Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 71, No 4, 2018

AGRICULTURAL SCIENCES

Plant breeding

DETECTION OF PVY, PLRV AND PVX POTATO VIRUSES IN SOME REGIONS OF SERBIA

Slavica Ćirić, Gordana Cvetković*, Slaviša Stojković, Slaviša Gudžić

(*Submitted by Academician A. Atanassov on December 12, 2017*)

Abstract

During a one-year period, the testing of samples of seed potatoes from the territories of Moravica, Zlatibor, Raška and Mačva districts of the Republic of Serbia was carried out. The presence of the three most important viruses: PVY, PLRV and PVX, was determined using serological DAS-ELISA test. Out of all tested samples, 34.62% were positive for some of these viruses. The greatest frequency was shown by PVY virus (86.36%), and significantly lower frequencies were shown by PLRV (11.62%) and PVX (2.02%) of all infected samples. PVY was detected in all districts, PLRV in all except Mačva district, and PVX was detected in the Moravica district, only. The results obtained indicate that the virus diseases could be one of the reasons for the lower potato yield in Serbia compared to Europe. This imposes the need for the application of integrated protection of potatoes.

Key words: seed potato, PVY, PLRV, PVX, DAS-ELISA

Introduction. In Serbia, the potato is grown on about 100 000 ha [1] and is one of the most important crops. In recent years, the yield of potatoes has shown a slight increasing trend, but still lower than the actual European average by roughly 45%. There are a number of reasons, one of them is numerous parasites. Losses in yield are estimated at around 21.8%. Potato diseases significantly decrease the yield during the growing period, and after removal of tubers during storage in a warehouse.

DOI:10.7546/CRABS.2018.04.17

Among the causal agents of diseases of potato, viruses have an important place given their wide distribution in the world, expansion and impact on yield and quality. In Serbia, viruses cause enormous damage to the production of potatoes, more than any other group of disease-causing agents. It is estimated that the average potato yield is three times lower due to the use of seeds infected by viruses. Plants infected by viruses give lower yields and smaller tubers. The biggest problem is that such tubers can not continue to be used for reproduction or, if they are used, they give sick plants, degenerate crop and, depending on the virus, reduced tuber yield to a certain extent, due to the systemic infection [2].

Economically important potato viruses are: Potato Virus Y (PVY), Potato Leaf Roll Virus (PLRV) and Potato Mosaic Virus (PVX).

In recent years, PVY virus has gained in importance because it is responsible for the decrease in yield and quality and is a major problem in the production of seed potato [3]. This virus causes serious disease in potato called mosaic or curled mosaic. The symptoms are variable depending on the viral strain and the host cultivar, the climatic conditions and on the fact whether it is primary (via a vector) or secondary (infected tuber) infection [4]. In previous studies, it has been shown that the Y virus was the most important and economically the most damaging potato virus in Serbia [5].

PLRV virus is also economically extremely harmful. It reduces the yield of the infected plants by more than 70%. The symptoms of viral infection occur on the leaves that curl toward the face, but can also occur on tubers as necrosis.

PVX virus is less frequent because, although it spreads easily due to mechanical transmission and not through aphids, it is easier to fight. Appearance of the symptoms can range from very weak to the strong mosaic followed by the plication of the lamina; the leaves are smaller and the plants are more stunted. Except with infected seeds, both PVY and PLRV in general are more spread compared to PVX since they are aphid transmissible in non-persistent and persistent manner, respectively.

Assuming that the potato viruses are becoming one of the most important pathogens, it is necessary to establish their presence and intensity of infection in Serbia. Therefore, the objective of the study was the detection of the PVY, PLRV and PVX viruses on seed potato from different altitudes on the territory of the Republic of Serbia using laboratory methods in accordance with internationally recognized procedures. The ultimate goal of this work constitutes the grounds for the application of appropriate measures and activities to prevent further spread of the viruses on potatoes.

Materials and methods. In Serbia, potato viruses began to receive full attention at the end of nineties, and DAS-ELISA as detection method began to be used relatively recently for this purpose. Earlier, viral infections of potato have been identified by applying field inspections based on symptoms. However, in many plants the virus is in latent form and there are no visible symptoms, so a

false picture of the condition of the crop is obtained. Modern methods have been applied rarely and in small areas.

In this paper, for the first time, the whole area of the Republic of Serbia where seed potatoes are produced was examined by a modern method in a laboratory.

