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EURL-Virology

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

PCR TESTS FOR BEGOMOVIRUSES THAT CAN INFECT *SOLANUM TUBEROSUM*

VALIDATION REPORT

VERSION SEPTEMBER, 23 2025

The validation was performed by the National Institute of Biology, Department of Biotechnology and Systems Biology, Microbiology Unit, Laboratory for detection of viruses, viroids and phytoplasmas, Večna pot 121, Ljubljana, Slovenia, a partner of the European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas.

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Contents

1 Scope of validation

Detection of begomoviruses that can infect *Solanum tuberosum* (potato).

Method	PCRs with the sets of oligonucleotide primers according to: - Wyatt and Brown (1996) (EPPO PM7/152, Appendix 4) - Li et al. (2004) (EPPO PM7/152, Appendix 5) - Saison and Gentit (2015) (EPPO PM7/152, Appendix 6)
Harmful organism	• chilli leaf curl virus (ChiLCV) • potato yellow mosaic virus (PYMV) • tomato leaf curl New Delhi virus (ToLCNDV) • tomato mosaic Havana virus (ToMHaV) • tomato mottle Taino virus (ToMoTV) • tomato severe rugose virus (ToSRV) • tomato yellow vein streak virus (ToYVSV)
Sample type	Plant material (leaves) of potato

Note:

Sequence analysis of amplicons from PCRs can be used for identification of the begomovirus. This analysis should be done according to the guidelines described in EPPO Standard PM 7/129(2), Appendix 7. The obtained consensus sequences can be compared with sequences from the GenBank database, using the Basic Local Alignment Search Tool, BLASTN, available at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/).

2 Description of the method

2.1 DNA extraction

Leaflets collected from young shoots of potato plants were cut into small pieces. 200 mg of tissue was homogenized in 1 mL extraction buffer (lysis buffer from QuickPick™ SML Plant DNA kit, Bio-Nobile) using a tissue homogeniser (FastPrep®-24, MP Biochemicals). Total DNA was extracted using the QuickPick™ SML Plant DNA kit (Bio-Nobile) and a magnetic particle processor (KingFisher® mL; Thermo Scientific) (Mehle et al., 2013). Total DNA extracts were eluted in 200 µL elution buffer (from the QuickPick™ SML Plant DNA kit, Bio-Nobile). Undiluted DNA was used for testing.

To monitor the DNA extraction procedure, an 18S rRNA endogenous control (Applied Biosystems) was included in the analysis (reagents used: TaqMan Universal PCR Master Mix (Applied Biosystems); equipment used: Applied Biosystems 7900HT Fast or ViiA7 or QuantStudio7 Pro).

2.2 PCRs

Chemicals used:

- Platinum™ Taq DNA Poymerase (Invitrogen; catalogue no. 10966034):
  - Platinum Taq DNA Polymerase (10 U/µL)
  - 10x Taq polymerase® buffer
  - 50 mM $MgCl_2$
- dNTP mix (10 mM each) (Invitrogen; catalogue no. R0192) (working solution of dNTP mix at a concentration of 5 mM each (dATP, dTTP, dGTP, dCTP))

Equipment used: ProFlex PCR system (Applied Biosystems), T100 Thermal Cycler (Bio-Rad) or GeneAmp PCR 9700 (Applied Biosystems).

A. Conventional PCR adapted from Wyatt and Brown (1996)

Primers	Sequence	Amplicon size
AV494	5'- GCC YAT RTA YAG RAA GCC MAG -3'	580 bp
AC1048	5'- GGR TTD GAR GCA TGH GTA CAT G -3'	

Master Mix:

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	14.8	N.A.
Taq polymerase® buffer (*Invitrogen*)	10 x	2.5	1x
$MgCl_2$ (*Invitrogen*)	50 mM	1.0	2 mM
dNTPs (*Invitrogen*)	5 mM each	0.5	0.1 mM
Forward primer AV494	10 µM	2.0	0.8 µM
Reverse primer AC1048	10 µM	2.0	0.8 µM
Platinum Taq® DNA polymerase (*Invitrogen*)	10 U/µL	0.2	2 U

Subtotal	23.0
DNA extract	2.0
Total	25.0

PCR conditions:
2 min at 94°C followed by 10 cycles (15 sec at 94°C, 20 sec at ramping 65°C -1°C to 56°C (annealing T down 1°C/cycle) and 30 sec at 72°C), 30 cycles (15 sec at 94°C, 20 sec at 55°C, 30 sec at 72°C) and 10 min at 72°C.

