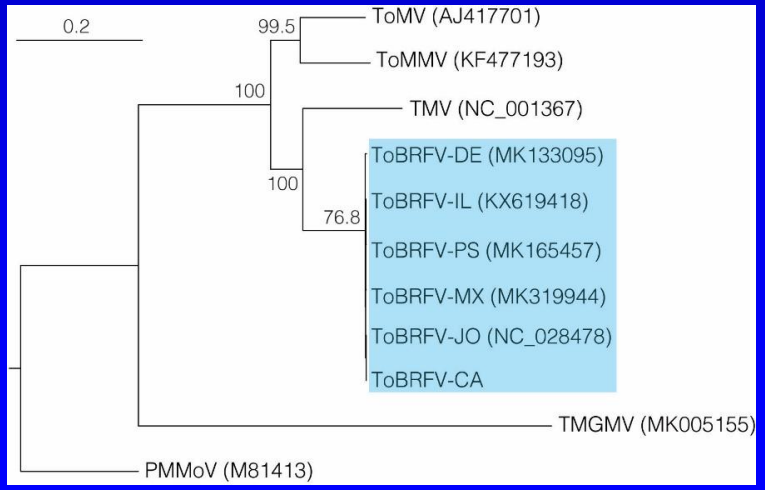
LAMP assay for *Beet curly top virus*

- Sample preparation: grind leaves in buffer in a plastic bag
- Results are obtained in 30-40 min and based on a color change from red to yellow for a positive test
- Requires minimal laboratory equipment

What is different about ToBRFV?

- More **rapid spread**-plants maintain a higher level of virus?
- Higher levels of **seed contamination**?
- **Breaks Tm-2^2**
- **Symptoms**: mild mosaic and distortion in leaves and discoloration, malformation and necrotic lesions in fruit
- **Sequence** of the viral genome (RNA)- relatively **divergent** and may be **recombinant**
- Fair to assume that ToBRFV shares many properties with other tomato **tobamoviruses** and that similar management tools can be used

Photo: N. Salem



IPM of ToBRFV

- **Before the growing season**
  - -Virus-free seeds/transplants
  - -Resistant/tolerant varieties*
  - -Disinfestation within the production system
- **During the growing season**
  - -Monitor for symptoms and remove infected plants
  - -Worker and other sanitation
  - -Minimize touching of plants
  - -Effective diagnostics
  - -Removal of infected plants
- **After the growing season**
  - -Sanitation, sanitation, sanitation
  - -Rotation
- **Long term**
  - -Identify sources of resistance
  - -Cross protection
  - -Grafting on resistant rootstocks (eggplant)

Greenhouse disinfectant-another product is Virkon

Cross protection of tomato with a mild ToMV strain

Comparison of methods for detection of ToBRFV from tomato (and other) seed

- Tests for ToBRFV associated with seed
  - -Bioassay
  - -ELISA (prescreen)
  - -qPCR
  - -RT-PCR (Mexico)
- Need for standardization
- ASTA-supported comparison of selected lots with different tests
- Share results make conclusions

