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РАЗПРОСТРАНЕНИЕ И ИКОНОМИЧЕСКО ЗНАЧЕНИЕ НА РАСТИТЕЛНИТЕ ВИРУСИ ПО КАРТОФИТЕ ЗА СЕМЕПРОИЗВОДСТВО В РЕГИОНА НА КЮСТЕНДИЛ

DISTRIBUTION AND ECONOMIC IMPACT OF PLANT VIRUSES IN POTATOES FOR THE SEED PRODUCTION IN THE REGION OF KYUSTENDIL

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Abstract

Potato is the world's fourth widespread food crop, following maize, wheat, and rice. Tubers play an important role in the spread of virus diseases in different seed production regions in Bulgaria. The most important viruses in potatoes in Bulgaria include Potato virus Y (PVY), Potato leaf roll virus (PLRV), Potato virus X (PVX), Potato virus S (PVS), Potato virus M (PVM), and the Tobacco rattle virus (TRV). Most of the viruses in potatoes have no symptoms on the tubers and the fact plays an important role for their easy dissemination by the growers. In that way, after planting the tubers, the quality and yield of the production are severely reduced. The use of virus-free tubers by growers is very important for the disease control and reduction of the production loss.

Key words: potatoes, viruses, economic impact.

INTRODUCTION

Potato (*Solanum tuberosum* L.) and other tuber bearing *Solanum* species first time originate in the highland regions of the Andes in Peru and Bolivia (Burton, 1966). Potato is the fourth most important crop in production and fifth in area among crop plants grown for human consumption worldwide. Potato is being appreciated for its nutritional value as well as its uses in the starch and food processing industry (Ross, 1986). It is now a valuable cash crop in almost all countries of this region and ranked as the 2nd to 4th most important crop in relation to other crops (Niederhauser, 1993).

Potato is susceptible to many diseases (viruses, bacteria and fungi) and pests, and the amount of chemical pesticides applied annually to this crop is

greater than that of any other food crop. About 40 viruses are known to affect the potato crop (Valkonen, 2007). Potato viruses cause remarkable reductions in yield quality and quantity of potato crop (Gildemacher et al. 2009)The six important viruses in many countries 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 causes the most significant yield loss in potato, wherever it is cultivated (De Bokx and Huttinga 1981). The yearly transfer is mainly via potato tubers. Different strains show different symptoms. PVY isolates have been categorized into several distinct groups of strains. These include the common (ordinary) group PVY^{O}, the tobacco vein necrosis group PVY^{N} , the stipple streak group PVY^{C}, PVY^{Z}, $PVY^{N\text{-}Wilga}$ and the tuber necrosis strain group PVY^{NTN} (Kerlan 2006, Singh et al. 2008). PVY^{N} induces vein necrosis on *Nicotiana tabacum* leaves but does not elicit hypersensitive resistance response in potato. $PVY^{N\text{-}Wilga}$ and PVY^{NTN} are known as new pathotypes. PVY^{Z} strains are able to overcome both *Ny tbr* and *Nc* resistance genes. PVY^{N}-^{Wilga} pathothypes are PVY^{N} strains serologically related to PVY^{O} but inducing less severe symptoms in potato than PVY^{N} strains. Primary infection with PVY^{O} and PVY^{C} strain group isolates induces necrosis, mottling or yellowing, necrotic leaf spots or rings, leaf drop and premature death of stems.

Sometimes necrosis in leaf, dwarfing and crinkling symptoms causes in potato by secondary infection of PVY^{O} and PVY^{C} (Hooker 1981). PVY^{N} produces milder form of leaf mottling. Plants infected with PVY^{O}, PVY^{C} and PVY^{N} produces the tubers with no symptoms. However with PVY^{NTN} produces tubers with irregular brownish colored rings on skin, which forming necrotic arc in the flesh and cracking the skin at the surface. PVY^{NTN} pathotypes are the main causal agents of potato tuber necrotic ringspot disease (PTNRD).

They were directly obtained from tubers exhibiting PTNRD and, apparently, originated from recombination between PVY^{O} and PVY^{N}. However, some non-recombinant isolates from North America (NA-PVY^{N} and NA-PVY^{NTN}) caused PTNRD when inoculated to potatoes (Singh et al., 2008). The virus is transmitted also in non persistent manner by aphids.

