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Molecular identification of *Potato X virus* in Lithuanian varieties of *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill. crops

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Plants of potato (*Solanum tuberosum* L.) cultivars 'Venta', 'Goda' and 'Vokė', exhibiting symptoms of greenish-yellow mosaic, were detected at the Vilnius State Plant Varieties Testing Station and private plots. Samples of diseased tomato (*Lycopersicon esculentum* Mill.) plants of cultivars 'Laukiai' and 'Aušriai' were found in Kaunas and Vilnius regions. Naturally infected tomato plants had symptoms suggestive of virus infection. Symptoms of infection were exhibited by the general interveinal-yellowing of leaves, bright mottling, reduced leaf size and slightly stunted plant growth. These potato and tomato samples were analysed using transmission electron microscopy (EM) and the reverse transcriptase-polymerase chain reaction (RT-PCR) technique. The identification of the virus was based on results of symptomatology on host-plants, the morphology of virus particle filaments (about 500 nm in lenght) and a specific size of cDNA amplification fragments (360 bp) of virus RNA in RT-PCR. The obtained results indicated that the virus isolated from Lithuanian varieties of potato and tomato plants had properties characteristic of *Potato X potexvirus.*

Key words: potato, tomato, identification, RT-PCR, *Potato X virus*

INTRODUCTION

Potato X potexvirus (PXV) is the most widespread of all the potato viruses. This virus is the main member of the *Potexvirus* group. PXV infects several solanaceous crops including potato, tomato, pepper and tobacco. Systemic infection occurs in these crops. The virus can also infect members of the *Chenopodiaceae* Vent. and *Amaranthaceae* Juss, producing local lesions. Several strains of PXV have differing host ranges, symptoms, serological properties, tryptophan and tyrosin contents in the coat protein, isoelectric points, and pH stability [1]. PXV symptoms vary. Plants affected with some strains are symptomless, other strains induce necrotic streaks, latent in many varieties or showing interveinal to barely perceptible mosaic. A combination of PXV, *potato Y potyvirus* (PYV) and *potato A potyvirus* (PAV) can cause more severe symptoms. In tomatoes, PXV causes mosaic and slight stunting.

PXV is mechanically transmitted by plant-to-plant contact (leaves, shoots, roots, machinery, cutting tools and animals). Grasshoppers and soil fungi are able to transmit PXV [2]. PXV can be soil-transmitted by the fungus *Synchytrium endobioticum* (Schilb) Percival zoospores. Literature data show the *Spongospora subterranea* Lange as a possible soil vector of PXV. It can be transmitted by dodder (*Cuscuta campestris* Yunek.) [3].

PXV virions are filamentous, not enveloped, usually flexuous, with a clear modal length of 480–580 nm and about 13 nm wide. Virions contain 6% of nucleic acid and 94% of protein. Their genome consists of single-stranded RNA [2, 4].

PXV causes a mild disease and thus is less damaging, and it reduces yields by 10 to 20%, in rare cases up to 50% depending on virus strain–cultivar interaction [3]. In

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Lithuania, the potato tuber yield was decreased by 7.0–52.6%, 5.6–31.1% and 1.7–17.1% by the high-, moderate- and low- virulency strains, respectively, depending on potato variety [5]. In developing countries, virus diseases are one the major causes of lower yields of potatoes, and their control requires the development of appropriate, sensitive and reliable detection methods [6].

Virus infection in tomato plants causes mild leaf mosaic and a slight growth reduction. Rare severe symptoms cause reduced yields [3]. This virus is one of the principal viruses infecting tomatoes in Italy [7], Tunisia [8], Algerie [9]. In Lithuania, PXV was detected in potatoes [5] and ornamental plants [10]. PXV – the agent of mild mosaic of potato – is one of the most widespread viruses in Lithuania. It has been identified by test-plant reaction, virus particle morphology and serological precipitation test. PXV strains were classified into three different virulence (high, moderate and low) groups on the basis of their biological, physical and electrophoretical properties [5].

The aim of the present work was to identify the agent of natural virus infection in Lithuanian potato and tomato varieties by the molecular method.

MATERIALS AND METHODS

Naturally infected symptomatic potatoes (*Solanum tuberosum* L.) showing viral symptoms – light green to yellow mosaic – were collected at the Vilnius State Plant Varieties Testing Station and private plots. Leaf samples of tomato (*Lycopersicon esculentum* Mill.) plants showing symptoms typical of virus infection were collected in Vilnius and Kaunas regions.

The experimental work was carried out at the Laboratory of Plant Viruses of the Institute of Botany.

