ACTA MICROBIOLOGICA BULGARICA Volume 39 / 3 (2023)

Potato Viruses of Economic Importance for Production of Planting Material in Bulgaria

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Abstract

Potato is the world's third most widespread food crop, following wheat and rice. Tubers play an important role in the spread of virus diseases in different regions in Bulgaria for seed production. The most important viruses in potatoes in Bulgaria include Potato virus Y (PVY), Potato leaf roll virus (PLRV), Potato virus S (PVS), and Potato virus M (PVM). Most of the viruses in potatoes do not induce symptoms in tubers, which plays an important role in viral dissemination by the growers. In this way, after planting the tubers, the quality and yield of the production may be severely reduced. The use of virus-free tubers by growers is of key importance for the control of diseases and the reduction of loss of production.
Keywords: potato planting material, viruses, PVY, PLRV, PVS, PVM

Резюме

Картофът е третата най-разпространена хранителна култура в света, след пшеницата и ориза. Клубените играят важна роля в разпространението на вирусните заболявания в различни региони на България за семе производство. Най-важните вируси по картофи в България са картофеният вирус Y (PVY), вирусът на картофеното листно завиване (PLRV), картофеният вирус S (PVS) и картофеният вирус M (PVM). Повечето вируси по картофи нямат симптоми по клубените, което играе важна роля за лесното им разпространение от страна на производителите. По този начин след засаждане на клубените качеството и добивът на производството са силно намалени. Използването на свободни от вируси, клубени от производителите е много важно за контрола на болестите и намаляване на загубата на продукция.

Introduction

Potato (*Solanum tuberosum* L.) originates from the highland regions of the Andes in Peru and Bolivia (Burton, 1966), and is in the top five of the most consumed crops in the world (Ross, 1986). Potatoes can be affected by many pathogens and pests, which is the reason for being treated with chemical pesticides more than any other food crop. Potatoes are susceptible to about 40 viruses, which can significantly reduce the quality and quantity of the yield (Valkonen, 2007; Gildemacher *et al.*, 2009). The most important viruses worldwide include *Potato virus Y* (PVY, genus *Potyvirus), Potato leaf roll virus* (PLRV, genus *Polerovirus*), *Potato virus X* (PVX, genus *Potexvirus*), *Potato virus S* (PVS, genus *Carlavirus*), *Tobacco rattle virus* (TRV, genus *Tobravirus*) and *Potato virus M* (PVM, genus *Carlavirus*) (Singh, 1999).

PVY is the most economically important virus of potatoes causing great yield loss, wherever the crop is cultivated (De Bokx and Huttinga, 1981; Petrov and Gaur, 2015). Potato tubers serve as the main source of infection every year. The virus comprises different strains, which are grouped in several distinct groups based on the interaction with the hypersensitive resistance genes *Nc, Ny*, and *Nz* in potato cultivars. The strain groups induce different symptoms: the common (or Ordinary) group PVY^{O} induces symptoms of the upper part of the plants, PVY^{N} induces vein necrosis in tobacco but does not elicit hypersensitive resistance response in potato, PVY^{NTN} induces tuber necrosis and PVY^{C} is the stipple streak group. PVY^{NTN} and PVY^{Wilga} are comparatively new pathotypes. PVY^{Wilga} is a PVY^{N} strain serologically related to PVY^{O}, however, the

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symptoms, which they induce in potatoes, are less severe compared to the typical PVY^N strains. PVY^O and PVY^C induce symptoms on leaves, such as mottling, yellowing, necrotic spots or rings, leaf necrosis, and leaf drop, as well as premature death of stems at primary infection (Kerlan 2006; Singh *et al.*, 2008; Petrov, 2012). Secondary infection with these two strains can cause leaf necrosis, dwarfing, and crinkling of the plants (Hooker, 1981). PVY^N induces less severe mottling of the leaves. Infection with PVY^O, PVY^C, and PVY^N is characterized by a lack of symptoms on tubers. On the contrary, plants infected with the PVY^{NTN} strain produce tubers with irregular brownish rings, which prolong in depth in the form of necrotic arcs. The skin of the tubers may also crack. The strain is the main inducer of the so-called potato tuber necrotic ringspot disease (PTNRD) and was considered a recombination between the strains PVY^O and PVY^N. However, in North America were recovered some non-recombinant isolates (NA-PVY^N and NA-PVY^{NTN}) also cause PTNRD in potatoes. In Bulgaria were recovered other devastating PVY strains ($PVY^{N/NTN}$ causing concentric necrotic rings on potato tubers), which were similar to PVY^{NTN} but not identical (Petrov *et al*., 2008). PVY strains are transmitted by aphids in a non-persistent manner (Singh *et al*., 2008).

