

Tomato infecting viroids: West African Example



Question: What is a viroid?

- ❖ Viroid is the smallest known pathogen of plants and the first one was discovered by T.O. Diner in 1971 (*Potato spindle tuber viroid*; PSTVd)
- ❖ Viroids are classified in a distinct group of virus-like agents that replicate autonomously in plants and infect systemically.
- ❖ Viroids consist of a covalently closed single-stranded circular RNA molecule, whose length varies between 246 and 401 nucleotides.
- ❖ Because of internal base pairing between the nucleotides, viroids look rod-shaped in vitro.
- ❖ Viroids are 'naked' and differ from viruses as they lack a protein shell
- ❖ Viroids can be classified as non-coding RNAs as there is no evidence of RNA translation and viroids do not encode for any protein.
- ❖ Viroids are classified into two families, the *Pospiviroidae* and the *Avsunviroidae*.

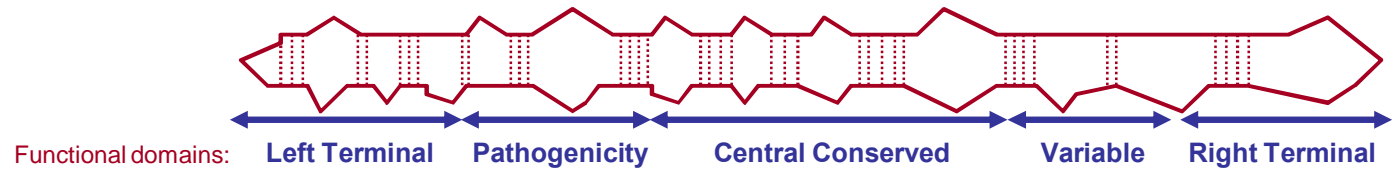
Introduction

Species and they are divided into two families:

-Pospiviroidae, with central conserved regions (CCR) but without hammerhead ribozymes (type member **Potato spindle tuber viroid, PSTVd**)



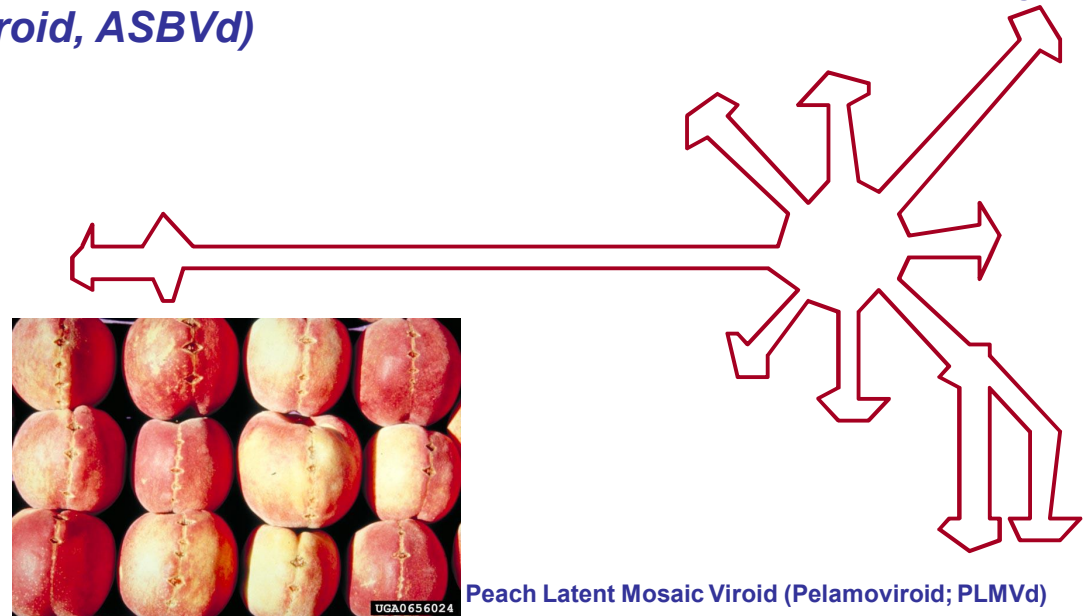
Image source: <http://www.sciencemag.org>



-Avsunviroidae, that lack CCR and are able to self-cleave, with their hammerhead ribozymes (type member **Avocado sunblotch viroid, ASBVd**)



Image source: <http://www.avocadosource.com>



Peach Latent Mosaic Viroid (Pelamoviroid; PLMVd)