Virus particles and their morphology were determined by investigation of negatively stained with 3% uranyl acetate dip preparations, using a JEOL JEM-100S transmission electron microscope at the instrumental magnification of 25000X [11–13].

For PXV detection by RT-PCR from infected test plants, frozen plants were used. Total RNA extraction was carried out according to the "Quick Prep total RNA extraction kit for the direct isolation of total RNA from most eukaryotic tissues or cells" instruction (Amersham Pharmacia Biotech.).

RT-PCR was done according to Technical Sheet No. 18 (developed at Agriculture Research Center, Giza, Egypt and University of Wisconsin, Madison). Two specific nucleotide primers for a part of the coat protein gene of PXV were designed by Soliman et al. [14] using the lineoup of published sequences for PXV from GenBank (accession numbers X88781, X88783, X88784, X88786, X88788 and Z23255). These primers were called PPVXv1 and PPVXc2. Nucleotide sequences of primers for RT-PCR amplification were the sense primer 5'-GAY ACN TGG CNC ARG CNG CNT GG-3' and the antisense primer 5'- YTG NGC NGC RTT CAT YTC NGC YTC-3' (N = A, C, G, T; R = A, G; Y = C, T). The thermal cycling profile (40 cycles) was 94 °C for 30 s, 60 °C for 1 min, 68 °C for 2 min with a final extension step of 68 °C for 7 min. The first-strand cDNA synthesis was carried out at 48 °C for 45 min and at 94 °C for 2 min. DNA amplification was performed in reaction mixtures containing 5× reaction buffer, RevertAid™ M-MuLV reverse transcriptase, dNTP mixture, both primers, magnesium chloride and recombinant *Taq* polymerase (MBI Fermentas) using the Eppendorf Mastercycler Personal.

PCR products were analysed by electrophoresis through 5% polyacrylamide gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator. DNA fragment size standard was : PhiX174 RFI DNA Hae III digest (MBI Fermentas) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 and DNA ladder – Gene Ruler™ 50 bp digest, fragment sizes (bp) from top to bottom: 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 50.

RESULTS AND DISCUSSION

The PXV was isolated from naturally infected potato cultivars 'Venta', 'Goda' and 'Vokė' bearing viral symptoms of greenish-yellow mosaic on leaves (Fig. 1) and from plants of tomato cultivars 'Laukiai' and 'Aušriai' that exhibited bright symptoms ranging from a mild yellowish-greenish mottle to a severe mottling with roughening of leaves. Plants were slighly stunted and had reduced leaves (Fig. 2).

Electron microscopic observation of negatively stained preparations revealed filamentous virus particles with a normal length of about 500 nm in preparations made from