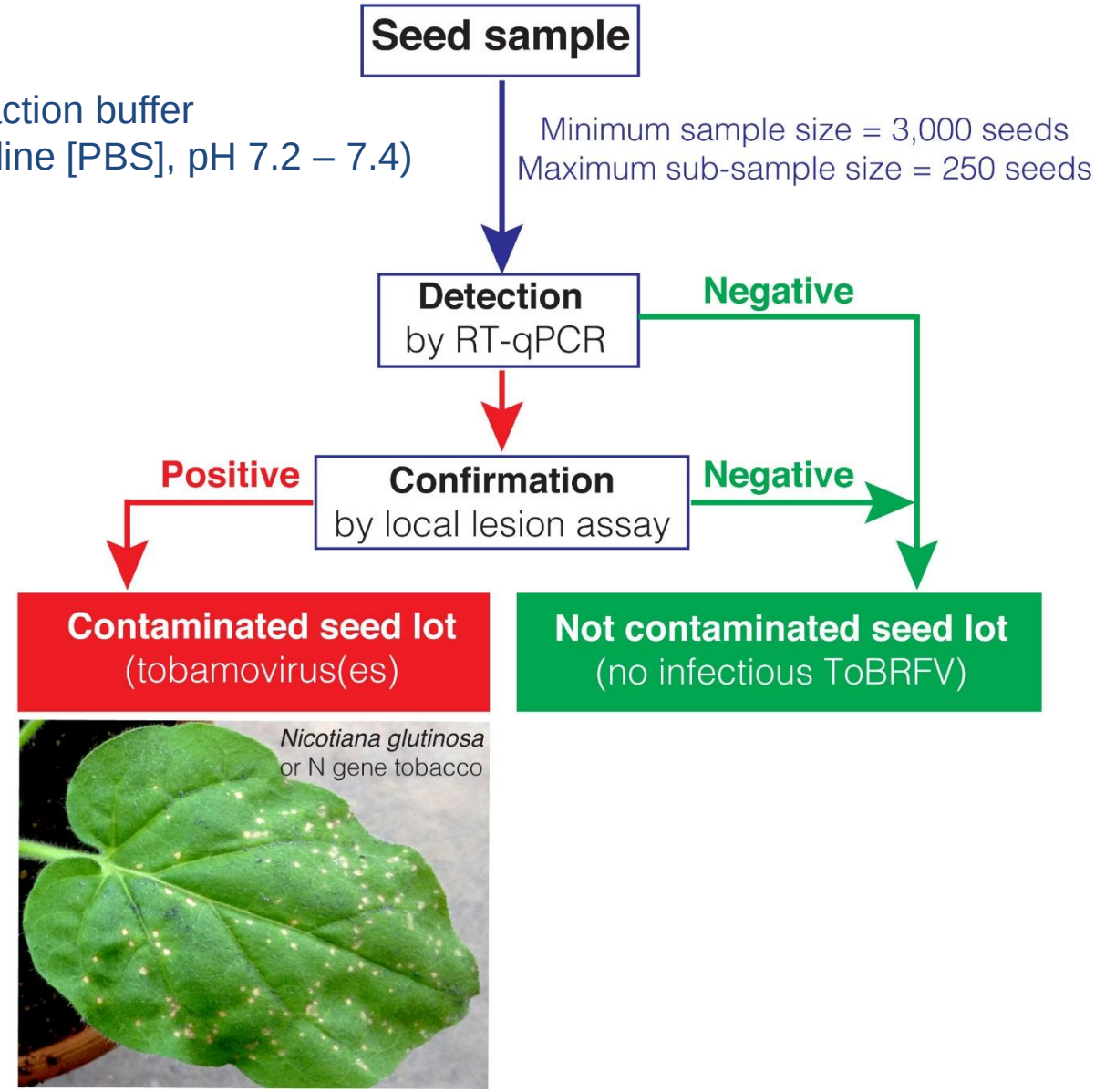
Local lesions induced by *Tomato mosaic virus* (ToMV in *N* gene tobacco

Detection of *Tomato brown rugose fruit virus* (ToBRFV) in tomato and pepper seed (ISHI-Veg)

Seed extraction buffer
(phosphate buffered saline [PBS], pH 7.2 – 7.4)

