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Zhimin Yin*, Krystyna Michalak, Renata Lebecka

Plant Breeding and Acclimatization Institute – National Research Institute, Młochów
Research Center, 05-831 Młochów, Platanowa 19 Str. Poland;

*Corresponding author: z.yin@ihar.edu.pl

COLLECTION OF POTATO VIRAL PATHOGENS, ITS VALIDATION AND MAINTENANCE

ABSTRACT

IHAR – PIB collection of potato viral pathogens has been maintained since 1970s. Currently there are 258 isolates in the collection representing 12 potato viruses, named PVY, PVM, PVS, PLRV, PVA, PVX, PAMV, BMV, TBRV, AMV, CMV and TRV. The viruses are maintained in potato plants *in vivo* in the greenhouse (246 isolates), in potato plantlets cultured *in vitro* (12 isolates), in frozen leaves (two copies each of the 246 isolates) and in freeze-dried tissue (234 isolates).

Keywords: potato viruses, *in vitro* plantlets, freeze-dried leaves, frozen leaves

INTRODUCTION

The maintenance of a virus and keeping its variability is a fundamental requirement for various purposes: virus diagnostics (e.g. antibody production), virology study (e.g. virus population, strain identification, virus-host interaction), and potato breeding for virus resistance.

The Agriculture Research Service's (ARS) Schultz Potato Virus Collection in the United States started in 1916 (Webb, 1958; Weaver-Missick, 2000). A plant virus collection at Plant Research International (PRI) in the Netherlands was established in 1950s (Dullenmans *et al.*, 2011). The collection of potato viruses in Czech in Potato Research Institute in Havlíčkův Brod was founded in the 1970s and has been maintained since (www.vurv.cz/mikroorganismy/Potato%20Viruses.html). In Scotland, diagnosis of potato viruses has been conducted by The Science and Advice for Scottish Agriculture (SASA).

In Poland, a history on plant virology studies (Kryczyński, 2010) and diseases caused by viruses in potato (Świeżyński, 1968) has been summarized. A collection of potato viral pathogens at IHAR-PIB Młochów Research Center has been maintained since 1974 (Chrzanowska *et al.*, 1996, 2001). Currently, there are 258 isolates representing 12 potato viruses named PVY, PVM, PVS, PLRV, PVA, PVX, PAMV, BMTV, TBRV, AMV, CMV and TRV in the collection (Table 1). Some virus isolates are sequenced (Table 2).

The viruses are maintained in potato plants *in vivo* in the greenhouse (246 isolates), in potato plantlets cultured *in vitro* (12 isolates), in frozen leaves (two copies each of the 246 isolates) and in freeze-dried tissue (234 isolates) (Table 3).

The detailed methods for the validation of the presence of viruses are referred to in Yin and Michalak (2018). Briefly, the virus isolates maintained in the collection in different forms have biologically, serologically and/or molecularly confirmed identities.

Table 1
Potato viruses maintained in potato plants *in vivo* in the greenhouse and cultured *in vitro* at IHAR-PIB Młochów

Name of virus	Abbreviation	Genus	Number of isolates	
			<i>in vivo</i>	<i>in vitro</i>
<i>Potato virus Y</i>	PVY	<i>Potyvirus</i>	190	4
<i>Potato virus M</i>	PVM	<i>Carlavirus</i>	10	
<i>Potato virus S</i>	PVS	<i>Carlavirus</i>	13	
<i>Potato leafroll virus</i>	PLRV	<i>Polerovirus</i>	7	2
<i>Potato virus A</i>	PVA	<i>Potyvirus</i>	1	6
<i>Potato virus X</i>	PVX	<i>Potexvirus</i>	2	
<i>Potato aucuba mosaic virus</i>	PAMV	<i>Potexvirus</i>	3	
<i>Beet mild yellowing virus</i>	BMV	<i>Polerovirus</i>	2	
<i>Tomato black ring virus</i>	TBRV	<i>Nepovirus</i>	2	
<i>Alfalfa mosaic virus</i>	AMV	<i>Alfamovirus</i>	3	
<i>Cucumber mosaic virus</i>	CMV	<i>Cucumovirus</i>	1	
<i>Tobacco rattle virus*</i>	TRV	<i>Tobravirus</i>	12	
SUM	12	8	246	12

*: Maintained in the tobacco cv. Samsun.