PLRV induce symptoms first on the lower leaves and later gradually progress upward. The resulting symptoms include, rolling of the upper leaves at the top of the plants that assume a brittle, leathery texture, marginal yellowing and thickness of apical leaves (Khalid et al., 2000). PLRV has worldwide distribution in persistent manner by aphids and probably occurs wherever potato is grown. Incidence of PLRV, however, remains low when seed stocks are frequently replaced and systemic insecticides are used (Radcliff et al., 1993).

PVX with association of other viruses like PVY and PLRV induce more significant damages. PVX is often a latent virus i.e. the symptoms are not clearly visible to the naked eye. It may show symptoms ranging from a mild mottling of the leaf to a severe mottling of the plant with roughening and reduced leaflet size. Mottling may be more visible in the cloudy weather, and may be not existent after a

few days of sunny weather. The overall growth of plant may be stunted with small leaves. In some cases, the tips of the plant may die (Rich, 1983). The PVX is distributed worldwide in potato grown areas. It is transmitted mechanically by plant to plant contact (leaves, shoots and roots), machinery, cutting tools and animals. There must be wounding and an exchange of plant sap for infection to occur.

PVM is considered to be one of the most common potato viruses distributed worldwide and is an economically important pathogen of potato. PVM causes a yield reduction in potatoes of 15–45%. The virus is transmitted non-persistently by several aphid species and by mechanical inoculation with sap from young leaves. PVM causes slight mottle and mild abaxial rolling of leaves and stunting of shoots (Kerlan, 2008). Symptoms of potato plants caused by PVM infection are similar to those caused by several other common potato viruses, including PVS, PVX and PVY^{O}. The severity of symptoms varies greatly depending on the combination of potato cultivars and PVM isolates (Ruiz de Galarreta et al., 1998).

PVS has two recognized strains, PVS^{O} (Ordinary) and PVS^{A} (Andean), and has a worldwide distribution. The virus is transmitted by several aphid species in a nonpersistent manner as well as by contact (Hooker, 1981). PVS has a narrow natural host range, and is highly restricted to species of *Sonalaceae* and *Chenopodiaceae* family. Infection of potato plants is often symptomless, but mild symptoms can occur. The yield reductions due to PVS are normally considered moderate, but can be up to 20% (Kerlan, 2008).

TRV is primarily a soil borne pathogen transmitted by root-feeding nematodes. Seed transmission is possible in some plant species, such as *Solanum lycopersicum* and *Nicotiana benthamiana* (Senthil-Kumar and Mysore, 2011). Natural infection has been reported in more than 100 plant species (Brunt et al., 1996). Inoculation with sap, plants of about 400 species in more than 50 families can be infected (Harrison and Robinson, 1978). TRV has continuously been a significant potato pathogen, which causes spraing or corky ring spot in potato tubers, which renders the crop unmarketable (MacFarlane, 2010).

The aim of this study is to identify distribution of plant viruses in main potato cultivars in Kyustendil potato seed production region. In order to be helpful for growers and seed producers we set ourselves the following objectives for increasing quality and yield of potato production. They include identification of potato viruses in these cultivars from samples taken from plants and tubers with symptoms and symptomless and to find if possible virus free cultivar.

MATHERIALS AND METHODS

We tested three potato cultivars (Sante, Trezor and Riviera) for seed production in Kyustendil region. All potato cultivars were tested for six viruses PVY, PLRV, PVX, PVM, PVS and TRV after germination and growing the plats with **DAS-ELISA** (Clark and Adams, 1977; Petrov, 2012) using sap from homogenized potato leaves. Tissue samples from healthy and infected plants were used as negative and positive controls. Positive results are these that exceed three time optical density of the negative control.

