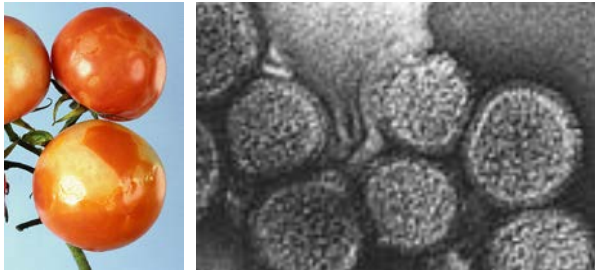




Netherlands Food and Consumer  
Product Safety Authority  
*Ministry of Economic Affairs*



## Generic RT-PCR tests for detection and identification of Tospoviruses

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Mehraban<sup>1</sup>, Ko Verhoeven<sup>1</sup>, Bart van de  
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<sup>1</sup>Dutch National Plant Protection Organization

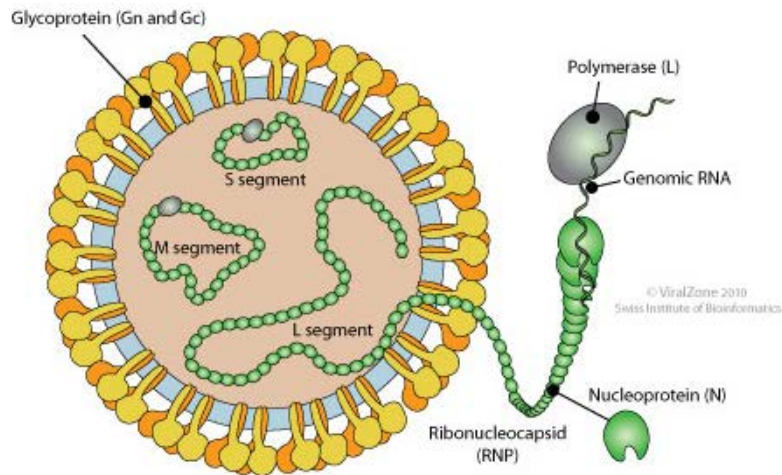
<sup>2</sup>Wageningen University and Research Centre



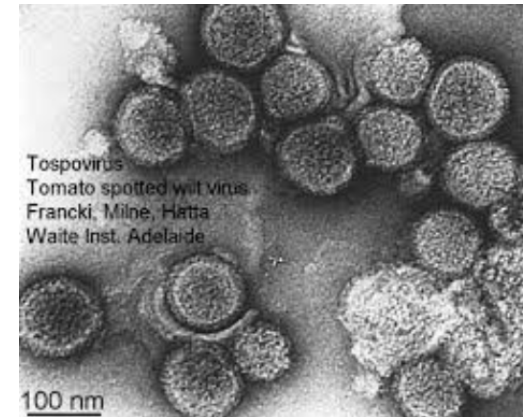
# Genus Tospovirus

## Family Bunyaviridae

### VIRION



Enveloped, spherical. Diameter from 80 to 120nm.



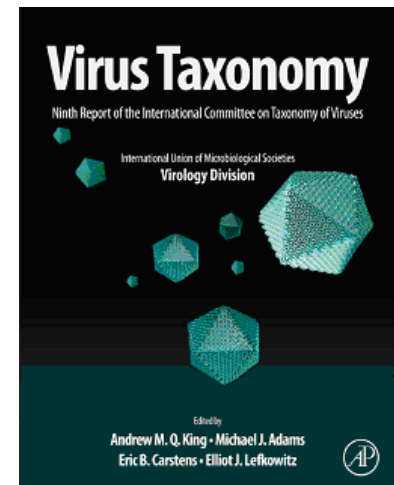
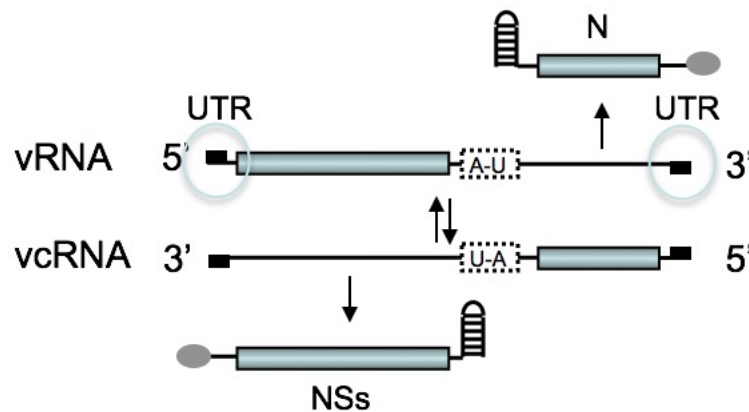
### RNA segments

