

European Union
Reference Laboratory
for Pests of Plants on
Viruses, Viroids
and Phytoplasmas



Co-funded by the
European Union

Tomato mild mottle virus (Ipomovirus) – Test Performance Study outcomes and upcoming Proficiency Testing

Nataša MEHLE, Jakob BRODARIČ, Irena BAJDE, Ana VUČUROVIĆ

14.1.2026, Wageningen

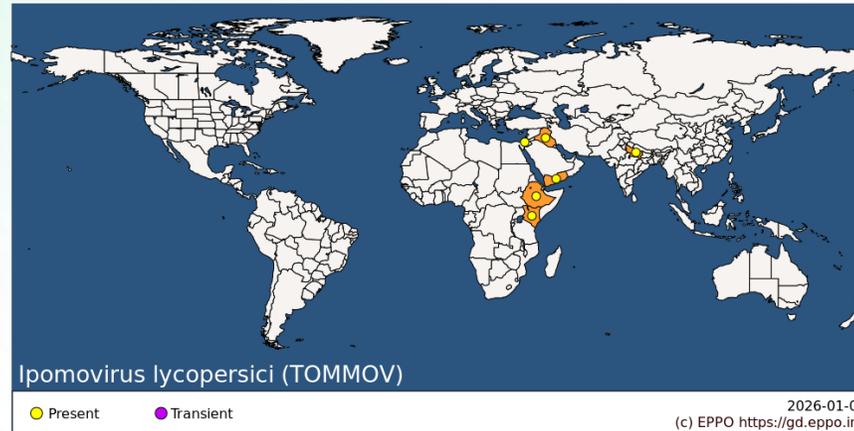
Tomato mild mottle virus (TMMoV)

Taxonomy:

- Family: Potyviridae
- Genus: **Ipomovirus**
- Species: *Ipomovirus lycopersici*

EU Categorization:

- **A1 Quarantine pest (Annex II A)**



Transmission:

- Mechanical inoculation
- Grafting
- Whitefly (*Bemisia tabaci*)
- ? Aphids ? (mix infection with aphid-transmitted potyvirus)
- ~~Seeds~~

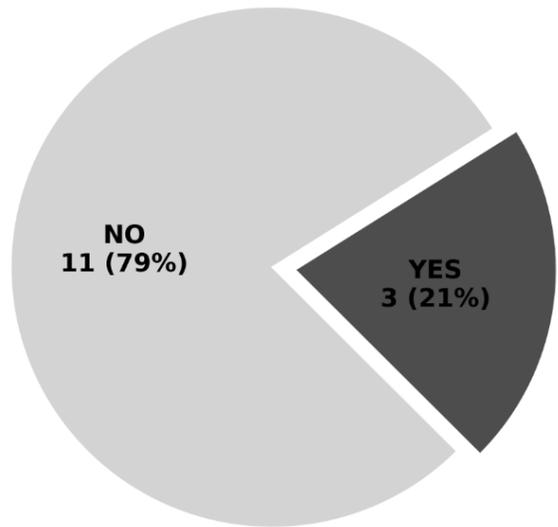
Hosts:

- ***Solanum lycopersicum*** (tomato) → Mottling symptoms on the leaves, slightly stunted plants
Mix infection with PVY: severe mosaic, leaf deformation, plant stunting
 - *Datura stramonium* (jimson weed)
 - *Solanum nigrum* (black night-shade)
 - *Nicandra physalodes*
 - ***Solanum betaceum*** (tamarillo) → Mix infection with PVY: leaf mosaic, mottling, malformation
 - ***Solanum melongena*** (eggplant) → Mild mottling of leaves (sometimes also mosaic, blistered areas, and downward curving leaves) & fruit distortion, sometimes accompanied by the formation of blisters on the fruit surface (-> in some cases huge yield loss).
- ↙ Eggplant mild leaf mottle virus (EMLMV) (TMMoV strain; 81% identities at the nucleotid level)

TMMoV – methods for detection and identification

NRLs from EU
Survey in August/September 2025

Have you previously used any tests for the detection and identification of TMMoV in your lab?



