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WEED RESERVOIRS OF PVY IN BULGARIA AS A MAIN SOURCE OF DISEASES OF POTATO

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Abstract

Potato virus Y (PVY) is the type species of the genus *Potyvirus*, family *Potyviridae*. It has the widest host range of the family. The virus infects 495 species from 72 genera and 36 botanical families. The main range of natural hosts is concentrated mainly in three botanical families – *Solanaceae*, *Chenopodiaceae* and *Commelinaceae*, including potatoes, tomatoes and peppers as the most cultivated crops. Weeds that have been identified and analyzed in the plantations of potatoes are *Chenopodium album*, *Solanum nigrum*, *Datura stramonium*, *Xanthium strumarium*, *Capsella bursa-pastoris*, *Cirsium arvense*, *Convolvulus arvensis*, *Sonchus arvensis* and others. Some of them are asymptomatic virus reservoirs by which vectors propagate viral infections to cultivated crops.

Key words: weeds, PVY, potatoes

Potato virus Y (PVY) Potyvirus and Potato leaf roll virus (PLRV) Polerovirus are the two most important viruses infecting potato crops in Bulgaria. PVY is transmitted to potato plants by many different aphid species, but the most important vector of both viruses is the green peach aphid, *Myzus persicae* (Harrison, 1984). Aphids transmit the virus in a non-circulative non-persistent manner, which means that virus acquisition from an infected plant and inoculation to a healthy one can be performed during short feeding probes made by aphids in the epidermal tissue to assess plant suitability as a host (Powell et al., 2006).

Potato virus Y (PVY), the type species of the genus Potyvirus in family *Potyviridae*, is a highly damaging virus affecting crops of potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum* spp.) and tomato (*Solanum licopersicum*) worldwide. PVY isolates represent three major strain groups: ordinary (PVY^O), tobacco veinal necrosis (PVY^N) and aphid non-transmissible stipple streak (PVY^C) (Petrov, 2012). Numerous recombinant as well as non-recombinant forms of PVY have been reported (Kerlan 2006; Petrov, 2012).

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 also 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.

The aim of this study is to find out which weed species in potato crops are PVY reservoirs.

MATERIAL AND METHODS

Plant material

Fourteen weed species were found in potato plantations and analyzed for viruses: *Sonchus arvensis* (*Asteraceae*), *Cirsium arvense* (*Astera-ceae*), *Chenopodium album* (*Chenopodiaceae*), *Xanthium strumarium* (*Asteraceae*), *Solanum nig-rum* (*Solanaceae*), *Convolvulus arvensis* (*Convolvulaceae*), *Anthemis arvensis* (*Asteraceae*), *Sinapis arvensis* (*Brassicaceae*), *Polygonum convolvulus*

(*Polygonaceae*), *Amaranthus retroflexus* (*Amaranthaceae*), *Portulaca oleraceae* (*Portulacaceae*), *Datura stramonium* (*Solanaceae*) and *Physalis floridana* (*Solanaceae*).

DAS ELISA serologic test for PVY virus detection

The serologic analysis was conducted by the method of Clark and Adams (1977). We used kit from the company LOEWE Biochemica GmbH, Sauerlach, Germany. ELISA plates are coated with antiserum (IgG) for PVY, with dilutions (according to the instructions of the manufacturer) in 0.05 M carbonate buffer. We incubated plates for 4 hours at 37 °C. All samples were crushed in extraction buffer containing 1% PVP (polyvinyl pyrrolidone) in a ratio of 1: 10. Plates stayed 16 hours at 4 °C. After the third washing step was added alkaline-phosphatase conjugate for PVY and the plates were incubated for 4 hours at a temperature of 37 °C. The used substrate is p-nitrophenyl phosphate (Sigma) in diethanolamine buffer (pH 9.8) at a ratio of 1 mg/1 ml. The reaction proceeded in the light and at a room temperature. Adsorption of coloration is measured on DTX 880 Multifunction detector (Beckman, USA) at a wavelength of 405 nm. The samples were considered positive for which the OD (optical density, absorption) is more than twice the value of the negative control, called threshold or Cut Off.

RNA extraction from weed plants

Extraction of total RNA was performed with RNEasy Plant Mini Kit (Qiagen, Germany). Extraction was carried out according to the instructions of the manufacturer.

Touch-Down RT-PCR for PVY identification and virus strain differentiation

We used primers PVY Primer 1, 7 and 8 for P1 gene region of the virus (Petrov, 2012), with program modification touch-down. 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 Ч MMLV-buffer, 2 µl of dNTPs (2 mM), 0.5 µl of M-MuLV Reverse transcriptase (200 U/µl), 7.5 µl H_2O. Incubation step at 42 °C for 60 min. Master mix for the PCR is: 1 µl cDNA, 2.75 µl 10 Ч PCR buffer, 2.2 µl $MgCl_2$ (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 sycles 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 min 72 °C.

The PCR products were visualized by agarose gel electrophoresis.