PLRV affects the lower leaves first and later gradually progresses upward. The infection causes marginal yellowing, rolling, and thickness of the upper and apical leaves and changes in their texture to a leathery feeling (Khalid *et al*., 2000; Petrov and Gaur, 2015). PLRV spreads in a persistent manner by aphids probably in all potato-producing regions on the globe. However, the frequent replacement of seed stocks and the systemic use of insecticides are able to keep the incidence low (Radcliff *et al*., 1993).

PVM is one of the most common and economically important among potato viruses. It is distributed all over the globe and causes a reduction in potato yield of 15–45%. Several aphid species transmit the virus in a non-persistent manner; mechanical transmission with sap from young leaves is also possible (Kerlan, 2008). Symptoms after infection with PVM are not specific and resemble the ones caused by several other common viruses of potatoes like PVS, PVX, and PVY^O (Ruiz de Galarreta et *al*., 1998). Slight mottle and mild abaxial rolling of the leaves, as well as stunting of the shoots, are common (Kerlan, 2008). Depending on the plant cultivar and the virus isolate, the severity of manifestation fluctuates greatly (Ruiz de Galarreta et *al*., 1998).

PVS is also spread worldwide and includes two strains, PVS^O (Ordinary) and PVS^A (Andean). Transmission occurs nonpersistently by several aphid species and by contact (Hooker, 1981). The host range of PVS is limited to *Sonalaceae* and *Chenopodiaceae* plant species. Potatoes often do not manifest the infection with PVS, however, the occurrence of mild symptoms can be observed. The reduction of yield is usually moderate and occasionally can be up to 30% (Kerlan, 2008). Upon coinfection with other viruses, such as PVM, the losses can reach 40-75% (Loebenstein, 2009).

The focus of this study is to elucidate the occurrence of the plant virus species, distributed in the prioritized potato cultivars grown for seeds in the region of Kyustendil. With the perspective to help the reduction of production losses, we aimed *a*) to identify the potato viruses present in samples taken from symptomatic and symptomless tubers and possibly, *b*) to find a virus-free cultivar.

Material and Methods

Objects of our study were five potato cultivars: Agria, Riviera, Trezor, Sante, and Djeli, grown for seed production in the region of Kyustendil. Tubers from all potato cultivars were screened for four viruses: PVY, PLRV, PVM, and PVS. After germination and growing, sap from homogenized potato tubers was tested with DAS-ELISA (Clark and Adams, 1977; Petrov, 2012). Samples from healthy and infected plants served as negative and positive controls, respectively. Tubers infected with PVY were additionally subjected to RT-PCR for strain differentiation.

Detection of viral infection by DAS-ELISA

A kit from LOEWE Biochemica GmbH, Sauerlach, Germany was used. ELISA plates were loaded with antiserum (IgG) for the specific virus (PVY, PLRV, PVS, and PVM), diluted in 0.05M carbonate buffer according to the manufacturer‘s instructions. The plates were incubated at 37°C for 4 h and the unbound components were washed three times with PBS-T buffer for 5 minutes. All samples were ground in extraction buffer containing 1% PVP (polyvinyl pyrrolidone) at a ratio of 1:10. The plates were incubated at 4°C for 16 h. After washing three times, alkaline phosphatase conjugate for the specific virus (PVY, PLRV, PVS and PVM) was added and the plates were additionally incubated at 37°C for 4 h. Para-nitrophenyl phosphate (p-nitrophenyl phosphate, Sigma) in diethanolamine buffer (pH

9.8) at a ratio of 1 mg:1ml was used as a substrate. The reaction was carried out at room temperature in the light. 3N NaOH was used to stop the reaction. Color adsorption was measured in ChroMate® 4300 (Awareness Technology Inc., USA) at a wavelength of 405 nm. The samples with optical density (OD, absorption) exceeding three times the value of the negative control (called the cut-off value) were considered positive (Petrov, 2012).