L:	8.8 kb
M:	4.8 kb
S:	2.9 kb



# Species demarcation criteria Tospoviruses (ICTV)

- Vector specificity
- Plant host range
- Serological relationship Nucleocapsid (N) protein
- N-protein sequence less than 90% aa identity (S segment)





## Impact of Tospoviruses

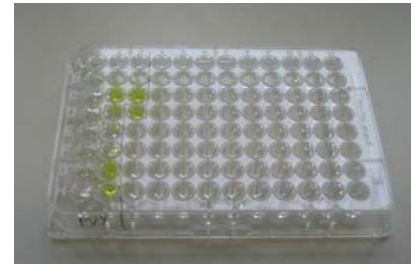
- So far 29 species described
- Infect a broad range of plant species
- Cause serious damage in important crops worldwide
- Transmitted by various difficult to control thrips species
- Natural resistance limited
- EU-regulated tospoviruses
  - *Chrysanthemum stem necrosis virus* (CSNV)
  - *Tomato spotted wilt virus* (TSWV)
  - *Impatiens necrotic spot virus* (INSV)





# Diagnosis of Tospoviruses

- Indicator plants - *Nicotiana benthamiana*
  - › Detection by symptoms
  - › Identification not possible
- Serological tests – ELISA
  - › Cross reactions between species
- Molecular tests - RT-PCR and sequence analysis
  - › Developed for a small range of tospoviruses
  - › Poor knowledge about specificity





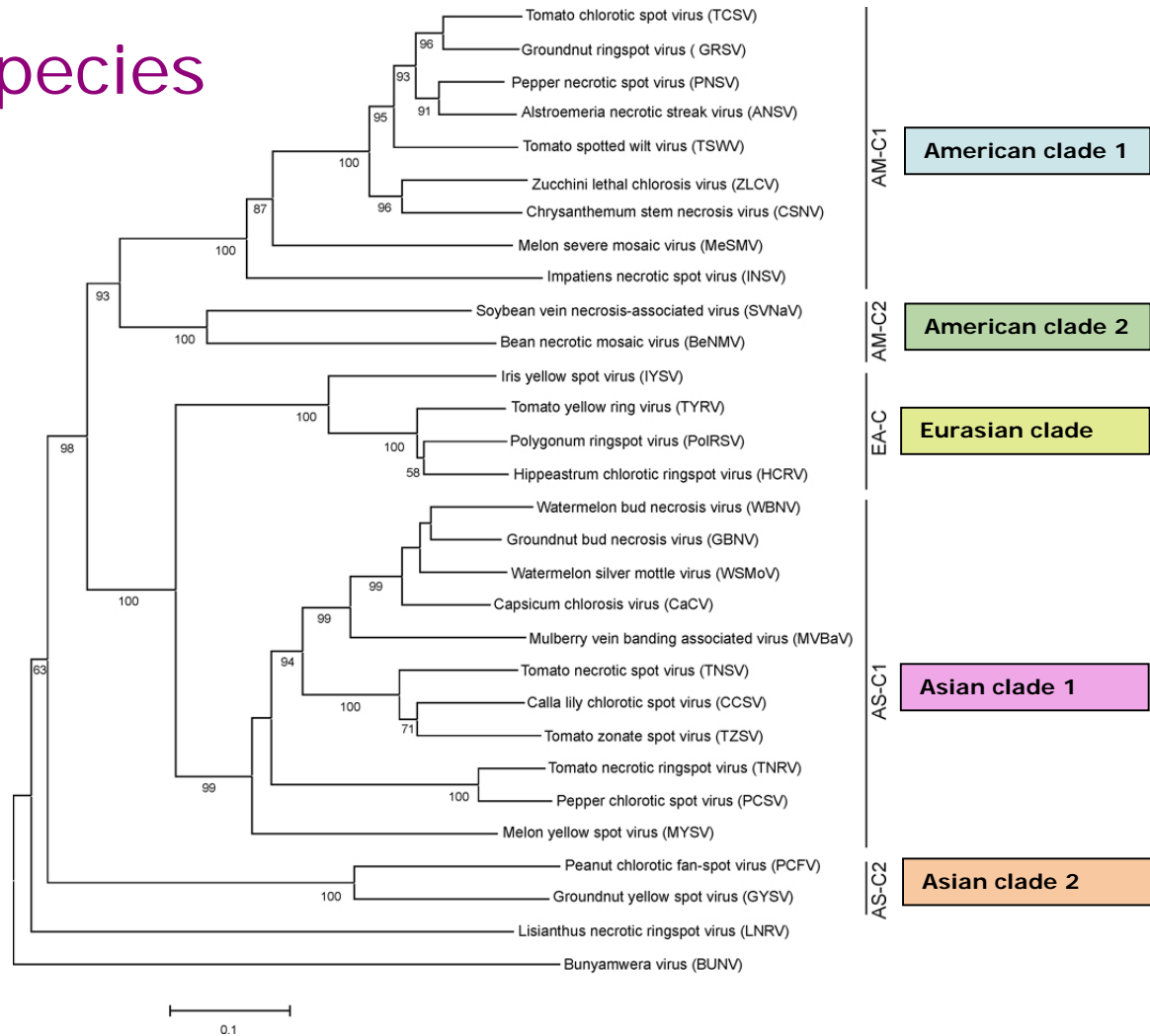
## Objective

- Generic RT-PCR test(s) for detection of Tospoviruses
- Identification by sequencing and analysis of amplicons