B. Conventional PCR adapted from Li *et al*. (2004)

Primers	Sequence	Amplicon size
SPG1	5'- CCC CKG TGC GWR AAT CCA T -3'	912 bp
SPG2	5′- ATC CVA AYW TYC AGG GAG CTA A -3'	

Master Mix:

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	22.76	N.A.
Taq polymerase® buffer (*Invitrogen*)	10 x	3.00	1x
$MgCl_2$ (*Invitrogen*)	50 mM	1.20	2 mM
dNTPs (*Invitrogen*)	5 mM each	0.60	0.1 mM
Forward primer SPG1	10 µM	0.60	0.2 µM
Reverse primer SPG2	10 µM	0.60	0.2 µM
Platinum Taq® DNA polymerase (*Invitrogen*)	10 U/µL	0.24	2.4 U
Subtotal		29.00	
DNA extract		1.00	
Total		30.00	

PCR conditions:
2 min at 94°C followed by 11 cycles (40 sec at 94°C, 40 sec at ramping 61°C +1°C to 71°C (annealing T up 1°C/cycle) and 90 sec at 72°C), 24 cycles (40 sec at 94°C, 40 sec at 60°C, 90 sec at 72°C) and 10 min at 72°C

C. Conventional PCR according to Saison and Gentit (2015)

Primers	Sequence	Amplicon size
Beg-CP-F	5′-GCC CAT GTA YMG RAA RCC-3′	580 bp or 950 bp
Beg-580-R	5′-GGR TTA GAR GCA TGM GTA CA-3′	

Master Mix:

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	17.50	N.A.
Taq polymerase® buffer (*Invitrogen*)	10 x	2.50	1x
$MgCl_2$ (*Invitrogen*)	50 mM	0.80	1.6 mM
dNTPs (*Invitrogen*)	5 mM each	0.50	0.1 mM
Forward primer Beg-CP-F	10 µM	0.75	0.3 µM
Reverse primer Beg-580-R	10 µM	0.75	0.3 µM
Platinum Taq® DNA polymerase (*Invitrogen*)	10 U/µL	0.20	2 U
Subtotal		23.00	
DNA extract		2.00	
Total		25.00	

PCR conditions:
3 min at 94°C followed by 35 cycles (30 sec at 94°C, 35 sec at 58°C, 30 sec at 72°C) and 7 min at 72°C

3 Validation procedure

Validation was performed according to EPPO PM7/98 Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity.

3.1 Analytical specificity

In-silico analyses were performed to assess the potential for detecting begomoviruses that can infect potatoes, using three different generic begomovirus PCR tests. Viral sequences were retrieved from the GenBank repository of NCBI, and primers from all three PCR tests were aligned. The number of mismatches for each primer set was recorded to evaluate the likelihood of detecting the selected begomoviruses with each of the three PCRs.

The steps taken to select accession numbers of begomoviruses for the *in-silico* analysis are briefly described below:

- A list of “Member Species” of all begomoviruses was compiled using the ICTV database (https://ictv.global/report/chapter/geminiviridae/geminiviridae/begomovirus), accessed in August 2025. In total, 648 begomovirus accessions (accession numbers of monopartite viruses and DNA-A of bipartite viruses) were retained from the ICTV database. Metadata for all these begomoviruses was collected from the GenBank database.
- The table was then filtered to include all accessions that list potato as a host and/or contain the word "potato" in the virus name.
- Additional begomoviruses that may potentially infect potato were identified through a literature review and a search of the NCBI nucleotide database (August 2025). The NCBI database was queried using a Python script that searched both the “Virus name” and “Species” to avoid missing accessions due to inconsistent naming. The script filtered for sequences longer than 2,000 base pairs (to ensure the presence of primer binding sites for all three PCR tests) and associated with the host plant potato (*Solanum tuberosum*). For each virus name or species, the script retrieved the first matching sequence and extracted relevant metadata (organism, host, segment, accession number), saving the results to an Excel file. These hits were then manually reviewed.
- For each identified begomovirus species, the accession numbers listed in the ICTV database were included in Table 1. For some begomoviruses, additional accession numbers available in the NCBI database were also included (Table 1).
  Note: Potato yellow mosaic Panama virus (PYMPV) is not included in Table 1, as it has never been reported from potato. The virus received its name because it was previously

considered a strain of potato yellow mosaic virus (PYMV), but there are no records of PYMPV infecting potato.

Inclusivity (tested targets):

- A synthetic DNA fragment (gBlock) corresponding to tomato severe rugose virus (ToSRV) based on the sequence from the NCBI database (Accession No. KC706617), was used in the study. The gBlock encompassed a 1997-nucleotide-long region of the viral genome that is targeted by three generic begomovirus PCRs. This region includes fragments corresponding to the core part of the capsid protein (CP) gene and portions of the open reading frames AC2 and AC1. A 10^{-2} dilution of the gBlock was prepared using nuclease-free water (10^{-2} dilution had 4.88 x 10^{7} copy of target DNA per one µL) and tested (test IDs: 5/23, 6/23 and 7/23).
- DNA of begomovirus isolates, included in the test performance study within Euphresco BegomoVal (2016-A-212), relevant for potato: potato yellow mosaic virus (PYMV, origin: Martinique, collection: ANSES), chilli leaf curl virus (ChiLCV, origin: India, collection: ANSES) and tomato leaf curl New Delhi virus (ToLCNDV, origin: Spain, collection: ANSES).
- Begomovirus isolates included in the validation of PCR tests for begomoviruses that can infect tomatoes and plants of the Cucurbitaceae family (see Mehle et al., 2023), and which are also relevant for potato, include ToLCNDV isolates PV-1109, PV-1111, and PV-1285

Since these are generic begomovirus PCR tests, we also refer to the data presented in Mehle et al. (2023), Validation report on the testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR, for the evaluation of inclusivity. In that study, all three generic begomovirus PCRs were tested on a broad panel of begomoviruses.

Exclusivity (tested non-targets):
For evaluation of exclusivity, we refer to the data from the test performance study within Euphresco BegomoVal (2016-A-212). In that study, all three generic begomovirus PCRs were tested on five viruses from other genera.

3.2 Analytical sensitivity

To determine the analytical sensitivity of the PCR tests, a dilution of gBlock KC706617 (ToSRV; undiluted gBlock had 4.88 x 10^{9} copy of target DNA per one µL) in a homogenate of healthy potato leaves was prepared (dilutions of gBlock prepared: from 10^{-3} to 10^{-10}). The DNA

was then extracted and tested. Each dilution was tested in two parallels (test IDs: 10/23, 11/23, 12/23).

The procedure for assessing the analytical sensitivity on serial dilutions of gBlock is schematically presented in Figure 1.

Note: This synthetic gBlock was selected because its sequence spans the regions targeted by all three PCR tests evaluated in this study. By using a single synthetic DNA fragment compatible with all tests, we were able to directly compare the analytical sensitivity of each PCR test on the same material. The choice of this gBlock was therefore based on its suitability for simultaneous evaluation of all three tests, rather than on any specific biological property of the virus.

3.3 Selectivity

To evaluate the influence of different potato cultivars on test performance five samples of different cultivars of *Solanum tuberosum* were used (sample IDs: D583/25, D585/25, D587/25, D589/25, D591/25; specific information on the cultivars tested is not available). For each sample, leaf tissue was homogenized in lysis buffer. A volume of 810 µL of each homogenate was spiked with 90 µL of the synthetic DNA fragment (gBlock) corresponding to ToSRV (GenBank Acc. No. KC706617), previously diluted to 10^{-4}. This resulted in a final gBlock concentration equivalent to 10^{-5} (4.88×10^4 copy of target DNA per one µL) in the homogenate. DNA was extracted from the spiked homogenates and tested using all three generic begomovirus PCR tests described in section 2.2 (test IDs: 32/25, 33/25, 34/25).