Total RNA extraction

RNA was extracted with the use of RNEasy Plant Mini Kit (Qiagen, Germany), according to the instructions of the manufacturer (Petrov, 2012).

Touch-Down RT-PCR

We carried out RT-PCR program with touch-down modification using PVY-specific Primers 1, 7, and 8 for the P1 gene region of the virus (Petrov, 2012). The synthesis of copy DNA was performed as follows: 1) denaturation of total RNA (0.05-0.5 µg) at 95ºC for 5 min with 10 µl PVY Primer1 in a final volume of 10 µl; 2) cooling on ice to avoid renaturation; 3) preparation 15 µl of master mix, including 5 µl of 5 × MMLV-buffer, 2 µl of dNTPs (2 mM), 0.5 µl of M-MuLV Reverse transcriptase (200 U/µl) and 7.5 µl H_2O; *4*) incubation step at 42°C for 60 min. The master mix for the PCR was: 1 µl cDNA, 2.75 µl 10 × PCR buffer, 2.2 µl MgCl2 (25 mM), 2.2 µl dNTPs (2 mM), 1 µl PVYPrimer1 (10 µM), 1 µl PVYPrimer7 (10 µM), 1 µl PVYPrimer8 (10 µl), 1 µl Taq DNA-Polymerase (5 U/µl), 12.85 µl H_2O. PCR was carried out in thermo cycler StepOnePlus (Applied Biosystems, ThermoFisher Scientific, USA) using the following program: 1) initial denaturation step at 95°C for 3 min; 2) five cycles at 92°C for 30 sec, 62°C for 30 sec, 72°C for 90 sec; 3) five cycles at 92°C for 30 sec, 60°C for 30 sec, 72°C for 90 sec; 4) five cycles at 92°C for 30 sec, 58°C for 30 sec, 72°C for 90 sec; 5) ten cycles at 92°C for 30 sec, 55°C for 30 sec, 72°C for 90 sec; 6) final elongation at 72°C for 10 min. The DNA fragments were separated by gel electrophoresis in 1.5% agarose gel in TAE buffer with ethidium bromide (0.2 µg/ml) at 80-150V for 1 h (Petrov, 2012). The products were observed in the gel documentation system Nu Genius (SynGene, USA) at a wavelength of 315 nm.

Results and Discussion

Potato cultivars from the region of Kyustendil showed different results from the ELISA screening. The OD values of the samples from Agria and Sante cultivars were under the cut-off value for all the four tested plant viruses PVY, PLRV, PVM, and PVS (Fig. 1). However, the samples from potato cultivars Trezor and Djeli revealed the presence of all four viruses (Fig. 1). The batches from Riviera cultivar were free form PVM, but carried PVY, PLRV or PVS. It is important to note that most of the potato plants and tubers infected with PVY, PVM, or PVS were symptomless. Moreover, some of the symptomless samples carried PVY and PVM simultaneously. Some of the potato plants from cultivars Riviera, Trezor, and Djeli that were infect-