Fig. 1. Symptoms on potato naturally infected with PXV

Fig. 2. Symptoms on tomato naturally infected with PXV

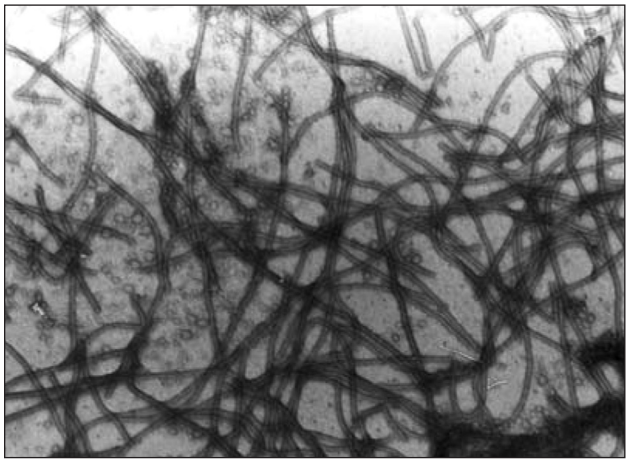

Fig. 3. Electronomicrograph of PXV particles

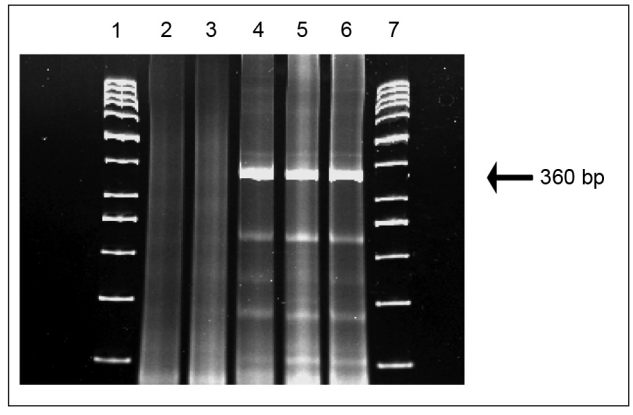

Fig. 4. RT-PCR DNA amplification of PXV from potato

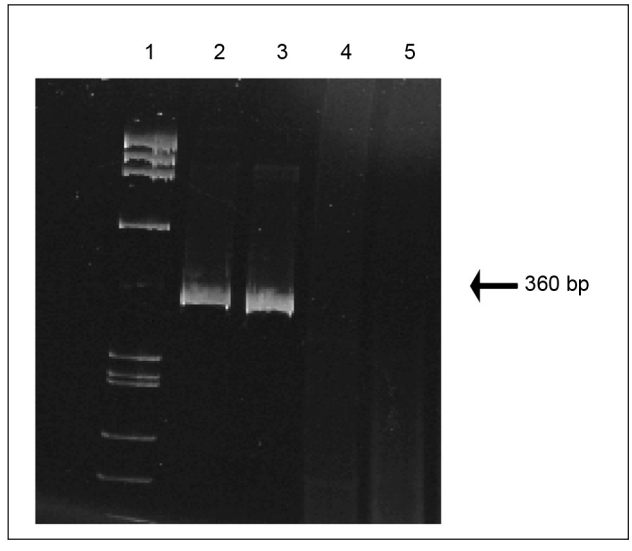

Fig. 5. RT-PCR DNA amplification of PXV from tomato

naturally infected potato and tomato leaves. The morphology of such particles was characteristic of viruses from the genus *Potexvirus* [15, 16]. According to host range, specific symptoms and virion morphology, this virus clearly differed from the *Pepino mosaic potexvirus* detected in tomato fruits imported from Spain to Lithuania [17].

RT-PCR amplification of double-stranded DNA from PXV was carried out on the total RNA isolated from infected plants using specific primers (PPVXv1 and PPVXc2) designed to amplify 360 bp of coat protein. Electrophoresis of RT-PCR DNA products showed that a single amplified fragment with a size of about 360 bp was obtained from the coat protein gene of PXV from infected test plants. No fragments were amplified from total RNA extracted from healthy potato and tomato plant tissue and water control (Figs. 4, 5).

The primer pair designed by Soliman et al. [14] for this virus on the basis of published sequences specifically amplified cDNA templates in RT-PCRs and confirmed infection of PXV isolated from potato and tomato samples found in Lithuania.

The results presented in this article indicate that the virus detected in Lithuanian varieties of potato and tomato crops is the potexvirus which has been identified as a typical strain of PXV [2].

PXV infects several solanaceous crops. It is most damaging when found in combination with PYV or *Cucumber mosaic cucumovirus* [18, 19]. A more severe yield loss can occur when PXV is present in mixed infections with other potato viruses. The disease can be controlled to some extent by the use of certified PXV-free tubers for seed, by avoiding contamination when cutting seed and using PXV-resistant cultivars.

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BULVIŲ X VIRUSO MOLEKULINIS IDENTIFIKAVIMAS LIETUVIŠKOSE VEISLĖSE *SOLANUM TUBEROSUM* L. IR *LYCOPERSICON ESCULENTUM* MILL.

Santrauka

Vilniaus augalų veislių tyrimo stoties bandyminiuose laukeliuose ir privačiuose sklypuose bulvių veislėse ‘Venta’, ‘Goda’ ir ‘Vokė’ aptikti virusinės ligos požymiai – sisteminė žalsvai gelsva mozaika. Virusuotų pomidorų ‘Laukiai’ ir ‘Aušriai’ pavyzdžiai rasti privačiuose sklypuose Vilniaus ir Kauno rajonuose. Natūraliai pažeistų pomidorų lapai buvo gelsvai margi, banguoti, deformuoti ir smulkūs, augalai neišsivystę ir žemaūgiai. Virusas buvo identifikuotas elektroninės mikroskopijos ir atvirkštinės transkriptazės bei polimerazės ciklinės reakcijos (AT-PCR) metodais. Pagal simptomus infekuotuose augaluose šeimininkuose, virionų morfologiją (apie 500 nm ilgio) bei AT-PCR duomenis (viruso specifinio cDNR fragmento apie 360 bp dydžio amplifikaciją) buvo nustatytas bulvių paprastosios mozaikos sukėlėjas – bulvių X virusas (*Potato X potexvirus*).

Raktažodžiai: bulvės, pomidorai, AT-PCR, bulvių X virusas