In addition, we tested leaves from potato plants assigned to be free of begomovirus infection based on the absence of any symptoms potentially associated with begomovirus infection and the fact that the samples were collected from a region where begomoviruses have not been reported to date (10 samples collected in 2023, 10 in 2024 and 10 in 2025) (test IDs: 36/23, 37/23, 38/23, from 13/24 to 18/24, 35/25, 36/24, 37/25). Among these 30 samples, several different cultivars were included; however, specific information on the cultivars tested is not available. Moreover, for the evaluation of the selectivity, we refer to the data presented in Mehle et al. (2023), Validation report on the testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR. In that study, all three generic begomovirus PCRs were tested on plant material from various other host species.

3.4 Repeatability

Repeatability was evaluated by testing three replicates of gBlock KC706617 spiked into homogenates of healthy potato leaves. The following dilutions were used for each PCR test:
Wyatt and Brown (1996): 10^{-7} and 10^{-8}
Li et al. (2004): 10^{-3} and 10^{-4}
Saison and Gentit (2015): 10^{-6} and 10^{-7}
Each dilution was tested in triplicate within the same PCR run (test IDs: 40/25, 41/25, 42/25).

3.5 Reproducibility

Reproducibility was assessed by testing selected gBlock dilutions by different operators (IB and TJ), using different PCR instruments (ProFlex PCR system, T100 Thermal Cycler or GeneAmp PCR 9700), on different days. The test were performed using the same gBlock dilutions as described in section 3.4, with PCRs carried out independently across multiple runs (test IDs: 10/23, 11/23, 12/23, 32/25, 33/25, 34/25, 40/25, 41/25, 42/25).