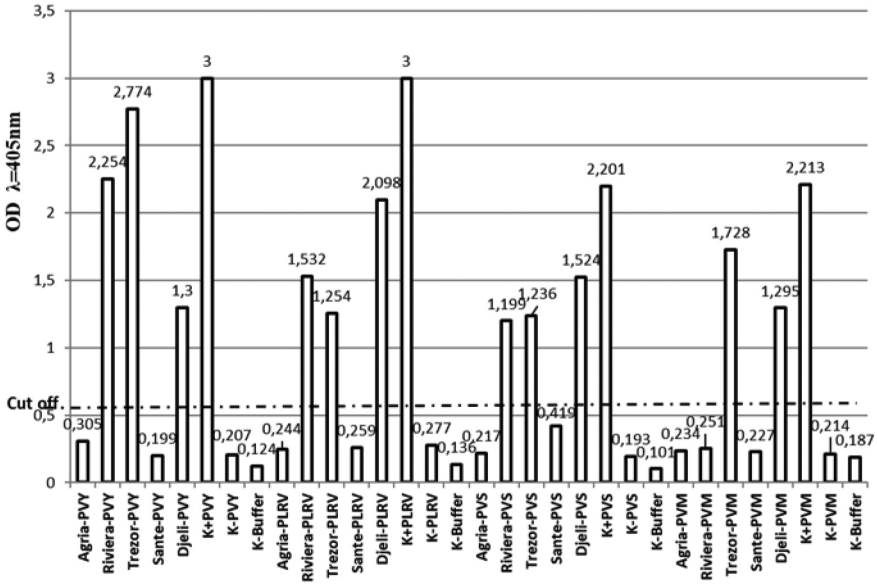

Fig. 1. DAS-ELISA results for virus infection of the tested potato cultivars

Legend: /Cultivar/-PVY, the specified cultivar tested for infection with PVY; /Cultivar/-PLRV, the specified cultivar tested for infection with PLRV; /Cultivar/-PVS, the specified cultivar tested for infection with PVS; /Cultivar/-PVM, the specified cultivar tested for infection with PVM; K+, positive control for the specified virus from the LOEWE kit; K-, negative control for the specified virus from the LOEWE kit; K-Buffer, negative control from the buffer

ed with PLRV expressed leaf rolling of the apical leaves, but, again, most of them including the tubers were symptomless.

The performed RT-PCR differentiated three PVY strains, carried by potato cultivars Riviera, Trezor, and Djeli. The polymerase reaction of the $PVY^{N/NTN}$ strain amplified one fragment of 445 bp, from the P1 region of the RNA virus genome. PVY^{O} gave one smaller fragment of 280 bp. PVY^{NTN} was identified by its two fragments of 450 bp and 640 bp. $PVY^{N/NTN}$ was the most common strain among the samples, followed by the strains PVY^{O} and PVY^{NTN}. All RT-PCR-tested potato cultivars in the Kyustendil region were infected with $PVY^{N/NTN}$. Most of the plants were symptomless, but sometimes mosaics on the leaves and necrotic spots on the leaf lamina could be observed. Again, potato tubers exhibited necrosis only occasionally but the symptoms could be clearly visible (Fig. 2). PVY^{O} was established only in cultivar Riviera and the only carrier of PVY^{NTN} was cultivar Trezor. PVY^{O} occasionally induced leaf mosaic. Many potato plants infected with PVY^{NTN} expressed irregular necrotic rings and different degree of chlorosis and necrosis of the leaves. PVY^{NTN} was the main inducer of potato tuber necrosis disease, which greatly affects the quality of the production.

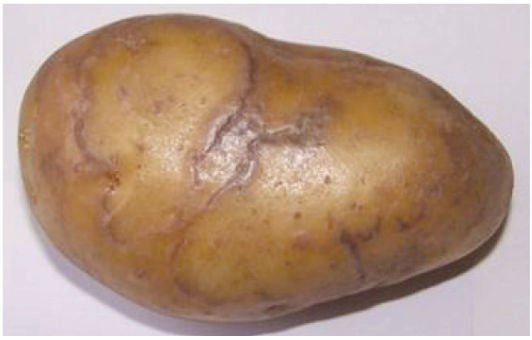

Fig. 2. Potato tuber with necrotic rings

The performed experiments revealed that many of the potato plants grown in the region are symptomless carriers of four economically significant plant pathogens – the viruses PVY, PLRV, PVM, and PVS, which is a serious problem for seed propagation. Infected and symptomless potato tubers used for crop production become sources of viral diseases, which can significantly reduce the quality and quantity of the yield. Cultivars Agria and Sante were virus-free of all four important viruses and their propagation does not treat the potato production.

Conclusion

Most of the plants from potato cultivars Riviera, Trezor, and Djeli grown for seed production in the region of Kyustendil region were symptomless carriers of viral infection with PVY, PLRV, PVM, and PVS, which are major plant pathogens in potato production. This raises the necessity of regular laboratory testing for economically important viruses to ensure the reduction of transmission and subsequent crop losses. Potato cultivars Agria and Sante were free from all tested viruses and their propagation could be considered as safe.

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