DNA fragments was separated on a 1% agarose gel in TAE buffer with ethidium bromide (0.2 µg/ml) at 80 – 150 V for 1 h. Products were visualized on a transilluminator GenoPlex (VWR) upon irradiation with UV light at a wavelength of 315 nm.

RESULTS AND DISCUSSION

We surveyed weeds in seed potatoes in the region of Sofia to establish the prevalence of PVY among them. Established necrotic isolate of PVY caused peripheral systemic chlorosis in the upper leaves and necrosis on the lower leaves of the late-spring weed *C. album* (Figure 1).

Parasitic plant *Cuscuta* sp. of the same area has been tested by DAS-ELISA for PVY and a virus infection was not detected. By RT-PCR was detected very low titer of PVY (Figure 6). Thus it was shown that *Cuscuta* sp. is one of the virus vectors.

On the leaves of *X. strumarium* we observed symptoms of systemic infection – peripheral and interveinal chlorosis, and later – necrosis (Figure 2). The presence of PVY was confirmed by RT-PCR assay (Figure 6), ELISA assay was with negative results (Figure 5). On the leaves of *A. retroflexus* we observed chlorotic, necrotic and mosaic symptoms of PVY (Figures 3 and 4).

It has been found that *C. album* and *X. strumarium* were infected with the same strain of PVY (Figure 6).

Fourteen weed species were identified in this study, including 10 annuals (broadleaf weeds) and 3 perennials. Almost 50 percent of all species identified were members of the *Asteraceae* and *Solanaceae*. *C. album* and *X. strumarium* showed distinct symptoms of PVY infection from *A. retroflexus*. *A. retrofelxus* showed small chlorotic and necrotic spots and mosaic (Figure 3) while *C. album* and *X. strumarium* had peripheral chlorotic and necrotic leaf area (Figures 1

Fig. 1. PVY symptoms in C. album

Fig. 2. Symptoms of PVY in X. strumarium

Fig. 3. Chlorotic, necrotic and mosaic symptoms of PVY in A. retroflexus

Fig. 4. Chlorotic symptoms of PVY in A. retroflexus

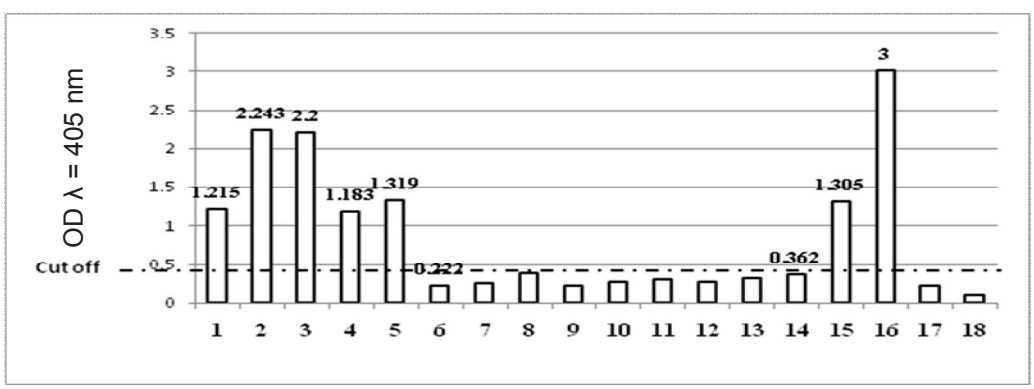

Fig. 5. DAS-ELISA results for PVY infection in weed species

Legend: **1** - *Solanum nigrum;* **2** - *Chenopodium album;* ***3*** - *Physalis floridana;* **4** - *Xanthium strumarium;* **5** - *Amaranthus retroflexus;* **6** - *Datura stramonium;* **7** - *Sonchus arvensis;* **8** - *Cirsium arvense;* **9** - *Convolvulus arvensis;* **10** - *Anthemis arvensis;* **11** - *Sinapis arvensis;* **12** - *Polygonum convolvulus;* **13** - *Portulaca oleraceae;* **14** - *Cuscuta* sp.*;* **15** - *Solanum tuberosum* cv. Mirabel*;* **16** - Positive control from kit; **17** - Negative control; **18** - Negative control of buffer.

and 2). Only *S. nigrum* was symptomless from the infected weeds. *C. album and S. nigrum* are well-known hosts for PVY (Shukla et al., 1994). Most of the 14 weed species are hosts of viruses known to infect potato. At least 5 species (*C. album*, *X. strumarium*, *S. nigrum*, *A. retroflexus*, and *P. floridana*) were susceptible to PVY (Figures 5 and 6). Some of these species are hosts