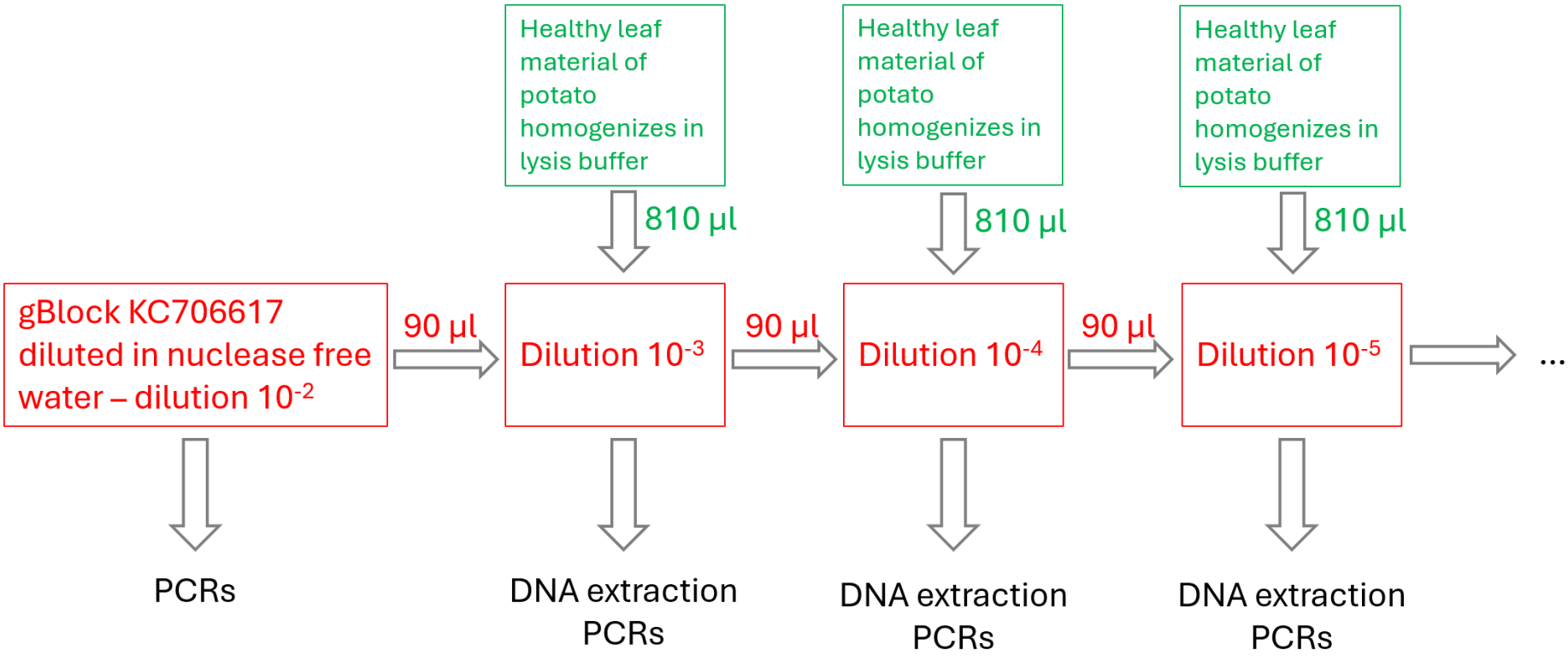

Figure 1: The procedure for testing of the analytical sensitivity on serial dilutions of gBlock KC706617 (ToSRV).

4 Results of validation

4.1 Evaluation of DNA extraction

DNA extraction performance was verified for each sample by real-time PCR using primers and a probe for 18S rRNA. DNA extraction was considered successful if 18S rRNA was detected with a Cq value below 25. This was the case in all instances (for samples of leaves from non-infected potato plants of different cultivars, 18S rRNA resulted with Cq between 13 and 21).

4.2 Analytical specificity

The in-silico analysis of begomoviruses that can infect potatoes showed that at least one of the examined PCR tests has no mismatches or only one mismatch between the primer and targeted sequences (Table 1). This suggests that all listed begomoviruses are likely to be detectable by at least one of the evaluated PCR tests, based on the results from our previous study (Mehle et. al, 2023), where we demonstrated that a single mismatch does not prevent detection. In fact, we successfully detected isolates with more than one mismatch (Mehle et. al, 2023).

Table 1. Number of primer mismatches for begomoviruses that can infect potato.

Virus	Accession No.	Wyatt and Brown, 1996	Li et al, 2004	Saison and Gentit, 2015
chilli leaf curl virus (ChiLCV)*	KM921669	2	0	>2
	AF336806	1	1	2
	GU136803	>2	0	>2
	AJ875159	>2	0	>2
	DQ673859	1	0	1
	FJ345402	2	0	1
	DQ116878	>2	2	1
potato yellow mosaic virus (PYMV)*	MN006828	1	0	0
	D00940	0	0	0
	AF039031	0	1	0
	AY965897	1	0	0
	EU518935	1	0	1
tomato leaf curl New Delhi virus (ToLCNDV)	U15015	1	0	>2
tomato mosaic Havana virus (ToMHaV)*	NC_003867	1	2	0
	Y14874	>2	>2	0
tomato mottle Taino virus (ToMoTV)*	NC_001828	1	1	0
	AF012300	1	1	0
tomato severe rugose virus (ToSRV)*	KC706617	0	1	0
	DQ207749	0	1	0
tomato yellow vein streak virus (ToYVSV)*	KJ413253	1	0	2
	EF417915	1	0	0

*virus species listed in Annex II/A of Commission Implementing Regulation (EU) 2019/2072.

The synthetic DNA fragment (gBlock) corresponding to ToSRV was successfully detected with all three generic begomovirus PCRs (Table 2).

Begomovirus isolates included in the Euphresco BegomoVal test performance study and in the validation of the PCRs for begomoviruses that can infect tomatoes and plants of the Cucurbitaceae family – PYMV, ChiLCV and ToLCNDV - were also reliably detected by at least one of three PCRs, confirming their ability to these relevant targets (EPPO, 2022; Mehle et al., 2023).