- **HTS – nanopore** (ISO 17025 accredited test for detection of TMMoV in tomato leaves)
- **RT-PCR for Potyviridae** (Gibbs & Mackenzie 1997, J. Virol. Meth. 63:9-16) (tested only with TMMoV from DSMZ)
- **RT-PCR for Potyviridae - CIFor/CIRv** (Ha et al. 2008, Arch Virol 153: 25-36) **& sequencing**

??Specific tests for detection and identification of TMMoV??



RT-PCRs for TMMoV

Primers	Genome part	Amplicon size (bp)	Reference
TF2/TR2	Nia, Nib	668	Kinoga et al. 2023
F-CP-8470/R-EM-8852	CP	508	Dombrovsky et al. 2013
F1/R1, F2/R2, F3/R3	Whole genome		Mishra et al. 2023

No validation data available!



RT-qPCR for TMMoV

F1, R1, P, P1	CP, 3'UTR	NIB
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TMMoV – methods for detection and identification

Tests optimization

In-house validation

Inclusivity

TMMoV isolate	RT-PCR <u>Potyviridae</u> Ha	RT-PCR <u>Kinoga</u>	RT-PCR <u>Dombrovsky</u>	RT-qPCR <u>NIB (Cq)</u>
DSMZ PV1015	Pos	Pos	Neg	Pos (14)
DSMZ PV0993	Pos	Pos	Neg	Pos (15)
DSMZ PV1459	Pos	Neg	Pos	Pos (14)
NVWA 6124591	Pos	Neg	Pos	Pos (13)
gBlock PQ072298.1	nt	nt	Pos	Pos (8)
gBlock MW537585.1	nt	nt (pos*)	nt	Pos (8)
gBlock HQ840786.2	nt	Neg	Pos	Pos (7)
gBlock HQ711860.1	nt	nt	nt	Pos (15)
gBlock OK558714.1	nt	nt	Pos	Pos (13)
gBlock OR082767.1	nt	nt	Pos	nt (pos*)
gBlock PV756015.1	nt	nt	Pos	nt (pos*)
gBlock AF359575.1	nt	nt	Pos	nt (pos*)
gBlock MW503940.1	nt	nt	Pos	nt (pos*)

Not tested (nt) but based on in-silico analysis it should be detected (pos)

TMMoV – methods for detection and identification – in-house validation

Exclusivity

Other ipomoviruses	RT-PCR Potyviridae Ha	RT-PCR Kinoga	RT-PCR Dombrovsky	RT-qPCR NIB
CBSV (DSMZ PV0957)	Pos	Neg	Neg	Neg
CBSV (DSMZ PV0949)	nt	Neg	Neg	Neg
CVYV (DSMZ PV0776)	Pos	Neg	Neg	Neg
CVYV (DSMZ PV1291)	nt	Neg	Neg	Neg
CVYV (DSMZ PV1292)	nt	Neg	Neg	Neg
CVYV (DSMZ PV0724)	Pos	Neg	Neg	Neg
CVYV (NVA 3264124)	Pos	Neg	Neg	Neg
SPMMV (DSMZ PV0900)	Neg*	Neg	Neg	Neg
SPMMV (JKI 686)	Pos	Neg	Neg	Neg
SPMMV (JKI 812)	Pos	Neg	Neg	Neg
SqVYV (DSMZ PV1348)	Neg	Neg	Neg	Neg
SqVYV (DSMZ PV1224)	Pos	Neg	Neg	Neg
SqVYV (NVA 5873919)	Neg	Neg	Neg	Neg
UCBSV (DSMZ PV0926)	Neg*	Neg	Neg	Neg
UCBSV (DSMZ PV0927)	nt	Neg	Neg	Neg
UCBSV (DSMZ PV0912)	nt	Neg	Neg	Neg
UCBSV (DSMZ PV0952)	nt	Neg	Neg	Neg

*The TPS participant who performed this test reported positive results for these two samples