Table 2

The sequenced virus isolates of the IHAR-PIB/Mlochów collection

Name of virus	Name of isolate	Strain	NCBI GenBank accession number	Whole genome or partial	References
PVY	PVY-3202	PVY ^{NTN}	KX356068	Whole genome	
PVY	PVY-3303	PVY ^Z -NTN	KX356069	Whole genome	Yin <i>et al.</i> , 2017
PVY	PVY-3411	PVY ^{N-Wi}	KX356070	Whole genome	
PVY	Wilga (Wi)	PVY ^{N-Wi}	EF558545	Whole genome	Kosakowski <i>et al.</i> , 2007 (unpublished)
PVY	Ditta	PVY ^{NTN}	AJ890344	Whole genome	
PVY	Gr99	PVY ^{NTN}	AJ890343	Whole genome	
PVY	34/01	PVY ^N	AJ890342	Whole genome	Schubert <i>et al.</i> , 2007
PVY	LW	PVY ^O	AJ890349	Whole genome	
PVY	12-94	PVY ^{NTN}	AJ889866	Whole genome	
PVY	E30	PVY ^{NTN}	HM991453	Whole genome	Golnik <i>et al.</i> , 2010 (unpublished)
PVY	Nysa	PVY ^N	FJ666337	Whole genome	Golnik <i>et al.</i> , 2010 (unpub)
PVY	FrKV15	Atypical PVY ^{N-Wi}	HM991454	Whole genome	Obtained from C. Kerlan, France
PVY	YE Epo	PVY ^{N-Wi}	JF804800	Partial, HC-Pro	
PVY	YE Epo	PVY ^O	JF804799	Partial, VPg	
PVY	YE Epo	PVY ^{N-Wi}	JF804798	Partial, VPg	
PVY	YE Epo	PVY ^O	JF804787	Partial, CP	
PVY	YE Epo	PVY ^{N-Wi}	JF804786	Partial, CP	
PVY	Cou8/03	PVY ^{NTN}	JF804780	Partial, CP	Golnik <i>et al.</i> , 2016 (unpublished)
PVY	YC Zb	PVY ^C	JF804797	Partial, VPg	
PVY	YC Zb	PVY ^C	JF804785	Partial, CP	
PVY	N-Gineke	PVY ^N	JF804793	Partial, VPg	
PVY	N-Gineke	PVY ^N	JF804781	Partial, CP	
PVY	New Zealand-N	PVY ^N	JF804789	Partial	
PVY	New Zealand-NTN	PVY ^{NTN}	JF804788	Partial	
PVY	47/96	PVY ^{NTN}	KY092173	Whole genome	
PVY	FrKv2	PVY ^{N:O}	KY112747	Whole genome	Grupa and Syller 2017, (unpublished)
PVY	PVY ^O Li	PVY ^O	KY112748	Whole genome	
PVY	PVY ^{N-Wi} Wy	PVY ^{N-Wi}	KY112749	Whole genome	
TRV	11r21	nd	KF758790	RNA1	
TRV	11r21	nd	KF758797	RNA2	
TRV	Deb57	nd	KF758791	RNA1	
TRV	Deb57	nd	KF758794	RNA2	
TRV	Mlo7	nd	KF758792	RNA1	Yin <i>et al.</i> , 2014 a, b
TRV	Mlo7	nd	KF758795	RNA2	
TRV	Slu24	nd	KF758793	RNA1	
TRV	Slu24	nd	KF758796	RNA2	
PVM	I38	nd	KJ365309	Partial, CP	Grupa and Syller, 2016

nd: not determined.

