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METHODS TO STUDY THE PVY POPULATION IN THE POTATO

ABSTRACT

The PVY population in the potato has been studied continuously using tobacco bait plants in potato fields at Młochów since 1980 at two-year intervals and in potato tuber samples collected from different regions of Poland since 2001 yearly. The paper presents the combined biological, serological and molecular assays for PVY identification and strain classification. Biologically, PVY strains are defined with respect to their ability to elicit hypersensitive resistance (HR) mediated by *N* genes in differential potato cultivars (King Edward, Desiree and Pentland Ivory) and to symptoms in the tobacco (cultivar Samsun). Serologically, an ELISA assay based on polyclonal or monoclonal cocktail antibodies recognizes all PVY strain types, while the specific monoclonal antibodies help to recognize PVY^{N} or PVY^{O}/PVY^{C} strains. Multiplex RT-PCR, Real-time RT-qPCR and sequencing-based assays are used to define the PVY genome structure. In the Polish population of PVY, the strains PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, PVY^{Z}-NTN and PVY^{E} were identified, while the PVY^{C} strain was not detected.

Keywords: differential potato cultivars, multiplex RT-PCR, PVY strains classification, real-time RT-qPCR, sequencing, tobacco bait plants

INTRODUCTION

Potato virus Y, a member of the *Potyviridae* family, is among the top ten plant viruses due to its scientific and economic importance (Scholthof *et al.*, 2011). It infects a wide host range including the potato. PVY is classified into different strains based on its ability to elicit hypersensitive resistance (HR) mediated by *N* genes in differential potato cultivars, symptoms in the tobacco and genomic information (Singh *et al.*, 2008; Kehoe and Jones, 2016). The PVY strains that elicit HR genes *Ny*, *Nc* and *Nz* are classified as PVY^{O}, PVY^{C} and PVY^{Z} strains, respectively; and they do not induce veinal necrosis (VN) in the

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tobacco (Singh *et al.*, 2008; Kerlan *et al.*, 2011). The PVY strains that overcome all these three HR genes are classified as PVY^{N} that causes VN in the tobacco and PVY^{E} that does not induce VN in it (Singh *et al.*, 2008; Galvino-Costa *et al.*, 2012). PVY^{N-Wi} and PVY^{NTN} belong to the PVY^{N} strain group, and PVY^{NTN} elicits potato tuber necrotic ringspot disease (PTNRD) in sensitive potato cultivars. Both strains possess a recombinant genome between PVY^{N} and PVY^{O} (Hu *et al.*, 2009, Green *et al.* 2018).

The recombinant PVY variants are found to be prevalent worldwide in potato -growing area and are replacing the non-recombinant strains PVY^{C}, PVY^{O} and PVY^{N} (Funke *et al.,* 2017, Davie *et al.,* 2017). In the UK, a survey of the molecular diversity of PVY indicated that 80-90% belong to the recombinant European PVY^{NTN} group (Davie *et al.*, 2017). In the United States, there is a rise in the recombinant PVY^{N-Wi} strain incidence, from less than 27% in 2011 to 53% in 2015. In Poland, the first PVY^{N-Wi} isolate (named Wi) was identified on the potato cv. Wilga collected from western Poland in 1984 (Chrzanowska, 1991) and the first PVY^{NTN} isolate (named 12/94) was detected on a tobacco bait plant grown in a potato field at Młochów in 1994 (Chrzanowska and Doroszewska, 1997). The recombinant PVY^{N-Wi} and PVY^{NTN} strains are the predominant forms among the isolates from the potato crop in Poland (Yin *et al.*, 2012, Yin *et al.*, 2016 and Figures 2 and 3).

The PVY population in the potato has been studied continuously using tobacco bait plants in potato fields at Młochów since 1980 at two-year intervals and in potato tuber samples collected from different regions of Poland since 2001 yearly (Chrzanowska, 1991; Chrzanowska and Doroszewska, 1997; Zimnoch-Guzowska *et al.*, 2013; Yin *et al.,* 2010, 2011, 2012, 2017).

MATERIALS AND REAGENTS

1) Potato tuber samples: 100 tubers per cultivar
2) Tobacco bait (cv. Samsun) plants: 300 plants per experiment
3) Virus-free potato differential cultivars: King Edward, Desiree, Pentland Ivory
4) Virus-free potato cultivar Nicola (for PTNRD assessment)
5) Soil mixed with peat in a proportion of 2:1 in trays.
6) Fertilizer PG mix (14-16-18) + Micro at a concentration of 0.5-0.8 kg × m^{-3}.
7) Pots (Ø=8cm)
8) Carborundum powder
9) Latex powder-free gloves "Protect clinic" (Semperit Technische Producte Gesellschaft)
10) Mortar and pestle
11) Antibodies (Ab):
    - PVY^{all} monoclonal cocktail Ab for all strains (Bioreba IgG, cat. No 112911, Bioreba Conjugate, cat. No 112921)
    - PVY^{N} (Bioreba Conjugate, cat. No 112722) monoclonal Ab specific to PVY^{N}
    - $PVY^{O/C}$ (Adgen Conjugate, cat. No 1052-04), monoclonal Ab specific to PVY^{O}