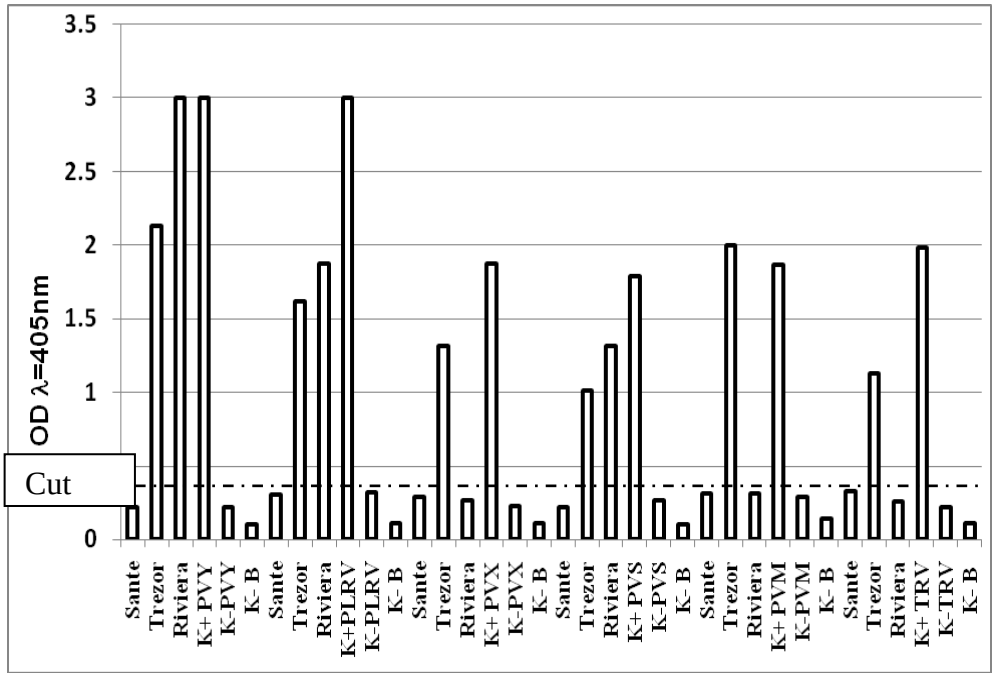
Total RNA extraction: RNA extraction was done by RNEasy Plant Mini Kit (Qiagen, Germany), according to the instructions of the manufacturer (Petrov, 2012). **Touch-Down RT-PCR**: We used primers PVY Primer 1, 7 and 8 for P1 gene region of the virus, with RT-PCR program modification touch-down (Petrov, 2012). Copy DNA synthesis: denaturation of total RNA (0,05-0,5 µg) at 95 C for 5 min with 10 µl PVY Primer1 primer in a final volume of 10 µl.; Cooling on ice to avoid renaturation; Preparation 15 µl of master mix: 5 µl of 5 × MMLV-buffer, 2 µl of dNTPs (2mM), 0.5 µl of M-MuLV Reverse transcriptase (200 U/µl), 7.5 µl H2O. Incubation step at 42°C for 60 min. Master mix for the PCR is: 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 done in thermo cycler Auto-Q Server (LKB, UK) with following programme: initial denaturation step 3 min 95°C; five cycles 30 sec 92°C, 30 sec 62°C, 90 sec 72°C; five sycles 30 sec 92°C, 30 sec 60°C, 90 sec 72°C; five sycles 30 sec 92°C, 30 sec 58°C , 90 sec 72°C,ten cycles 30 sec 92°C, 30 sec 55°C, 90 sec 72°C; final elongation 10 min72°C.

Gel electrophoresis: Visualizing the PCR fragments by agarose gel-electrophoresis DNA is separated in 1 to 2% agarose gel in TAE buffer with ethidium bromide (0,2 µg/ml) at 80-150V for 1 h. Products are displayed on a transilluminator GenoPlex (VWR) (Petrov, 2012) with UV irradiation at a wavelength of 315 nm.

RESULTS AND DISCUSSION

Potato cultivars expressing different symptoms were infected with PVY, PLRV, PVX, PVS, PVM and TRV (Fig. 2). Potato tubers infected with PVY^{NTN} expressed irregular necrotic rings (Fig. 1) and different degree of chlorosis and necrosis of the leaves. Most of potato plants and tubers infected with PVY were symptomless. Some potato plants infected with PLRV expressed leaf rolling of the top leaves but most of them including tubers were symptomless. All the samples which had PVS, PVM, PVX and TRV infection had no symptoms.