TMMoV – methods for detection and identification – in-house validation

Sensitivity

	RT-PCR Potyviridae Ha		RT-PCR Kinoga 			RT-PCR Dombrovsky 			RT-qPCR NIB (Cq) 		
	PV0993	PV1459	PV0993	PV1459	gBlock	PV0993	PV1459	gBlock	PV0993	PV1459	gBlock
10 ⁻¹	nt	Pos	Pos	Neg	nt	nt	Pos	nt	Pos (15)	Pos (16.5)	nt
10 ⁻²	Pos	Sus	Pos	Neg	Neg	Neg	Neg	Pos	Pos (18)	Pos (20)	Pos (10)
10 ⁻³	Pos	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Pos (21)	Pos (24)	Pos (14)
10 ⁻⁴	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Pos (24.5)	Pos (29)	Pos (17)
10 ⁻⁵	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Pos (28)	Pos (33)	Pos (20.5)
10 ⁻⁶	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Pos (31)	Pos (36)	Pos (24)
10 ⁻⁷	Neg	Neg	Sus	Neg	Neg	Neg	Neg	Pos	Pos (34.5)	Neg (undet)	Pos (27)
10 ⁻⁸	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos (37)	Neg (undet)	Pos (30)
10 ⁻⁹	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg (39-undet)	Neg (undet)	Pos (33)
10 ⁻¹⁰	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg (undet)	Neg (undet)	Pos (37)
10 ⁻¹¹	nt	Neg	nt	Neg	Neg	nt	Neg	Neg	nt	Neg (undet)	Sus (37-undet)

PV0993 = DSMZ isolate of TMMoV; dilutions in RNA of healthy tomato plants

PV1459 = DSMZ isolate of TMMoV; dilutions in RNase free water

gBlock for isolate HQ840786.2 targeting RT-PCR Kinoga & Dombrovsky and RT-qPCR NIB; dilutions in RNA of healthy tomato plants