To further support the evaluation of inclusivity, we also used other results from Mehle et al. (2023), which included 30 isolates/ corresponding gBlocks of begomoviruses capable of infecting other host plants. All of these isolates/ gBlocks were reliably detected by at least one of the three generic begomovirus PCR tests (Mehle et al., 2023).

Results of the evaluation of the exclusivity from Euphresco BegomoVal test performance study (see also Mehle et al. (2023)) showed that generic begomovirus PCRs do not detect the following viruses from different other genera: banana bunchy top virus (BBTV, origin: Reunion Island, collection: ANSES), maize streak virus (MSV, origin: Reunion Island, collection: ANSES), pea necrotic yellow dwarf virus (PNYDV, origin: Austria, collection: ANSES), pepino mosaic virus (CH2 PepMV, collection: ANSES), tomato chlorosis virus (ToCV, collection: ANSES).

4.3 Analytical sensitivity

PCR Li et al. (2004) was able to detect the target gBlock of ToSRV until dilution of 10^{-5} (4.88 x 10^4 copy of target DNA per one µL). PCRs Wyatt and Brown (1996) and Saison and Gentit (2015) were able to detect the same target until dilution 10^{-8} (48.8 copy of target DNA per one µL) (Table 2).

It is important to note that this difference in analytical sensitivity not necessarily are caused by the single mismatch present in one primer of Li et al. (2004) PCR. For ToSRV, both the Wyatt and Brown (1996) and the Saison and Gentit (2015) PCRs have no mismatches with the target sequence, while the Li et al. (2004) PCR has one mismatch. However, based on previous testing (Mehle et al., 2023), we know that even multiple mismatches do not necessarily reduce sensitivity. For example, TYLCTHV was detected using the Wyatt and Brown (1996) PCR despite having four mismatches, and this test was still 100 times more sensitive than the Li et al. (2004) test, which had no mismatches for that virus. These findings clearly demonstrate that mismatch count alone is not a reliable predictor of PCR performance, and other factors -

such as primer design, amplification efficiency, and reaction conditions - play a more decisive role.

Table 2. PCR results of testing serial dilution of gBlock of ToSRV.

Dilution	Copy of target DNA/ 1 µL	Dilutant	**Wyatt and Brown (1996)** (replicate 1/ replicate 2)	**Li et al. (2004)** (replicate 1/ replicate 2)	**Saison and Gentit (2015)** (replicate 1/ replicate 2)
10^{-2}	4.88 x 10^{7}	RNase free water	pos (+++) / pos (+++)	pos (+++) / pos (+++)	pos (+++) / pos (+++)
10^{-3}	4.88 x 10^{6}	Healthy potato extract	pos (+++) / pos (+++)	pos (+++) / pos (+++)	pos (+++) / pos (+++)
10^{-4}	4.88 x 10^{5}		pos (+++) / pos (+++)	pos (++) / pos (++)	pos (+++) / pos (+++)
10^{-5}	4.88 x 10^{4}		pos (+++) / pos (+++)	pos (+) / pos (+)	pos (+++) / pos (+++)
10^{-6}	4.88 x 10^{3}		pos (++) / pos (++)	neg / neg	pos (++) / pos (++)
10^{-7}	4.88 x 10^{2}		pos (++) / pos (++)	neg / neg	pos (++) / pos (++)
10^{-8}	48.8		pos (+) / pos (+)	neg / neg	pos (+) / pos (+)
10^{-9}	4.88		neg / neg	neg / neg	neg / neg
10^{-10}	0.488		neg / neg	neg / neg	neg / neg

pos – band of the expected size (from strong (+++) to weak (+)); neg – no band

The relationship between the number of gBlock copies and the virus concentration in infected plants is unknown. Therefore, these results primarily reflect the relative sensitivity of the three tests to each other. However, a previous study evaluating the analytical sensitivity of the same tests on infected leaf material from three other begomoviruses demonstrated that each virus could be detected by at least one test at dilutions ranging from 10^{-1} to 10^{-4} (Mehle et al., 2023). In addition, ToLCNDV was detected at a 10^{-2} dilution in the Euphresco BegomoVal study. Extrapolating these findings to begomoviruses infecting potato suggests that these tests are likely sensitive enough for detection in infected plant material.