Sampling of seed potatoes was carried out in such a way that the passage in the form of the letter W was made on the plot of 1 ha, from each of 120 dents two tubers were taken and two sacks, each with 120 tubers, were formed. One such sample was taken from each plot of 1 ha, two samples from each plot of 1–3 ha, three samples from the plots of 3–5 ha and four samples from the plots larger than 5 ha. The total of 572 samples of potatoes were collected from the part of the territory of the Republic of Serbia during the one-year investigation. Samples were submitted to the laboratory of Agricultural advisory and expert service, Niš, Serbia, in mesh bags, marked with a label with corresponding code, without designations of the variety, category and manufacturer. The samples were collected from the territory of the districts of Moravica, Zlatibor, Raška and Mačva (Fig. 1).

The presence of viruses that cause strong mosaic, PVY and PLRV, and the presence of the virus that causes milder mosaic, even in the cases of mixed infections, PVX, were determined. Detection of the possible presence of the viruses was done by the method of germination and cultivation of plantlets, as well as the application of ELISA test.

After removing, the tubers can not germinate and give plants so, it is necessary for tubers or parts of the tubers to be treated with hormones that stimulate growth. For this purpose, gibberellic acid is the most commonly used. Gibberellic acid was dissolved in distilled water at a concentration of 2 ppm, and in the resulting solution the parts of the potato tubers were immersed for a time interval of 20 min [6]. A hundred tubers, which represented a working sample, were separated from the formed sample. From each tuber, one bud, cone shaped, was extracted with a scalpel. The parts of tubers in the form of cone were immersed in gibberellic acid for a period of 20 min. The buds were planted in the substrate-ground in a particular plastic container with a drilled bottom, and the sample vessels were kept in a greenhouse. Under the influence of gibberellic acid, the buds were germinated and the plants were formed. After the sprouting, the green parts of the leaves and stems of the obtained plants were taken for testing. These samples were tested by DAS-ELISA method (double-antibody sandwich enzyme-linked immunosorbent assay), by using the commercial polyclonal antisera specific for the detection of PVY, PLRV and PVX viruses (Bioreba AG, Switzerland).

The green parts of the leaves and the stems were taken from four plats and sub-samples of 0.25 g were measured. In a sterile pestle and mortar the maceration of each sub-sample was carried out separately with the addition of extraction buffer in the ratio of 1:20. In this way, the macerates for all three

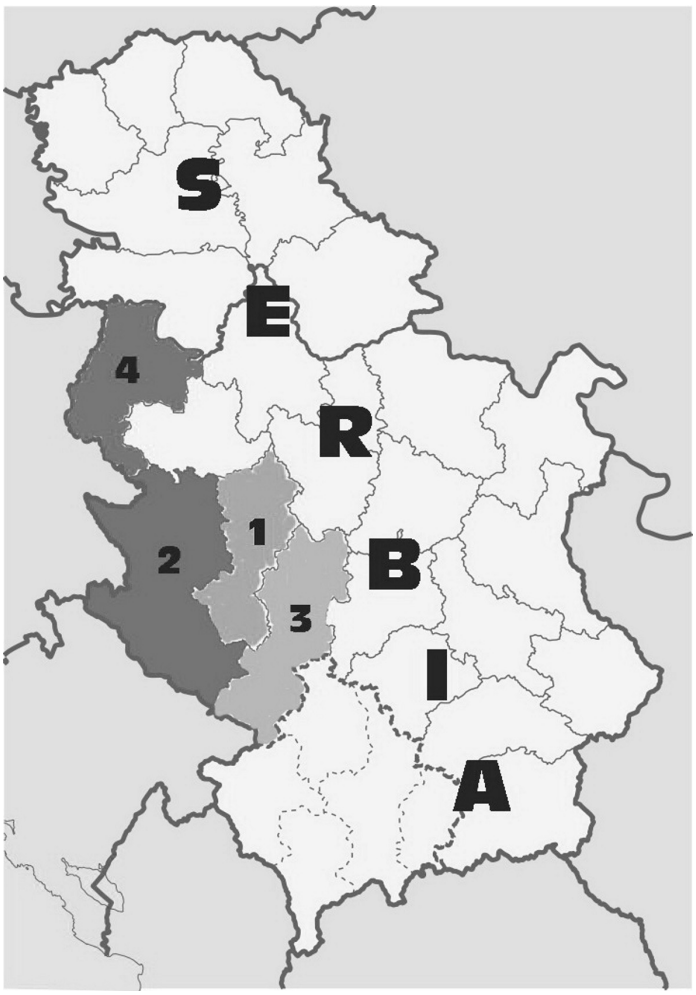

Fig. 1. Map of Serbia with marked areas of investigation:
1 – Moravica district; 2 – Zlatibor district;
3 – Raška district; 4 – Mačva district

viruses were obtained. One sample consisted of twenty-four sub-samples, each of the four units (0.25 g) of leaves and stem tops.