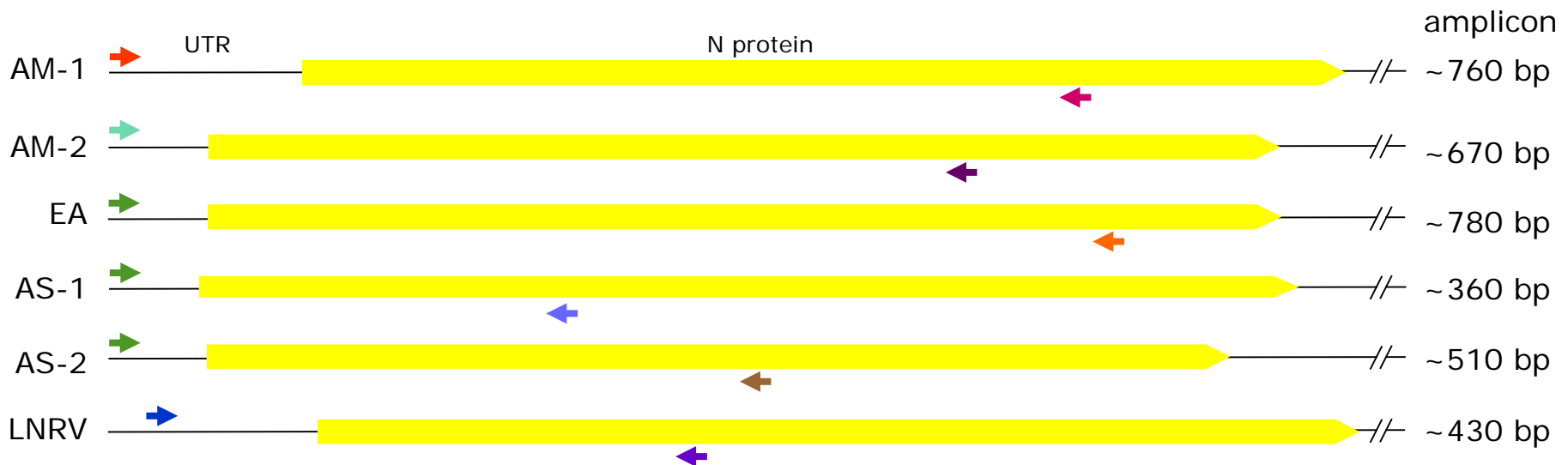
# Tospovirus species





## Primer design

- Nucleotide sequences of 28 different species from GenBank: N gene and 5' and 3' non-coding trailer sequences (S segment)
- Multiple sequence alignment (MAFFT)
- Selection of (sub)clade specific conserved region(s)
- (Sub)clade specific amplicon length







# Isolates used for testing

Species	Code	Origin
TCSV	BR-03	WUR
GRSV	SA-05	WUR
ANSV	Colombia	WUR
TSWV	BR-01	WUR
CSNV	Brazil	WUR
INSV	NL-07	WUR
MeSMV	Mexico	Istituto di Virologia Vegetale del CNR
BeNMV	Brazil	University of Brasilia
IYSV	IYSV-NL	WUR
TYRV	Iran	WUR
PoIRSV	PIg3	Istituto di Virologia Vegetale del CNR
AYSV	ALS-2000	WUR
WSMoV	Thailand	WUR
GBNV	India	WUR
CaCV	Thailand	WUR
CCSV	Taiwan	National Chung Hsing University
TNRV	Thailand	WUR
MYSV	Thailand-physalis	WUR
PCFV	Taiwan	National Chung Hsing University
LNRV	Japan	Kochi Agricultural Research Center