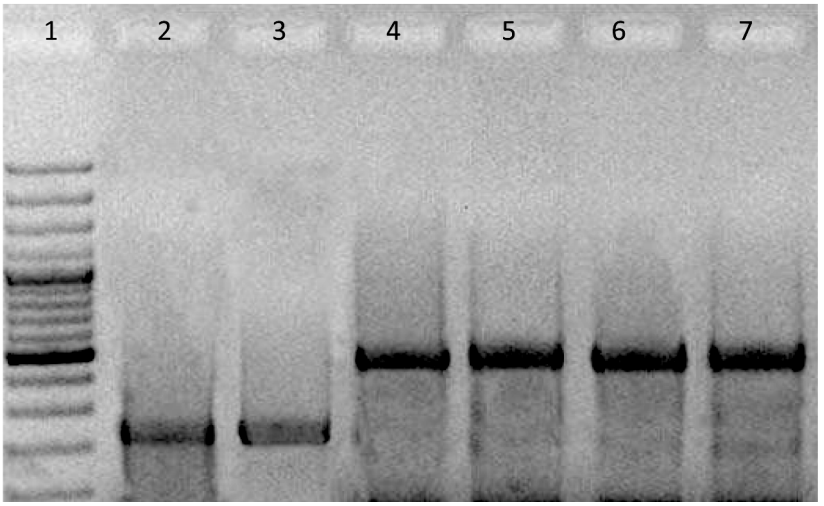

Fig. 6. Touch down RT-PCR for P1 gene region of PVY, 281 bp and 443 bp

Legend: **1** - M 100bp DNA ladder; **2** - 281 bp, PVY^{O} strain from *P. floridana*; **3** - 281 bp, PVY^{O} strain from *A. retroflexus*; **4** - 443 bp, $PVY^{N/NTN}$ strain from *X. strumarium*; **5** - 443 bp, $PVY^{N/NTN}$ strain from *S. nigrum*; **6** - 443 bp, $PVY^{N/NTN}$ strain from *C. album*; **7** - 443 bp, $PVY^{N/NTN}$ strain from *Cuscuta* sp.

for more than one virus. For example, *C. album* is the host for PVY and ToMV (Petrov, unpublished). Weeds bordering potato fields can act as important reservoirs for PVY (Kaliciak and Syller, 2009). In Syria were found PVY isolates in *S. nigrum* and *P. floridana* in the field. Weed hosts and tobacco plantations located in the neighborhood are natural reservoirs of PVY (Chich Ali et al., 2008). There is no latency period for acquisition and transmission of PVY (Bradley and Rideout, 1953). This means that the virus is carried on the aphid's mouthparts and is transmitted immediately as it probes leaves (Bradley and Rideout, 1953) in search of a suitable host (Powell et al., 2006). The most abundant hosts for PVY in the landscape near potato fields were also likely the most important sources for PVY.

CONCLUSIONS

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.

REFERENCES

Beemster, A. B. R. & de Bokx, J. A. 1987. Survey of properties and symptoms. In J. A. de Bokx & J. P. H. van der Want (Eds.). Viruses of potatoes and seed-potato production. Wageningen: Pudoc, p. 84-113

Harrison, B. D. 1984. Potato leafroll virus. Kew, Surrey, UK, CMI/AAB. Descr. Pl. Viruses 291. 6 p.

Kaliciak, A., Syller, J. 2009. New hosts of Potato virus Y (PVY) among common wild plants in Europe. *Eur. J. Plant Pathol.*, 124, 707-713

Kazinczi, G., Horvath, J., Takacs, A. P. 2004. Experimental and natural weed host - virus relations. *Commun. Agric. Appl. Biol. Sci.*, 69, 53-60

Kerlan, C. 2006. Potato virus Y. Descriptions of plant viruses, no. 414. Retrieved from http://www.dpvweb.net/dpv/showdpv.php?dpvno=414.

Nault, L. R. 1997. Arthropod transmission of plant viruses: a new synthesis. *Ann. Entomol. Soc. Am.*, 90, 521-541

Petrov, N. 2012. Potato virus Y (PVY) on crops of the family Solanaceae. PhD thesis. ISSAPP "N. Pushkarov", Sofia.

Powell, G., Tosh, C. R., & Hardie, J. 2006. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, 51, 309-330; doi:10.1146/annurev.ento.51.110104.151107.

Powell, G., Tosh, C. R., Hardie, J. 2006. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol.*, 51, 309-330

Shukla, D. D., Ward, C. W. & Brunt, A. A. 1994. The Potyviridae. Wallingford: CAB International.

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Плевелни резервоари на PVY в България като основен източник на заболявания по картофите

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Резюме

Картофеният вирус ипсилон (PVY) е типов представител на род *Potyvirus*, сем. *Potyviridae*. Той е с най-широк кръг от гостоприемници в семейството. Инфектира 495 вида от 72 рода и 36 ботанически семейства. Основният кръг от естествени гостоприемници е концентриран главно в 3 ботанически семейства – *Solanaceae*, *Chenopodiaceae* и *Commelinaceae,* сред които картофи, домати и пипер, като най-отглеждани културни видове. Плевелите, които са открити и анализирани в насажденията от картофи са: *Chenopodium album*, *Solanum nigrum*, *Datura stramonium*, *Xanthium strumarium*, *Capsella bursa-pastoris*, *Cirsium arvense*, *Convolvulus arvensis*, *Sonchus arvensis* и др. Някои от тях са безсимптомни резервоари на вируси, от които чрез вектори се разпространяват вирусните инфекции по културните растения.