DAS-ELISA is a sensitive serological test method used to determine the presence of antigens on the basis of the enzymatic reaction. The assay was performed according to the following procedure [7]:

1. Dilution of the specific IgG antibody in coating buffer and lining the wells of the microtiter plate. Microtiter plates were labelled (date, virus) and loaded by eight-channel micropipettor (200 µl in each well).

2. Incubation for 4 h at 30 °C.

3. After the incubation, the washing of the microtiter plates with washing buffer, three times at intervals of 3 min, was carried out.

4. The prepared plant samples were macerated, with the addition of 5 ml of extraction buffer. In this way, an antigen was obtained that was poured into the washed microtiter plates.

5. The microtiter plates were wraped in cellophane and stored in a refrigerator for 16 h.

6. The following day, the microtiter plates were washed three times for 3 min by the washing solution.

7. Prepared specific IgG conjugation antibody was added in the conjugation buffer and it was poured into labelled microtiter plates, 200 µl in each well.

8. Incubation for 5 h at 30 °C.

9. Flushing with buffer three times for 3 min. After 5 h of incubation, the microtiter plates were washed with washing solution three times for 3 min.

10. The microtiter plates were loaded with a substrate buffer with pNPP tablet which gave colour to the wells. It was incubated for 60 min at room temperature in a dark chamber.

11. The values of the results were read on ELISA reader by THERMO (Italy), at 405 nm.

If the yellow colour occurred in the wells, the presence of the virus was proved. The color intensity varies, and a reader gives accurate results. The value of positive well was calculated as follows: the average of the negative controls was subtracted from the average of the positive controls and multiplied by a constant of 0.06. Then, these values were added up to the average value of the negative control. All the values greater than obtained were considered positive for that ELISA plate.

Results and discussion. Examination of 572 samples of seed potato from different localities in Serbia showed the presence of the viruses at all localities and in all districts.

Visual inspection of the tubers of samples of seed potato, before the virus identification, showed the presence of a number of potato diseases. The most expressed symptoms on the tubers were the symptoms of the following causal agents: *Helminthosporium solani*, *Spongospora supterranea*, *Streptomyces scabies* and *Rhizoctonia solani*. In some samples *Erwinia carotovora* was recorded, too.

The appearance of a set of symptoms that are linked to the viral infection was recorded by examination of potato samples from different localities of Serbia in

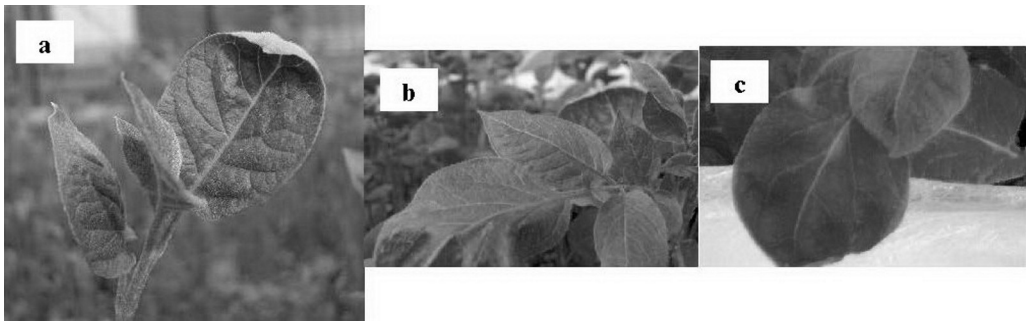

Fig. 2. The symptoms on the potato leaves caused by tested viruses in the greenhouse: a – PLRV virus; b – PVY virus; c – PVX virus

a greenhouse (Fig. 2). The symptoms were manifested as changes on the potato leaves. The most commonly observed symptoms on the leaves were: mosaic, curliness, plication and twisted lamina.