Image source: <http://www.forestryimages.org>

Introduction

REPLICATION SITE	FAMILY	SUBFAMILY	GENUS	SPECIES		
Nucleus (accumulates in nucleolus)	Pospiviroidae	Pospiviroidae	Pospiviroids	Potato spindle tuber (PSTVd) Mexican papita (MPVd) Tomato planta macho (TPMVd) Chrysanthemum stunt (CSVd) Citrus exocortis (CEVd) Tomato apical stunt (TASVd) Iresine 1 (IrVd 1) Columnnea latent (CLVd)		
			Hostuviroids	Hop stunt (HSVd)		
			Cocadviroids	Coconut cadang-cadang (CCCVd) Coconut tinangaja (CTIVd) Hop latent (HLVd) Citrus IV (CvD-IV)		
		Apscaviroidae	Apscaviroids	Apple scar skin (ASSVd) Citrus III (CvD-III) Apple dimple fruit (ADFVd) Grapevine yellow speckle 1 (GYSVd 1) Grapevine yellow speckle 2 (GYSVd 2) Citrus bent leaf (CBLVd) Pear blister canker (PBCVd) Australian grapevine (AGVd)		
				Coleviroidae	Coleus blumei 1 (CbVd 1) Coleus blumei 2 (CbVd 2) Coleus blumei 3 (CbVd 3)	
				Avsunviroids	Avocado sunblotch (ASBVd)	
		Chloroplasts (accumulates, too)	Avsunviroidae		Pelamoviroids	Peach latent mosaic (PLMVd) Chrysanthemum chlorotic mottle (CChMVd)

Tomato infecting Pospiviroids:

“Potato spindle tuber viroid (PSTVd)

“Tomato apical stunt viroid (TASVd)

“Columnea latent viroid (CLVd)

“Citrus exocortis viroid (CEVd)

“Tomato chlorotic dwarf viroid (TCDVd)

“Mexican papita viroid (MPVd)

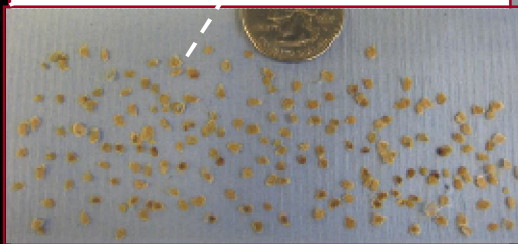
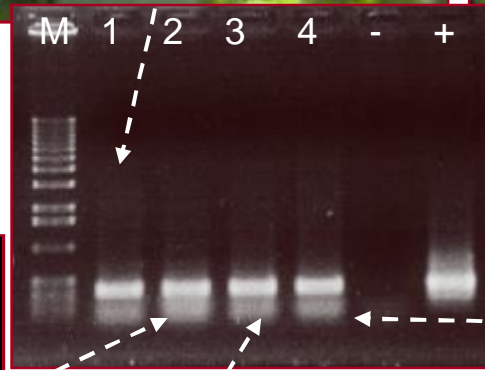
“Tomato planta macho viroid (TPMVd)

“Pepper chat fruit viroid (PCFVd)