4.4 Selectivity

All three PCR tests (Wyatt and Brown, 1996; Li et al., 2004; Saison and Gentit, 2015) successfully detected the ToSRV gBlock spiked into homogenates of five different potato cultivars and produced amplicons of the expected size. No differences in amplification efficiency or product intensity were observed between cultivars, indicating that the presence of different potato matrices did not interfere with the detection of the target DNA (Table 3).

Table 3. PCR results of testing serial dilution of gBlock of ToSRV.

Sample ID	**Wyatt and Brown (1996) (replicate 1/ replicate 2)**	**Li et al. (2004) (replicate 1/ replicate 2)**	**Saison and Gentit (2015) (replicate 1/ replicate 2)**
D583/25 + ToSRV gBlock (10^{-5} dilution)	pos (+++) / pos (+++)	pos (+) / pos (+)	pos (+++) / pos (+++)
D585/25 + ToSRV gBlock (10^{-5} dilution)	pos (+++) / pos (+++)	pos (+) / pos (+)	pos (+++) / pos (+++)
D587/25 + ToSRV gBlock (10^{-5} dilution)	pos (+++) / pos (+++)	pos (+) / pos (+)	pos (+++) / pos (+++)
D589/25 + ToSRV gBlock (10^{-5} dilution)	pos (+++) / pos (+++)	pos (+) / pos (+)	pos (+++) / pos (+++)
D591/25 + ToSRV gBlock (10^{-5} dilution)	pos (+++) / pos (+++)	pos (+) / pos (+)	pos (+++) / pos (+++)

pos – band of the expected size (from strong (+++) to weak (+))
Note: The negative amplification controls were always negative.

Testing of potato leaf samples (30 samples in total, collected in 2023, 2024 and 2025), which were not infected with begomoviruses according to prior assessment, yielded negative results in PCRs following the tests of Li et al. (2004) and Saison and Gentit (2015). The PCR test by Wyatt and Brown (1996), however, frequently produced non-specific amplification products. Because some of these bands migrated at or close to the expected size of a begomovirus-specific amplicon, sequencing was used to verify their identity, which showed that all such products were not related to begomovirus sequences. This underlines the importance of sequencing when non-specific bands are close in size to the expected product size in order to prevent misinterpretation. It should be noted that the samples were not tested for other pathogens.

Extending these observations beyond potato, and as reported in Mehle et al. (2023), cross-reactions of the generic begomovirus PCR tests were not observed with healthy tomato plant material (36 samples) nor with plant material from various Cucurbitaceae species (26 samples). Similar to the observations in potato, these matrices can nonetheless yield non-specific amplification products; when such bands migrate at or near the expected size of a begomovirus-specific amplicon. Therefore, sequencing was performed to verify their identity.

4.5 Repeatability

All three PCR tests produced amplicons of the expected size in all three replicates at each tested dilution, confirming a 100% repeatability (Table 4).

Table 4: Parallel testing of dilutions of DNA samples:

Sample		**Wyatt and Brown (1996)**	**Li et al. (2004)**	**Saison and Gentit (2015)**
ToSRV gBlock	dilution	10^{-7}	10^{-3}	10^{-6}
	replicate 1	pos (++)	pos (++)	pos (++)
	replicate 2	pos (++)	pos (++)	pos (++)
	replicate 3	pos (++)	pos (++)	pos (++)
	dilution	10^{-8}	10^{-4}	10^{-7}
	replicate 1	pos (+)	pos (+)	pos (+)
	replicate 2	pos (+)	pos (+)	pos (+)
	replicate 3	pos (+)	pos (+)	pos (+)
Repeatability		**100%**	**100%**	**100%**

pos – band of the expected size (from strong (+++) to weak (+))
Note: The negative amplification controls were always negative.