The results from microtiter plates were read on the ELISA reader. By using ELISA assay, the presence of PVY, PLRV and PVX viruses in the examined regions of Serbia was confirmed. In the samples, PVY virus dominated, and its presence was proven in 171 samples (29.90%), while the representation of PLRV virus was 4.02%, i.e. this virus has been identified in 23 samples. The presence of PVX virus was proven in four samples (0.70%).

The presence of individual potato viruses in the investigated regions of Serbia is shown in Table 1. Most examined samples were from Moravica district, 265 (46.32%), followed by Zlatibor district with 249 samples (43.53%), Raška district with 48 samples (8.39%) and Mačva district with ten tested samples (1.76%). In Moravica district, there were 93 samples (35.10%) positive to some of the tested

T a b l e 1

The presence of potato viruses in the examined regions of Serbia

	Moravica district	Zlatibor district	Raška district	Mačva district
Number of tested samples (%)	265 (46.32)	249 (43.53)	48 (8.39)	10 (1.76)
Samples positive for some of the viruses, number (%)	93 (35.10)	89 (35.74)	15 (31.25)	1 (10.00)
Samples positive for PVY, number (%)	80 (30.19)	77 (30.92)	13 (27.03)	1 (10.00)
Samples positive for PLRV, number (%)	9 (3.40)	12 (4.82)	2 (4.16)	–
Samples positive for PVX, number (%)	4 (1.51)	–	–	–

viruses; 80 samples (30.19%) were positive for the presence of PVY virus; nine samples (3.40%) were positive for the presence of PLRV virus, while the PVX virus was confirmed in four samples (1.51%). In the remaining three districts, PVX virus was not detected. In Zlatibor district, 89 samples (35.74%) were positive to some of the viruses; PVY virus was detected in 77 samples (30.92%) and PLRV virus in 12 samples (4.82%). In Raška district, 15 samples (31.25%) were infected; the presence of PVY virus was confirmed in 13 samples (27.03%), while the PLRV virus was detected in two samples (4.16%). In Mačva district, only PVY virus was identified in one sample (10.00%).

Out of the total number of tested potato samples in this part of Serbia (572), there were 198 samples (34.62%) infected with some of the examined viruses. The most common detected virus was PVY in 86.36% of the samples, followed by PLRV in 11.62% of the samples, and PVX in 2.02% of the samples (Fig. 3).

This representation of individual potato viruses is in accordance with the results of other studies in our country and in neighbouring countries. Milošević [5] found that in Western Serbia (which in part overlaps with the area of our examination), the highest intensity of spreading had PVY virus, while the intensity of spreading of PLRV was considerably weaker. In Montenegro [8], on the basis of serological analysis, it was established that out of the total number of the tested seed potato plants, 23.45% of the plants were infected with PVY virus, 2.74% of the plants were infected with PLRV, and in 0.52% of the plants PVX virus was confirmed.