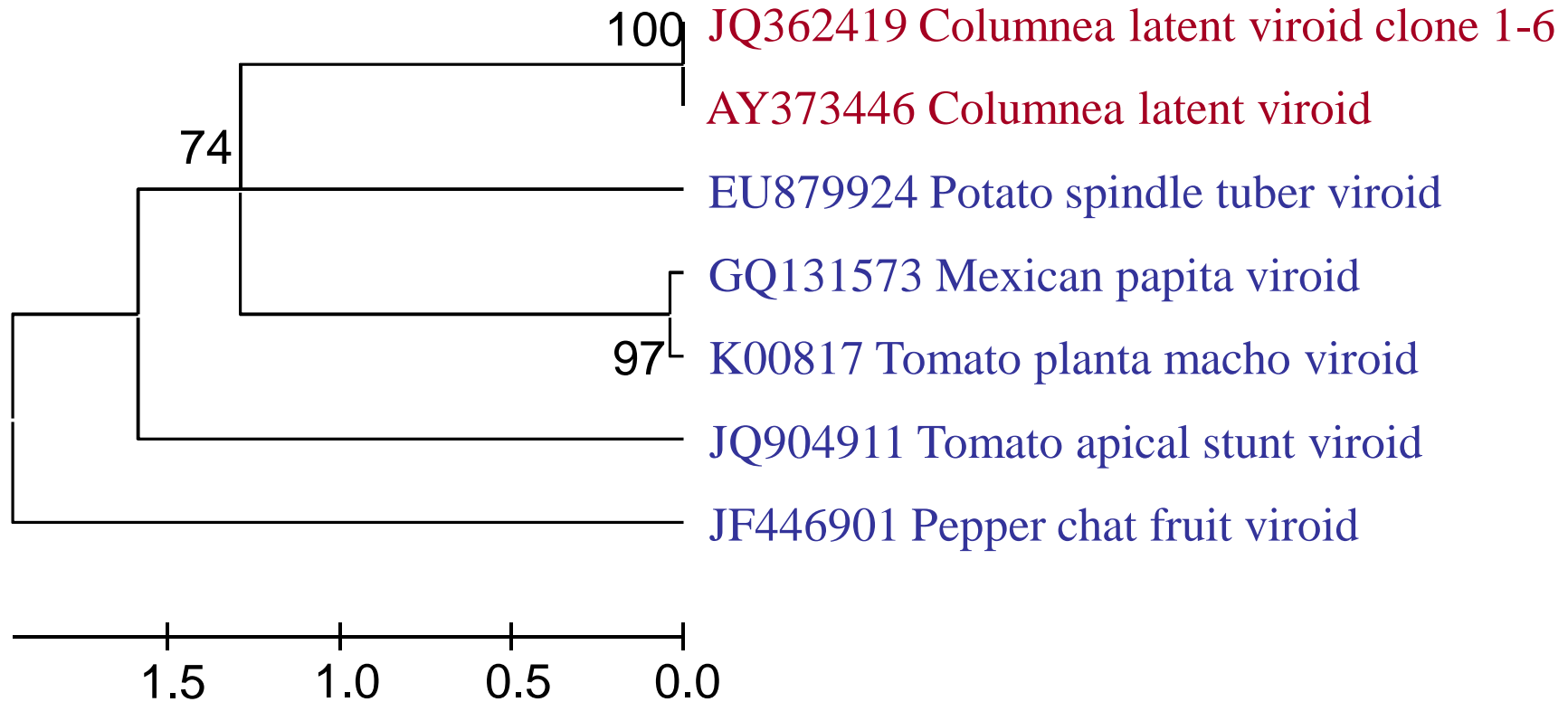
Tomato field surveys in Mali and Ghana in 2011

- ❖ Virus-like symptoms, including stunted growth, epinasty and chlorosis of leaves, and necrosis of leaf veins and stems were observed in 1-5% of tomato plants in field surveys conducted in Ghana and Mali in 2011.
- ❖ Symptomatic plants tested negative for known tomato-infecting DNA and RNA viruses, phytoplasma and '*Candidatus Liberibacter*'.
- ❖ A putative virus-like agent was transmitted to tomato seedlings following rub-inoculation with RNA extracts, and inoculated plants developed similar symptoms, indicating that the casual agent might be a viroid. Subsequent RT-PCR analyses revealed the presence of *Columnea* latent viroid!

Columnnea latent viroid (CLVd) in tomato in West Africa



CLVd with other pospiviroids based on sequence comparisons



The nucleic acid sequence of the CLVd 1-6 (GenBank Accession No. JQ362419) is 99% identical with that of an isolate of CLVd from the Netherlands.

Tomato field surveys in Ghana in 2012

- ❖ The 'rasta' disease of tomatoes in Ghana is associated with stunted growth, leaf epinasty and necrosis symptoms.
- ❖ A virus-like agent from plants with 'rasta' was seedborne and readily transmitted to tomato plants via sap inoculation.
- ❖ Tests for CLVd, a viroid that we previously have shown to infect tomatoes in Ghana, were negative.
- ❖ However, tomato seedlings rub-inoculated with total RNA extracts from leaves of symptomatic tomato plants developed similar symptoms, indicating that the casual agent might be a different viroid.

Tomato field surveys in Ghana in 2012

Bob: Check these samples for CLVd

Ozgur: Your samples are all negative for CLVd

Bob: I swear they were all looking alike CLVd-
infected, it must be a viroid!

You screwed something up in your tests!

Ozgur: I rub-inoculated some tomatoes with
RNA extracts and showing CLVd-like
symptoms!

Bob: Dang!, ... I knew those tomatoes were
infected with viroid!

Ozgur: But, they are still CLVd negative!