American clade 1

American clade 2

Asian clade 1

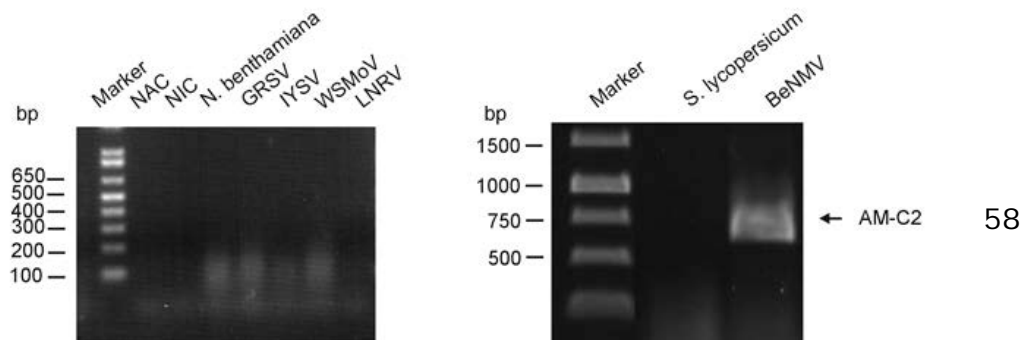
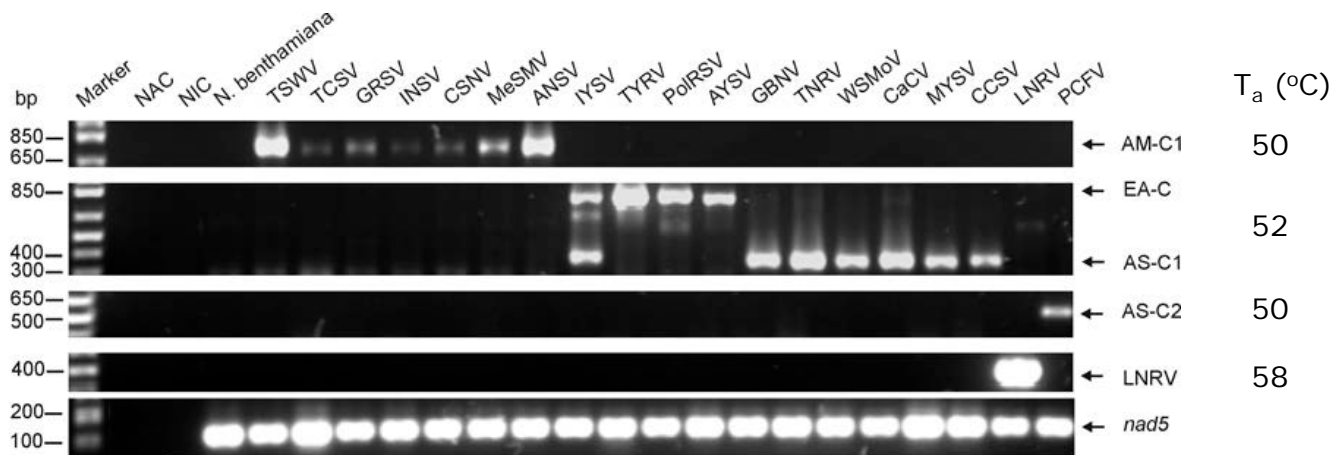
Asian clade 2

Eurasian clade

Identity confirmed by sequencing of the entire *N protein* gene



# Results testing different primer sets



## Identification by amplicon sequencing



## Conclusions

New generic and LNRV-specific primer sets allowed

- Detection of all included species
- Provisional identification by sequence analysis



## Results testing field samples

Species	Crop species	Origin (number of samples)
GRSV	<i>Capsicum annuum</i>	Brazil (1)
INSV	<i>Leontopodium</i> sp.	Switzerland (1)
TCSV	<i>Capsicum annuum</i>	Brazil (1)
TCSV	<i>Capsicum frutescens</i>	Dominican Republic (1)
TSWV	<i>Solanum lycopersicum</i>	Croatia (1)
TSWV	<i>Capsicum annuum</i>	South Africa (1), Turkey (1)
CaCV	<i>Capsicum annuum</i>	China (1), Vietnam (1)
CaCV	<i>Solanum lycopersicum</i>	Vietnam (2)
GBNV	<i>Solanum lycopersicum</i>	India (3)
TNRV	<i>Capsicum annuum</i>	Thailand (6), Vietnam (1)

American clade 1

Asian clade 1



## Conclusions and discussion

- Eurasian primer set might give background reaction in pepper (results not shown)
- Asian clade 1 primer set might react with IYSV (Eurasian clade)
- Amplicon sequences allow provisional identification
- American clade 1 and Asian clade 1 primers allow detection and identification of different species in various field samples
- Eurasian-primer set able to detect a 'new' tospovirus species (AYSV not used for primer design)
- Limited experience with American clade 2 and Asian clade 2 species so far (for both only one of two species tested)



## Summary

- Five RT-PCR's able to detect and provisionally identify the majority of known tospovirus species
- Fair chance to detect unknown species because of the generic primer design
- Important tool for diagnostic laboratories in plant health to prevent and/or control tospovirus infections





# Acknowledgements

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