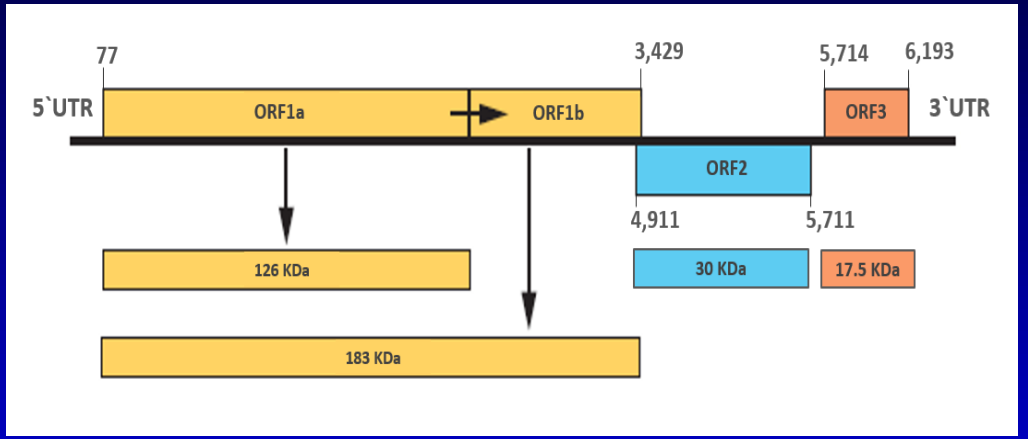
Name	Source
CaTa28 Fw	Enza Zaden B.V. Netherlands
CaTa28 Pr	
CaTa28 Rv	
CSP1325[1] Fw	CSP Labs USA
CSP1325 Pr	
CSP1325 Rv	
BaCV-F	Naktuinbouw Netherlands
BaCV-R	
BaCV-P	

Fw: Forward
Rv: Reverse
Pr: Labeled Fluorescent Probes



Aspects of Tm-2^2 breaking by ToBRFV

- Mechanisms
- Experimental systems
- Symptom determinants and mild strains
- Genetic diversity



Genome organization of ToBRFV (6393 nt)

-One +-sense ssRNA

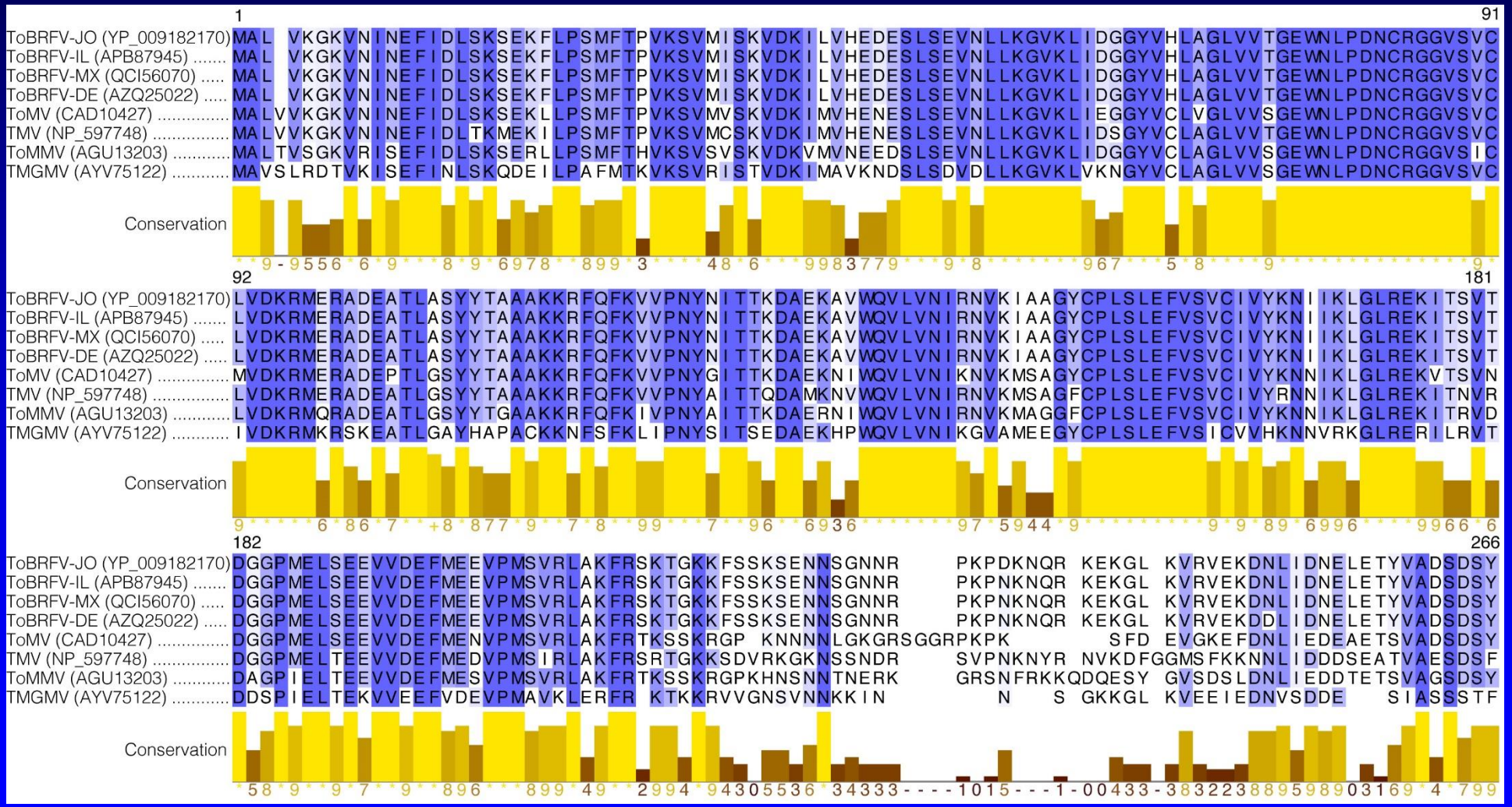
-Four genes:

ORF1a and 1b-126/183 K-replication

ORF2-30 K-movement and Tm-2^2 effector

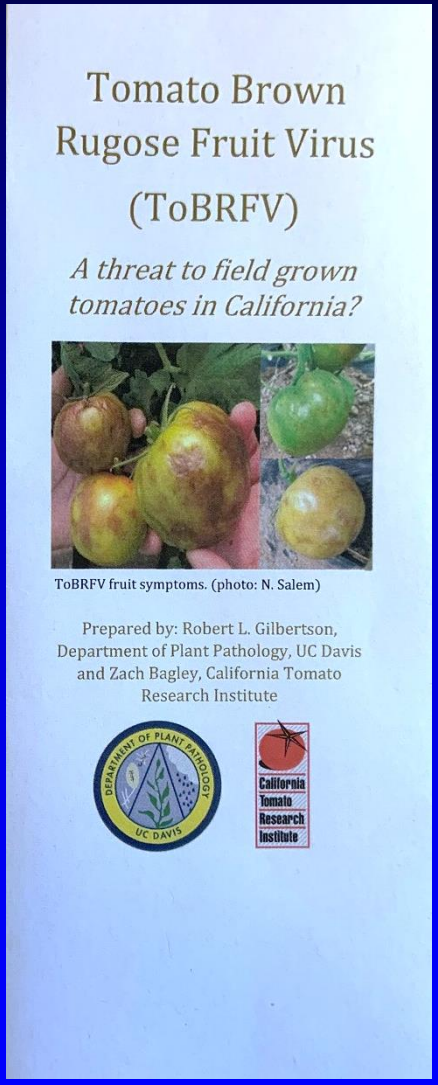
ORF3-17 K-capsid protein-virion formation

Alignment of the amino acid sequences of the movement protein of five selected tobamovirus that infect tomato



Integarated pest management of ToBRFV

- Site visits
- IPM strategy
  - -Identify tools
  - -General or site- or production-specific strategy
  - -Evaluate in pilot study with selected growers
- Outreach
  - -Information (e.g., ASTA and UCD flyers)
  - -Grower meetings
- Monitor awareness and uptake
  - -Surveys
  - -Grower meetings



Research team

UC Davis

- Dr. Tomas Melgarejo
- Ms. Marcela Vasquez
- Mr. Minor Maliano
- Dr. Maher Al Rwahnih
- Dr. Robert L. Gilbertson

CSP Labs

- Sukhi Pannu

ASTA

- Ric Dunkle
- Mary Scott