They show viroid (CLVd-like) symptoms!



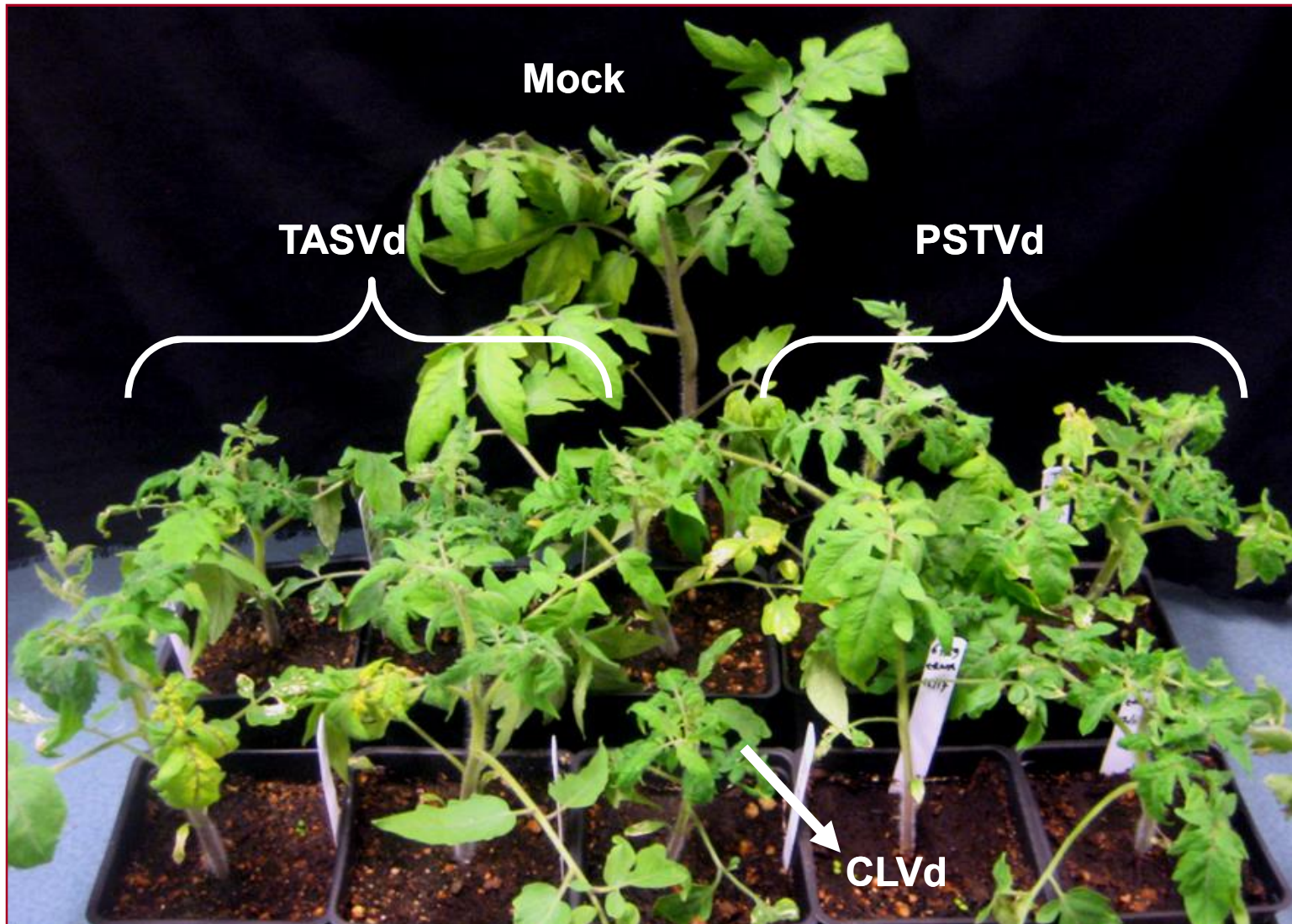
- ❖ RT-PCR tests for CLVd were negative!
- ❖ Pospiviroid universal primers did not pick a viroid
- ❖ However, RT-PCR tests with some of viroid-specific primer combinations yielded a small fragment!