12) RNeasy Plant Mini kit (Qiagen, cat. No 74904)
13) Superscript III one-step RT-PCR with Platinum Taq DNA polymerase (Invitrogen, cat. No 12574026)
14) TaqMan micro-RNA Reverse Transcription kit (Applied Biosystems, cat. No 4366597)
15) SYBR Select Master Mix (Applied Biosystems cat. No 4472908)
16) Primers (see Yin and Michalak, 2017)

EQUIPMENT

1) Insect-free greenhouse with natural light
2) ELISA reader (DYNATEX MRX II)
3) GeneAmp PCR System 9700 (Applied Biosystems)
4) LightCycler 480 real-time PCR instrument (Roche Diagnostics)
5) Pipette sets (Sartorius Poland Sp. z o.o.)
6) Deep freezer (- 80°C) (Frigor)
7) MiSeq sequencer (Illumina) (sequencing done by the DNA Sequencing Laboratory, IBB, PAS, Poland)

PROCEDURE

The combined biological, serological and molecular assays are used for PVY identification and strain classification.

1) The biological assay is conducted according to Singh *et al.* (2008). The potato differential cultivars King Edward, Desiree and Pentland Ivory are used for PVY strain differentiation. Potato cv. Nicola is used for the assessment of potato tuber necrotic ringspot disease (PTNRD).
2) The serological assay (ELISA) for PVY (all strains) is based on polyclonal or monoclonal cocktail antibodies. The specific monoclonal antibodies help to recognize PVY^{N} or PVY^{O}/PVY^{C} strains.
3) Multiplex RT-PCR for PVY strain typing is carried out according to Lorenzen *et al.* (2006), Rigotti and Gugerli (2007), and Chikh Ali *et al.* (2010).
4) Real-time RT-qPCR for detection of PVY RNA (*HC-Pro*) and sequencing of PVY whole genome are conducted according to Yin *et al.* (2017). The strains PVY^{O}, PVY^{NTN}, $PVY^{N\text{-}Wi}$, PVY^{Z}-NTN and PVY^{E} were identified. The PVY^{C} strain was not detected according to our survey.

The scheme of methods to study PVY population in the potato used at IHAR-PIB/Młochów is illustrated (Fig. 1). The details of biological, serological and molecular assays are referred to in Yin and Michalak (2017). The growing-on tests is according to Michalak and Chrzanowska (2017).

The strains PVY^{O}, PVY^{NTN}, $PVY^{N\text{-}Wi}$, PVY^{Z}-NTN and PVY^{E} were identified in the population of Polish PVY isolates, while the PVY^{C} strain was not detected in our survey (Figs 2 and 3). Since early 1980s, the recombinant $PVY^{N\text{-}Wi}$ strain has become the predominant form in the PVY population. The recombinant PVY^{NTN} strain appeared in the early 1990s, and it

became predominant in 2008, composing 62% of the population (Yin *et al.*, 2016). Later on, from 2009 to 2014, the PVY^{N-Wi} strain dominated in the population again, reaching up to 63-88% of the population (Yin *et al.*, 2016). Very low percent of PVY isolates induced vein clearing (VCl) in tobacco (0.3-8%), and among them, two isolates were confirmed as PVY^{O} (Yin *et al.*, 2016) and several others were identified as PVY^{E} based on the biological tests (Figs 2 and 3). One isolate was identified as PVY^{Z} strain based on the biological tests, however it groups with PVY^{NTN} based on the sequence feature (Yin *et al.*, 2017, Figs 2 and 3). This isolate is named as a PVY^{Z}-NTN strain. The mixed infection, as shown the positive reaction to both PVY^{N}- and PVY^{O}-specific antibodies, reached 2 to 20% of the tested samples (Yin *et al.*, 2016, Figs 2 and 3). We did not find differences in virus detection using serological and molecular methods among the samples tested.