EURL-Virology_TPS-2025-01-TMMoV

Invitation
August 20, 2025



Registration

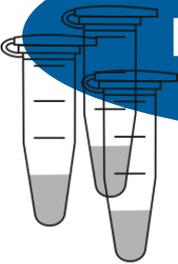
Registration by
September 10, 2025

13 NRLs

Protocols sent out
September 12, 2025



20 RNA samples +
NC + 2x PC sent out
November 11, 2025



Analysis

Deadline for results
submission
December 19, 2025



Report
by February 19, 2026



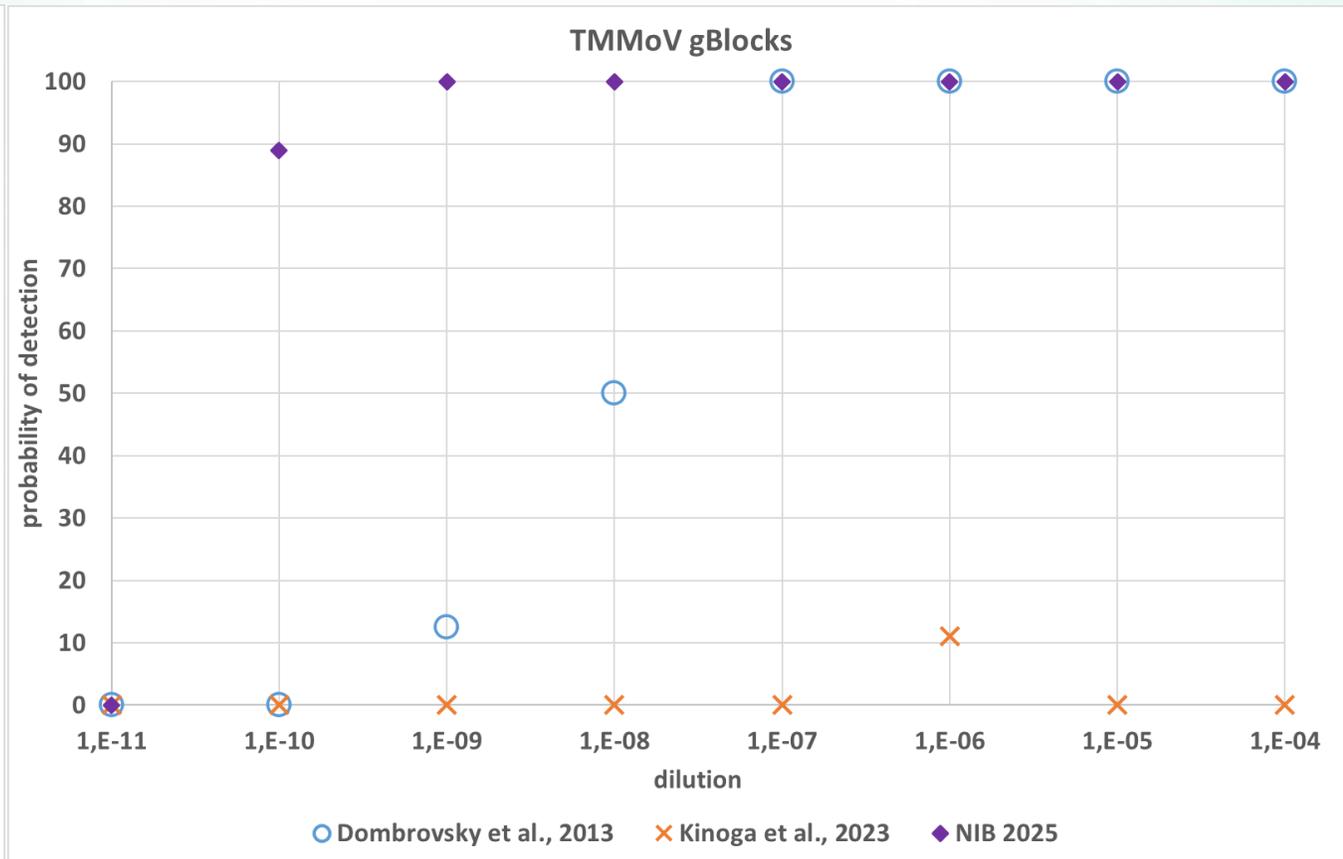
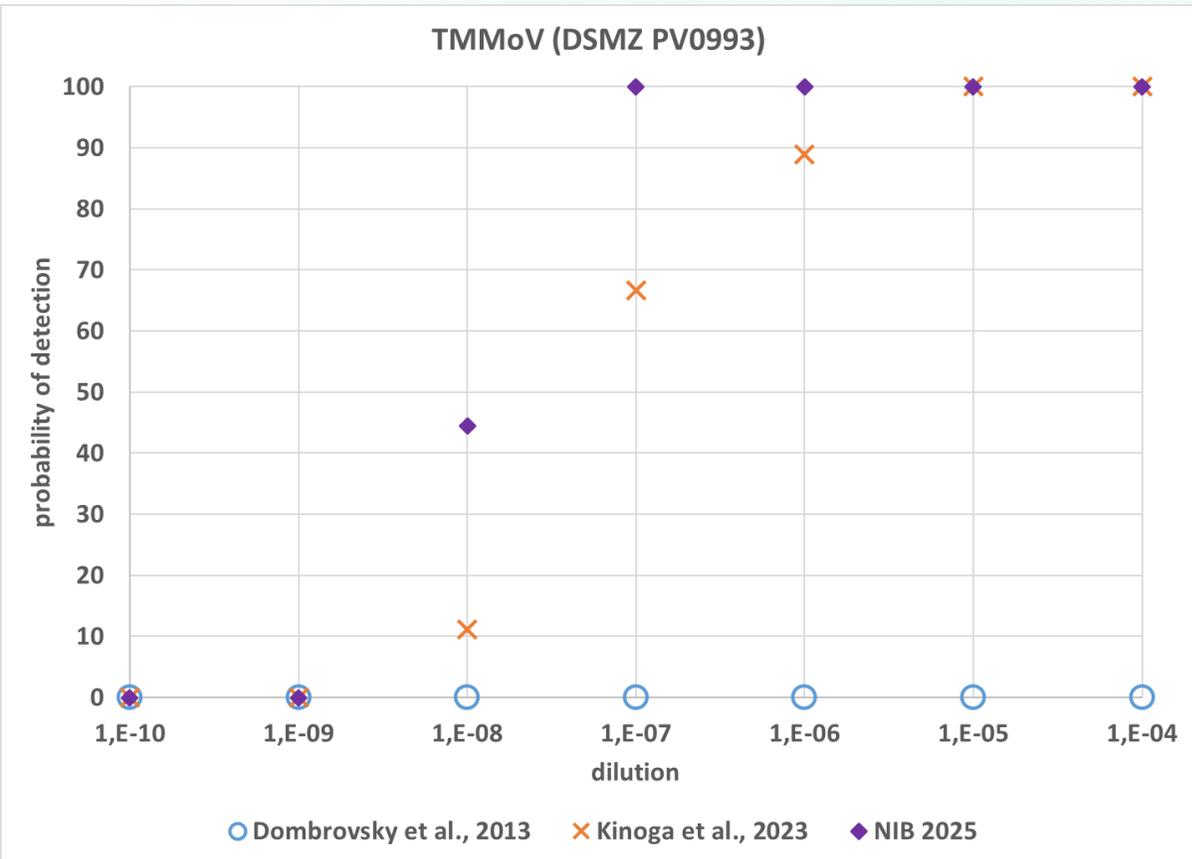
EURL-Virology_TPS-2025-01-TMMoV