Tomatoes that were identified in samples that were collected from Ghana in 2012

Tomatoes from Ghana	Collected Locality		
	Agago	Akomodan	Touboudan
1	PSTVd		
2	PSTVd		
3		TASVd	
4		PSTVd	
5		PSTVd	
6		PSTVd	
7		PSTVd	
8		PSTVd	
9		Not Detected	
10			TASVd
11			Not Detected
12			PSTVd
13			Not Detected

Sequencing confirmed the presence of TASVd (20%) and PSTVd (80%) in these samples, respectively.

Different viroids with similar symptoms!



Severe symptoms in tomato



Co-inoculated with TASVd and PSTVd RNA extracts

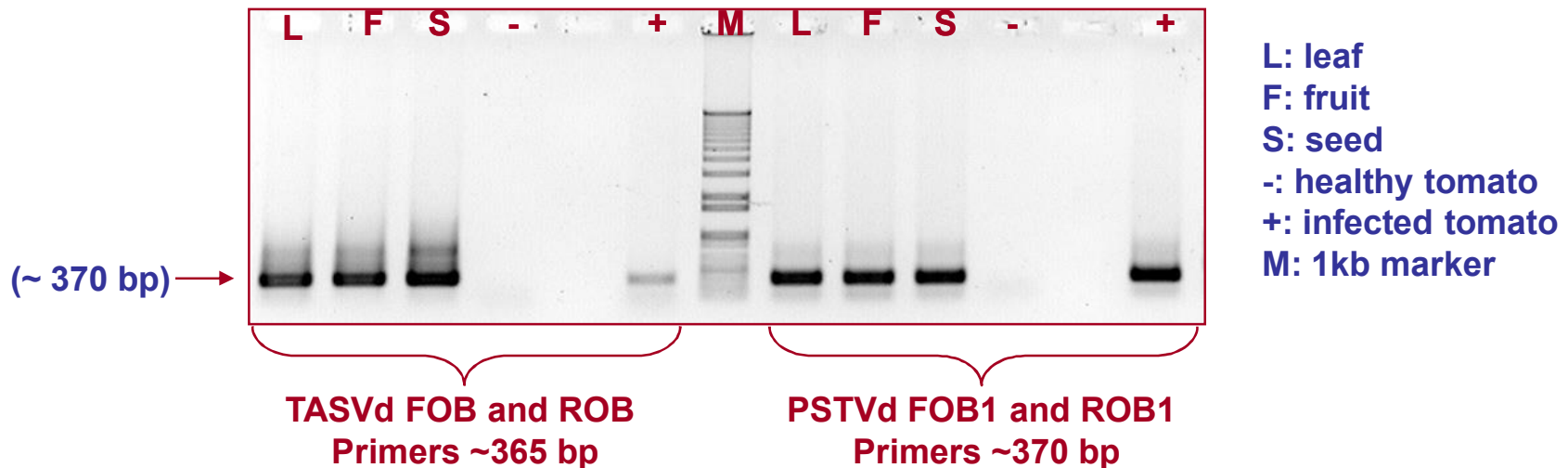
Host Range Studies



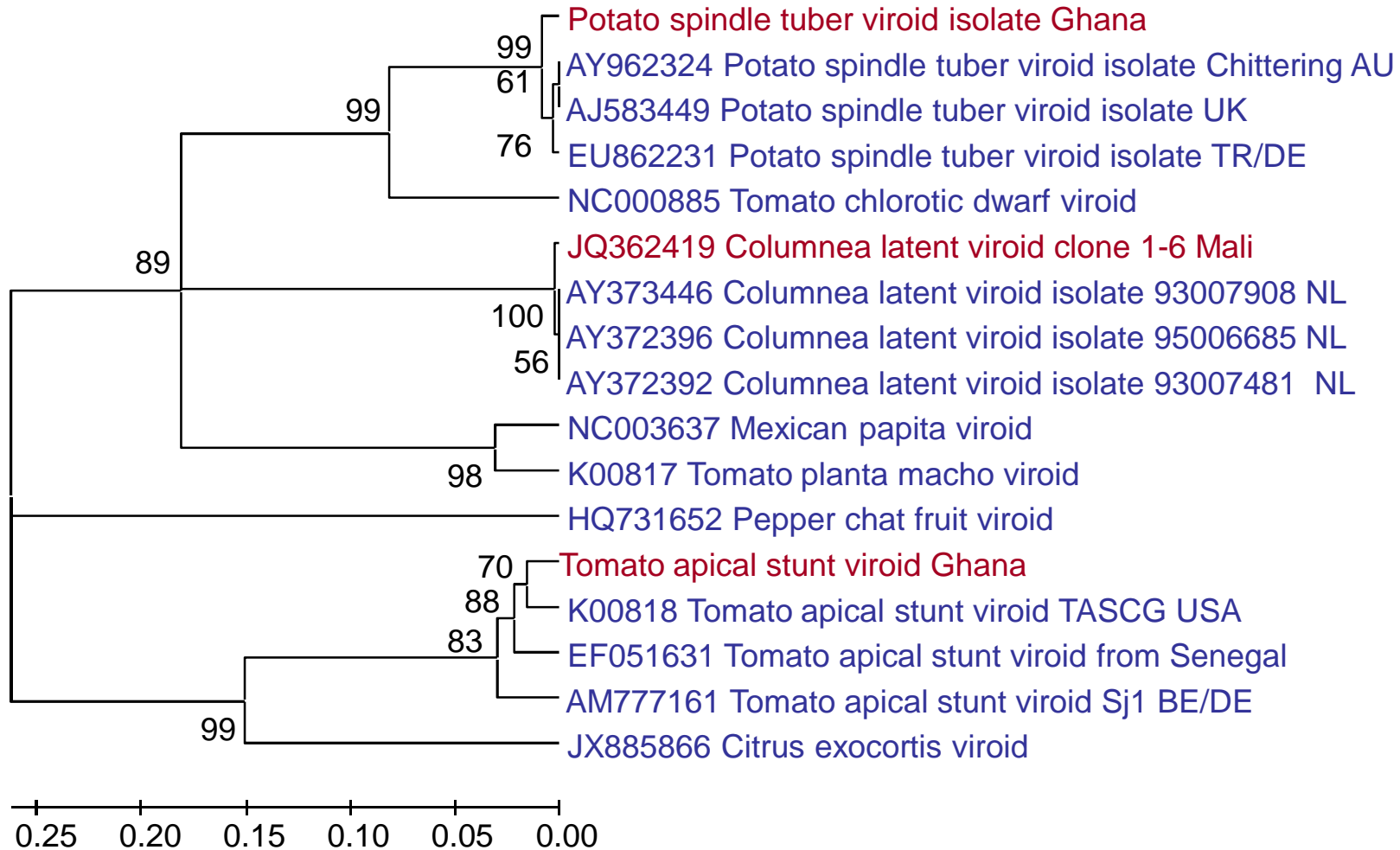
Lab-Host Plants	TASVd	PSTVd	CLVd
<i>Nicotiana benthamiana</i>	+	+	+
<i>Nicotiana tabacum</i> (cv. Havana)	+	+	-
<i>Nicotiana tabacum</i> (cv. Turkish)	+	+	+
<i>Nicotiana tabacum</i> (cv. Glurk)	-	+	-
<i>Nicotiana glutinosa</i>	+	+	-
<i>Chenopodium amaranticolor</i>	-	-	-
<i>Chenopodium quinoa</i>	-	-	-
<i>Datura stramonium</i>	-	+	-
Top crop	-	-	-
Small sugar pumpkin	-	-	-
Pepper	+	+	+
Tomato (only symptomatic lab host)	+	+	+
Cucumber	-	-	-
<i>Arabidopsis thaliana</i> (Col-0)	-	+	Not Tested
Pinto beans	-	Not Tested	Not Tested

Seed Transmission

- ❖ Tomatoes inoculated with TASVd or PSTVd were severely stunted and showed similar symptoms. These plants produced few fruits and these were small and had few seeds.
- ❖ RT-PCR tests revealed that seeds extracted from TASVd- and PSTVd-infected tomato fruits were positive for these viroids.



Phylogeny of viroids from Ghana with other pospiviroids



The nucleic acid sequence identities for the TASVd, PSTVd and CLVd viroid isolates from Ghana and other isolates from different part of the world ranged from 96 to 99%.

Conclusions

- ❖ This is the first report of CLVd, TASVd and PSTVd infecting tomato in Ghana, although these viroids have been previously reported from Africa.
- ❖ The TASVd and PSTVd isolates, similar to CLVd, are seedborne and highly infectious, making these pathogens a potential threat for tomato production in West Africa.
- ❖ The origin of these viroids are unknown but they may have been introduced in association with tomato seed.



Your complimentary
use period has ended.
Thank you for using
PDF Complete.

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

Thank You!

This research was supported
by
the USAID-funded IPM-CRSP
(Cooperative Agreement No. EPP-A-00-04-
0016-00).