

CHARACTERISATION OF BULGARIAN POTATO VIRUS Y ISOLATES IN POTATOES

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ABSTRACT

Potato virus epsilon (PVY) is wide spread in potatoes in Bulgaria and cause extensive damages to agricultural production. We identified several strains of the virus, of which the most common strain is PVY^{N/NTN}. Most of the strains showed no tuber symptoms in primary infection, which is essential for release of latent infections in seedlings, rapid spread of the virus in the crop and subsequent heavy losses for the use of infected tubers. Characterization of Bulgarian PVY isolates and determination of the virus strains is essential for the diagnosis of the virus in propagating seed material and timely control of its distribution.

Key words: PVY strains, Touchdown RT PCR, potato

Introduction

Potato virus Y (PVY) is among the most economically damaging viruses with a host range including major crops, such as potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum*, pepper (*Capsicum annuum* L.), tobacco (*Nicotiana* spp) and several species of weed mainly in the *Solanaceae* family (Rolland *et al.*, 2008; Kerlan and Moury, 2008). PVY is considered to have a relatively wide host range including mainly solanaceous crops as well as solanaceous and non-solanaceous weeds, and even ornamentals (Kerlan 2006). Susceptibility to PVY of some solanaceous weeds, such as *Physalis floridana*, *Solanum nigrum* and *Solanum dulcamara*, is commonly known, *P. floridana* being used to differentiate PVY strains (Beemster and de Bokx 1987). Arable weeds, especially biennial and perennial ones, can act as natural virus reservoirs for transmission by vectors. There are common weed species known as hosts for PVY including *Capsella bursa pastoris* and *Chenopodium album* (Kazinczi *et al.*, 2004). A comprehensive survey of common weed species, including known hosts for PVY, may be valuable for understanding the epidemiology and future management of the virus in cropping systems where potato is grown. Fourteen weed species were identified in potato crops in the region of Sofia. Most of them were annuals (broadleaf weeds). Five weed species species - *C. album*, *X. strumarium*, *S. nigrum*, *A. retroflexus*, and *P. floridana* were reservoirs of PVY. (Petrov *et al.*, 2015)

Potato virus Y isolates were originally divided into three main strain groups stained; PVY^O, PVY^N and PVY^C, based on systemic and local symptoms induced by specific cultivars of tobacco and potato - *Nicotiana tabacum* cv. Samsun and *Solanum tuberosum* ssp. *tuberosum*. Some strains do not belong to any of these strain groups (Delgado-Sanchez and Grogan, 1970). Unlike PVY^O and PVY^N strains some PVY^C strains are not transmitted by aphids (Watson, 1956; De Bokx, 1978).

PVY^O group or so called Ordinary strains are widespread and cause severe mosaic symptoms turning to necrosis in *Physalis floridana* and mosaic in tobacco (Van der Vlugt, 1993). PVY^N strains known to cause venous necrosis in tobacco, produce very little mosaic symptoms in the most potato cultivars and necrosis in *P. floridana* (Van der Vlugt, 1993). PVY^{NTN} was first found in Hungary in 1978 and since then it has been reported from most potato producing countries in Europe (Le Romancer, 1994; Beczner, 1984; Weidemann, 1996). PVY^{NTN} strain resembles PVY^N but cause characteristic necrotic ring spots on potatoes tubers. Today, this strain is considered as a substrain of PVY^N (van der Heuvel, 1994; Glais, 1996). PVY^{N-Wilga}, discovered in Poland in 1984, is described as differing in virulence and aggressiveness against the earlier discovered PVY^N strains,

and later it was found to be serologically related to PVY^O. It causes tobacco vein necrosis, which is typical for PVY^N, but its genomic RNA had nucleotide sequences more similar to PVY^O and PVY^{NTN} (Shukla, 1994). Similar virus strains have been reported in Canada (McDonald, 1996), Spain (Blanco -Urgoiti, Sanchez, 1998) and France (Kerlan, 1999). PVY^Z, first reported in Britain in 1984, differs from PVY^O and PVY^C with its ability to overcome hypersensitive genes Ny, Nc and Nz gene (Jones, 1990).

The aim of this research is to determine PVY strains in potatoes in Bulgaria and to characterize their epidemiology and transmission through potato tubers.

Material and methods

RNA extraction from potatoes infected with PVY. Extraction of total RNA was carried out by RNEasy Plant Mini Kit (Qiagen, Germany) according to the instructions of the manufacturer.