Table 3

Potato viruses maintained in freeze-dried potato tissue at IHAR-PIB/Młochów

Virus	Number of isolates in freeze-dried form			
	1996-1997	2003	2007	2016
PVY	31	11	145	190
PVM			9	10
PVS			3	13
PLRV			5	7
PVA			2	1
PVX			1	2
PAMV			1	3
BMV			2	2
TBRV			1	2
AMV			2	3
CMV			1	1
TRV	1			
SUM	32	32	172	234

MATERIALS AND REAGENTS

- 1) Virus infected potato tubers
- 2) Soil mixed with peat in a proportion of 1:2 in trays
- 3) Fertilizer PG mix (14-16-18) + Micro at a concentration of 0.5-0.8 kg × m⁻³
- 4) Pots (Ø=16 cm)
- 5) MS medium with 3% sucrose and 0.8% agar
- 6) Reclosable Bag (Plast Polska 150x200 mm)
- 7) Falcon tube (Medlab Products, 50 ml, Cat. No 24.3050.1s)
- 8) Latex powder-free gloves "Protect clinic" (Semperit Technische Producte Gesellschaft)

EQUIPMENT

- 1) Insect-free greenhouse with natural light
- 2) Cold dark room (4°C)
- 3) Cold chamber (6-8°C)
- 4) Laminar flow cabinet KL-21
- 5) Freezer (-20°C) (LIEBHERR)
- 6) Lyophilizing cabinet (LABCONCO, Freeze Dry/SHELL FREEZE SYSTEM, FreeZONE 18)

PROCEDURE

Maintaining viruses in potato plants in vivo in the greenhouse

- 1) The virus isolates are maintained in their host cultivars by replanting the infected potato tubers yearly in an insect-free greenhouse (day temp. 20-26°C and night temp. 14-16°C, day length 16 h) from May to June.

- 2) The harvested tubers are stored at 4°C in a dark cold room.
- 3) The selected virus isolates are checked for their pathogenicity in the potato or other proper test plants every 3-4 years.

The isolates which have been maintained for 40 years are still infectious. In total, 246 virus isolates are maintained in potato plants *in vivo* in the greenhouse.

Maintaining viruses in potato plantlets cultured in vitro

- 1) Potato plantlets of the originally collected virus-infected cultivar are maintained an *in vitro* culture in an MS medium (Murashige and Skoog, 1962).
- 2) The tubes with plantlets are kept in a growth chamber at 20-22°C with a photoperiod of 16 h with a monthly passage to a fresh medium.
- 3) For long-term maintenance, the MS medium is modified with a higher percentage of sucrose (3%).
- 4) The plantlets are kept at 20-22°C and the rooted plantlets are transferred to a cold chamber at 6-8°C under 1000 lx light intensity. Under such conditions, the plantlets may be maintained for two years without passage.
- 5) The detailed *in vitro* preservation of potato plants is referred to in Strzelczyk-Żyta (2018).

In the *in vitro* collection, 4 PVY isolates, 6 PVA isolates and 2 PLRV isolates have been maintained since 1990s (Zagórska and Kryszczuk, 1998).

Maintaining viruses in frozen leaves

- 1) Leaf tissues with characteristic symptoms are collected from 4- to 6-week-old potato plants which contain the respective virus.
- 2) The infected leaf tissues are kept in sterile plastic bags and stored at -20°C in a freezer.
- 3) Two copies of each of the 246 isolates, which are maintained in potato plants *in vivo*, are parallelly stored at -20°C.
- 4) The TRV isolates are maintained in frozen tobacco leaves at -20°C.

Maintaining viruses in freeze-dried tissues

- 1) One copy of the *in vivo* maintained viruses is also maintained in a freeze-dried form.
- 2) The leaf tissues with characteristic symptoms are collected into a falcon tube from 4- to 6-week-old potato plants containing the respective viruses, and stored at -20°C in a freezer.
- 3) Before lyophilisation the cap is removed and the tube is closed with a parafilm perforated with small holes.
- 4) The leaves in the tubes are freeze-dried in a lyophilizing cabinet for two days.
- 5) Finally, the tube containing the freeze-dried tissue is closed with the same cap and stored at 4°C in a cold room.

The number of virus isolates maintained in the freeze-dried form is 234 (Table 3).

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