Number of participants:

Total	11	12	13
TPS protocol	9	8	9
	Concordant %		

Sample	RT-PCR Kinoga	RT-PCR Dombrovsky	RT-qPCR NIB
TMMoV (DSMZ PV1015) (10 ⁻²)	100	0	100
TMMoV (DSMZ PV0993) (10 ⁻⁴) →	100	0	100
TMMoV (gBlocks HQ840786.2 for all 3 tests) (10 ⁻⁴) →	0	100	100
CBSV (DSMZ PV0957) (5x10 ⁻¹)	100	100	100
CVYV (DSMZ PV0776) (5x10 ⁻¹)	100	100	100
SPMMV (DSMZ PV0900) (5x10 ⁻¹)	100	100	100
UCBSV (DSMZ PV0926) (5x10 ⁻¹)	100	100	100
NC = healthy tomato (10 ⁻¹)	100	100	100
PC1 = TMMoV (DSMZ PV1015) + TMMoV gBlock PQ072298.1 for RT-PCR Dombrovsky (mix 1:1)	100	100	100
PC 2 = 50x dilluted PC1	100	100	100

EURL-Virology_TPS-2025-01-TMMoV



EURL-Virology_PT-2026-01-TMMoV

Invitation
March 2026



Registration

Registration
April 2026

LoA

Samples sent out
May 2026



RNA samples
and samples
with liophilized
plant material

Analysis

Deadline for results
submission
July 2026



RT-qPCR NIB

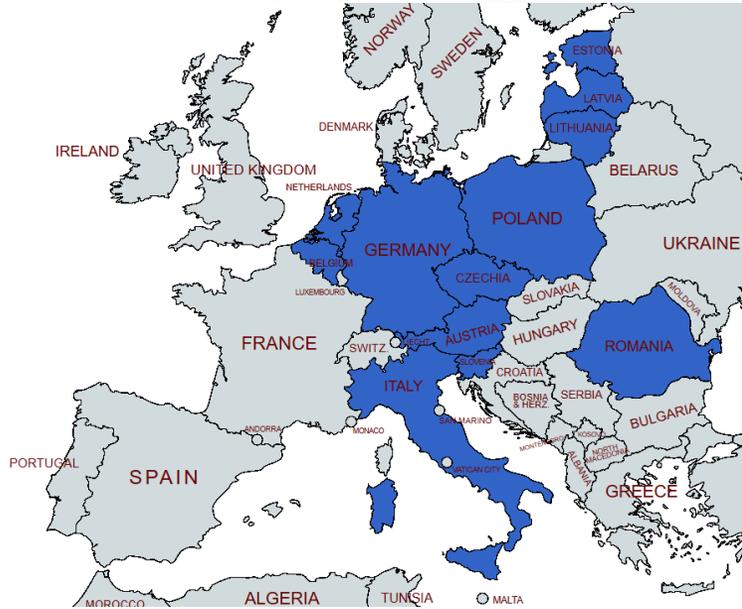
(If the RT-PCR Kinoga & RT-PCR
Dombrovsky is used, some isolates present
at low concentrations may fail to be detected!)

Report
September 2026



Special thanks to

- ❖ Everyone who shared Ipomovirus isolates with us: DSMZ, JKI, NVWA
- ❖ Everyone who contributed by answering the survey questions (14 NRLs)
- ❖ TPS participants (13 NRLs)



NRLs from:

- Austria
- Belgium (2x)
- Czech Republic
- Estonia
- Germany
- Italy
- Latvia
- Lithuania
- Netherlands
- Poland
- Romania
- Slovenia

- ❖ European Commission, Directorate General for Health and Food Safety

Future plan

Squash vein yellowing virus (SqVYV; Ipomovirus):

- literature search → test optimization/ development → in-house validation
→ TPS (end of 2026) → PT (2027)