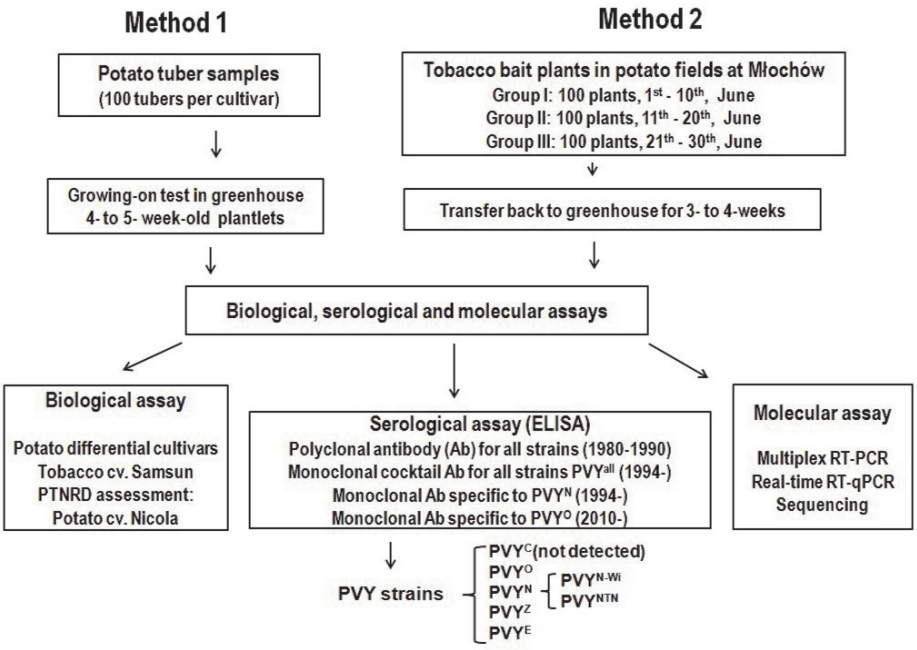

Fig. 1. A scheme of the methods to study PVY population in the potato used at IHAR-PIB/Młochów.

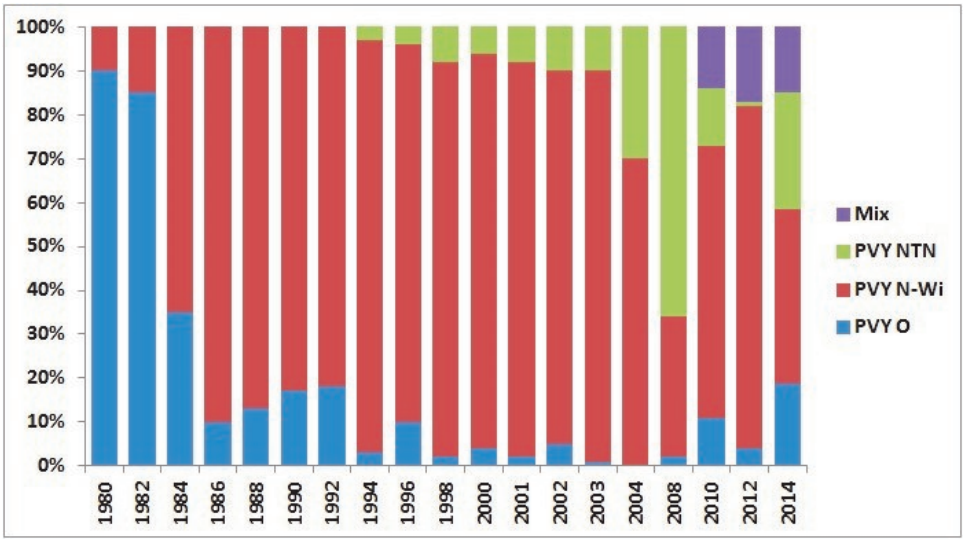

Fig. 2. Changes in the PVY population tested on tobacco bait plants at Młochów, 1980 – 2014

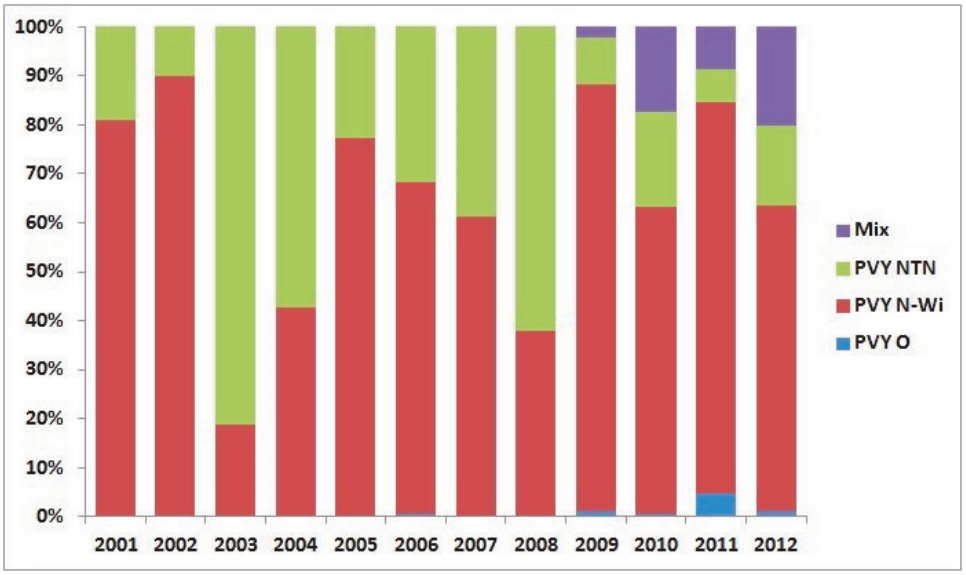

Fig. 3. The structure of the PVY population on potato samples collected in Poland from 2001 to 2012.

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