Фиг. 1. *Симптоми върху картофен клубен от вирусен щам* PVY^{NTN}
Fig. 1*. Symptoms of* PVY^{NTN} *infection in potato tuber*



Фиг. 2. *ДАС-ЕЛАЙЗА резултати за вирусна инфекция на тестирани картофени растения*

Fig. 2*. DAS-ELISA results for virus infection of the tested potato plants*

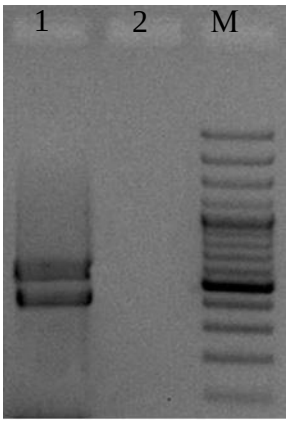
Legend: (K+) – Positive control for the relevant virus from the LOEWE kit; (K-) – Negative control for the relevant virus from the kit; (K-B) – Negative control from the buffer used for the tested samples

All the batches from potato cultivar Sante was virus free (Fig. 2). All the values of this cultivar from the tested six plant viruses were under the cut off value which is three times the value of negative control.

Different batches from the potato cultivar Tresor was infected with all six viruses (Fig. 2). Different batches from potato cultivar Riviera was infected with PVY, PLRV, PVS and TRV only.

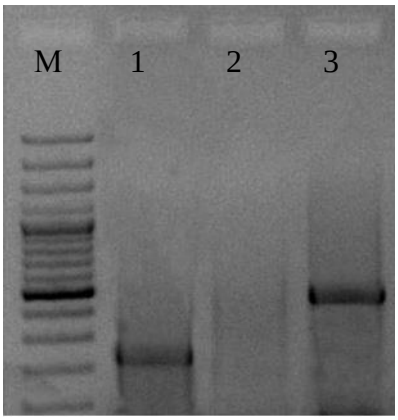
Samples from symptomless potato tubers were infected with a number of viruses – PVY, PLRV, PVM, PVS and TRV and is a serious problem for potato producers. Some of the symptomless leaf samples carried PVY and PVM simultaneously. Symptomless potato tubers are source for viral diseases when used for seed production. Using such infected with viruses symptomless seed tubers leads to significant reduction of quality and quantity of potato crop production.

The most spread PVY strain in the potato cultivars in this region is $PVY^{N/NTN}$, followed by PVY^{O} and PVY^{NTN} . From all these virus strains only PVY^{NTN} expressed tuber symptoms.



Фиг. 3. *Touch Down RT-PCR на вирусен щам PVY^{NTN} от картофи (450 bp and 640 bp)*
Fig. 3. *Touch Down RT-PCR of potato PVY^{NTN} strain (450 bp and 640 bp)*

Legend: (1) – Potato sample from PVY^{NTN} strain; (2) – Negative control; (M) – 100 bp ladder



Фиг. 4. *Touch Down RT-PCR на вирусен щам PVY^{O} от картофи (445 bp)*
Fig. 4. *Touch Down RT-PCR of potato PVY^{O} strain (280 bp) and $PVY^{N/NTN}$ strain (445 bp)*

Legend: (1) – Potato sample from PVY^{O} strain; (2) – Negative control; (3) – $PVY^{N/NTN}$ strain; (M) – 100 bp ladder

$PVY^{N/NTN}$ which gave one fragment of 445 bp (Fig. 4) from P1 region of the RNA virus genome was the most common strain in the population of PVY. It was established in all potato cultivars in Kyustendil region.This virus strain was symptomless mainly but sometimes expressed leaf symptoms of mosaic and necrotic spots on leaf lamina. On potato tubers $PVY^{N/NTN}$ not every time express visible symptoms of necrosis. PVY^{O} which gave one fragment of 280 bp (Fig. 4) was found only in potato cultivar Riviera. PVY^{NTN} was established by two fragments of 450 bp and 640 bp (Fig. 3) in potato cultivar Tresor and deteriorate food quality of potato tubers (Fig. 1) as the main causal agent of potato tuber necrosis disease.

CONCLUSIONS

1. Most of the potato cultivars grown for seed production in Kyustendil region were symptomless, concerning tubers and plant parts.
2. For this reason it is very important symptomless tubers to be tested for viruses to reduce virus transmission to the growers.
3. Potato viruses with great economic impact on potato yield production and food quality were PVY, PLRV, PVM and PVS. Mixed infections with PVY and PLRV gave great loss of production of potatoes.
4. Potato cultivar Sante was virus free.

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