Touch-Down RT-PCR. We used 7 primers for the P1, 6K2-NIa and HC/Pro-P genetic regions of PVY with program modification (Petrov, 2012). Copy DNA synthesis: denaturation of total RNA (0.05-0.5 µg) was held at 95°C for 5 min with 10 µl primer (PVY Primer 1) in a final volume of 10 µl, followed by cooling on ice to avoid renaturation. Master mix (15 µl) contains: 5 µl of 5x MMLV-buffer, 2 µl of dNTPs (2mM), 0.5 µl of M-MuLV reverse transcriptase (200 U/µl), 7.5 µl deionized H₂O. Incubation was held at 42°C for 60 min. Master mix for the PCR contains: 1 µl cDNA, 2.75 µl 10x PCR buffer, 2.2 µl MgCl₂ (25 mM), 2.2 µl dNTPs (2 mM), 1 µl PVY Primers (10 µM), 1 µl Taq DNA-Polymerase (5 U/µl), 12.85 µl H₂O. PCR was done in thermo cycler Auto-Q Server (LKB, UK) with the following program: initial denaturation step 3 min at 95°C; 5 cycles 30 sec at 92°C, 30 sec at 62°C, and 90 sec at 72°C; 5 cycles 30 sec at 92°C, 30 sec at 60°C, and 90 sec at 72°C; 5 cycles 30 sec at 92°C, 30 sec at 58°C, and 90 sec at 72°C; 10 cycles 30 sec at 92°C, 30 sec at 55°C, and 90 sec at 72°C; final elongation 10 min at 72°C. A combination of three primers - PVY Primers 1, 7 and 8 (Petrov, 2012) was used for distinguishing of PVY^{N/NTN} (443 bp) from PVY^O (281 bp). Primer pairs S9133/A9422 (641 bp) and S5585 A6032 (448 bp) distinguish PVY^{N:O} (only one product 641 bp) and PVY^{NTN} (two products 641 bp and 448 bp).

Results and discussion

From the South West corner of Bulgaria in the region of Smolyan we found potato tubers infected with four different PVY strains. Potato tubers with necrotic rings and irregular necrotic patterns were infected with PVY^{NTN} (Fig. 1, Fig.6). Potato tuber sample (Fig.1) first was identified as PVY^{N/NTN} (Fig. 5) and later was differentiated to PVY^{NTN} (Fig.6). Potato tuber sample (Fig.4) was also identified in the PVY^{N/NTN} strain group first and with the second PCR was differentiated to PVY^N strain remaining with only one fragment in contrast to PVY^{NTN} (two fragments). PVY^{NTN} virus strain caused the major damages in potato production affecting potato quality and quantity reducing the yield to almost 80 %. Fortunately this virus strain is not so common in Bulgaria. The most widely spread PVY strains were PVY^O (which did not induce symptoms on potato tubers) (Fig.2, Fig.5) and PVY^{N/NTN} strain group (Fig.4, Fig.5). PVY^{N/NTN} is widely spread not only on potatoes but also on tobacco and tomato in Bulgaria.

We differentiate relatively new for Bulgaria PVY strain – PVY^{N:O}, which is recombinant and induce small necrotic rings on potato tubers (Fig.3, Fig. 7).

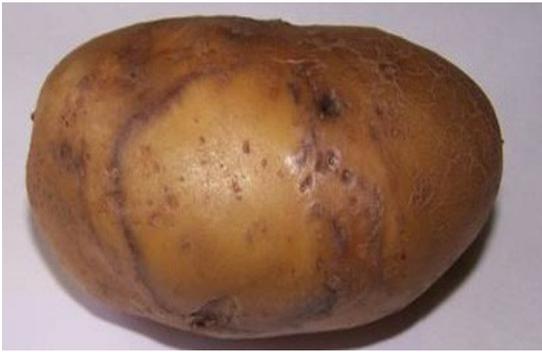


Fig. 1 Potato tuber infected with PVY^{NTN}



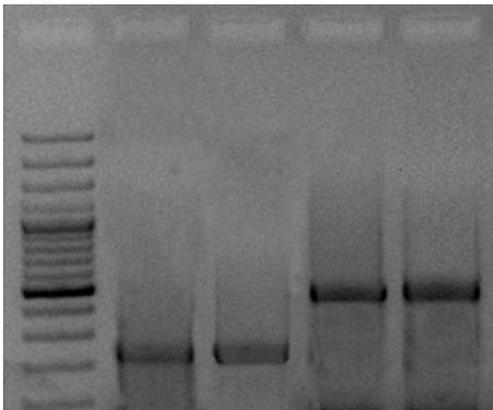
Fig. 2 Potato tuber infected with PVY^O



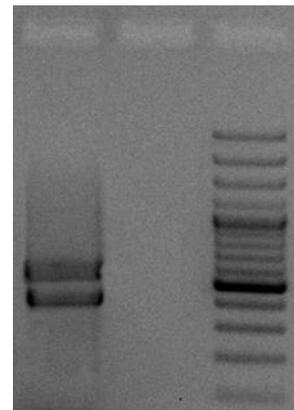
Fig. 3 Potato tuber infected with PVY^{N:O}



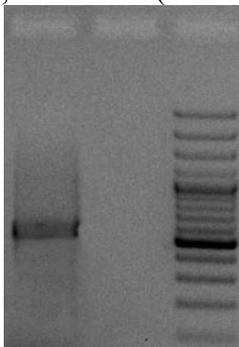
Fig. 4 Potato tuber infected with PVY^N



Фиг. 5 Touch down RT-PCR for PVY strains PVY^O (281 bp), PVY^{N/NTN} (443 bp)



Фиг. 6 Touch down RT-PCR for PVY strains PVY^{NTN} (two fragments - 448 bp and 641 bp)



Фиг. 7 Touch down RT-PCR for PVY strains PVY^{N:O} (641 bp)

In most of the cases of plants infected with PVY^{N/NTN} virus strain group had no symptoms on the tubers (Fig.4), leaves and seeds. This leads to the conclusion that in the strain group

predominate PVY^N strains, which did not induce symptoms. Due to the lack of visible virus symptoms on potato tubers many manufacturers can easily spread viral infection of major production areas by asymptomatic infected tubers. Therefore diagnosis and identification of the different viral strains is of great importance for control and containment of the disease and reduce damage in potato production.

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