4.6 Reproducibility

All three PCR tests produced reproducible results across runs performed on different days by different operators on different PCR instruments, demonstrating a 100% reproducibility for all tests (Table 5).

Table 5: Multiple testing of dilutions of DNA samples:

Sample		**Wyatt and Brown (1996)**	**Li et al. (2004)**	**Saison and Gentit (2015)**
ToSRV gBlock	dilution	10^{-7}	10^{-3}	10^{-6}
	Date: 30.3.2023 Operator: IB Instrument: ProFlex PCR system	pos (++)	pos (+++)	pos (++)
	Date: 24.6.2025 Operator: IB Instrument: ProFlex PCR system	pos (++)	nt	nt
	Date: 15.7.2025 Operator: TJ Instrument: • T100 Thermal Cycler (Li et al and Wyatt and Brown PCRs) • GeneAmp PCR 9700 (Saison and Gentit PCR)	pos (++)	pos (++)	pos (++)
	dilution	10^{-8}	10^{-4}	10^{-7}
	Date: 30.3.2023 Operator: IB Instrument: ProFlex PCR system	pos (+)	pos (++)	pos (++)
	Date: 24.6.2025 Operator: IB Instrument: ProFlex PCR system	pos (+)	pos (+)	pos (+)
	Date: 15.7.2025 Operator: TJ Instrument: • T100 Thermal Cycler (Li et al and Wyatt and Brown PCRs) • GeneAmp PCR 9700 (Saison and Gentit PCR)	pos (+)	pos (+)	pos (+)
Reproducibility		**100%**	**100%**	**100%**

pos – band of the expected size (from strong (+++) to weak (+)); nt – not tested
Note: The negative amplification controls were always negative

5 Conclusions

Criteria		Results
Analytical sensitivity	Tested concentrations	Dilutions of gBlock KC706617 (tomato severe rugose virus; ToSRV) in a homogenate of potato leaves
	LOD	Wyatt and Brown (1996): 10^{-8} (48.8 copy of target DNA/ 1 µL) Li et al. (2004): 10^{-5} (4.88 x 10^{4} copy of target DNA/ 1 µL) Saison and Gentit (2015): 10^{-8} (48.8 copy of target DNA/ 1 µL)
Analytical specificity	Number of tested samples	No. of targets tested: 3 virus species (ChiLCV, PYMV and four isolates of ToLCNDV) + 1 gBlock (ToSRV) + 30 isolates/ corresponding gBlocks of begomoviruses capable of infecting other host plants No. of non-target viruses tested: 5
	Inclusivity	For 3 PCRs together: 100% (each isolate would be detected by at least one of the PCRs)
	Exclusivity	For each of 3 PCRs by itself: 100%
	'*In silico*' analysis	The in-silico analysis showed that there is at least one PCR for all begomoviruses examined that has no mismatches or only one mismatch between primer and targeted sequences, similar to ToRSV of which a gBlock was successfully detected by all three PCRs -> all begomoviruses that can infect potato are likely to be detectable by at least one of the PCRs.
Selectivity	Impact of the matrix	The test was successfully used for the detection of gBlock of ToSRV (10^{-5} dilution) in different cultivars of potato plants. Cross-reaction of the PCRs with potato leaf samples - as well as with tomato and various species from the Cucurbitaceae family - was not observed. However, PCR tests can occasionally produce non-specific amplification products, which in some cases require confirmation by sequencing.
Repeatability	No. of replicates, percentage of identical results	No. of samples tested: 2 different dilutions of qBlock of ToRSV No. of replicates tested: 3 Percentage of identical results (positive replicates) is 100% for all three PCRs
Reproducibility	No. of replicates, percentage of identical results	No. of samples tested: 2 different dilutions of qBlock of ToRSV No. of operators: 2 No. of PCR instruments: 3 (2 different per each PCR) No. of different days: 3 Percentage of identical results (positive replicates) is 100% for all three PCRs

6 References

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