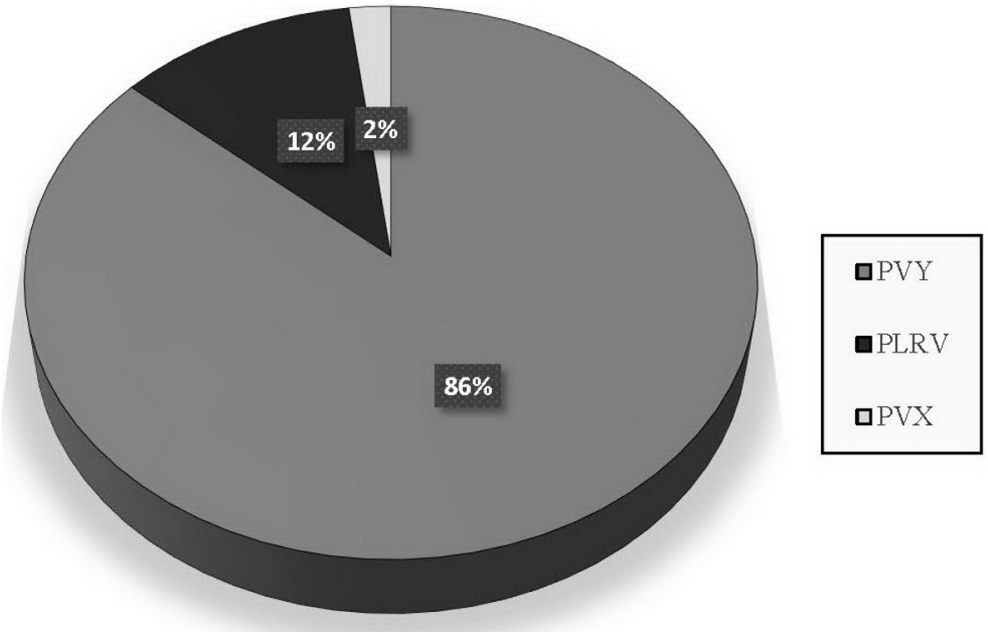

Fig. 3. The representation of individual potato viruses in the tested part of Serbia

More broadly in the world, similar results were obtained [9–11]. In the two-year study conducted by Baldauf et al. [12], which was related to determining the presence and spread of economically important potato viruses in North America, the dominant presence of PVY virus was noted. An interesting research was conducted at the University of Agriculture, Faisalabad, Pakistan [13]. Twenty-nine varieties of potato were examined for the presence of PLRV virus under conditions favourable to express the maximum of the viral infection. Using ELISA assay, the presence of the virus was confirmed in 25 samples (86.21%). By testing the plants in the field, based on the visible symptoms, in all varieties PLRV virus infection was manifested, while ELISA confirmed that in 25 samples. This demonstrates that, although symptomatology is the initial step for the diagnosis of diseases in the field, it is not a reliable criterion because the development of symptoms is influenced by many factors such as environmental conditions, presence of insects, lack of nutrients, growth phase, time of infection, host genotype, viral strains, etc.

For the successful control of viral diseases of potato it is necessary to constantly monitor and to have a good knowledge about the presence of the viruses at individual localities of cultivation. This is very important for the viruses that are transmitted by the aphids (*Myzus persicae*), such as PVY and PLRV viruses, but also for PVX which, despite the lack of vectors, is easily transmitted mechanically. Milošević [14] stated that only in high mountain regions, where there is no widespread production of the table-stock potato, it is possible to produce high-quality seed potato.

The results which confirmed the presence of potato viruses in Serbia impose the need for the application of integral protection of potato, which involves the simultaneous application of certain physical, chemical and agro-technical measures.

Conclusions. By examining 572 samples of seed potato from different localities of Serbia in the greenhouse, the appearance of a series of symptoms that are linked to a viral infection was recorded. The symptoms were manifested in the form of changes on the leaves: mosaic, curliness, plication and twisted lamina. Using the ELISA assay, the presence of the viruses was confirmed in 198 samples (34.62%). It was shown that PVY virus dominated in 86.36% of the samples, and much less was the presence of PLRV virus (11.62%) and PVX virus (2.02%). PVY virus was present in all investigated districts of Serbia, and it dominated everywhere. This was the only virus detected in Mačva district, but these results should be understood conditionally due to the relatively small number of tested samples. PVX virus was detected only in the Moravica district.

The results of this investigation enable the planning of effective protection measures in order to increase the quality of tubers and increase the yield of potatoes.

Viral infections of the seed potato can not be solved in the laboratory. It is necessary to develop awareness among the manufacturers about the need for using the declared and health tested seeds, respecting of crop rotation, application of spatial isolation, destruction of wild plants, removal of sick plants, monitoring the flight of aphids and their suppression.

REFERENCES

[1] Rekanović E., I. Potočnik, S. Milijašević-Marčić, M. Stepanović, B. Todorović, M. Mihajlović (2011) Sensitivity of Phytophthora infestans (Mont.) de bary isolates to fluazinam, fosetyl-Al and propamocarb-hydrochloride, Pestic. Phytomed., **26**(2), 111–116.

[2] Marković S. (2002) Plant quarantine in Yugoslavia: history and practices, Association of Agricultural Engineers and Technicians of Yugoslavia, Belgrade, 294 pp (in Serbian).

[3] Gray S., S. de Boer, J. Lorenzen, A. Karasev, J. Whitworth et al. (2010) Potato virus Y: an evolving concern for potato crops in the United States and Canada, Plant Dis., **94**(12), 1384–1397.

[4] Draper M. D., J. S. Pasche, N. C. Gudmestad (2002) Factors influencing PVY development and disease expression in three potato cultivars, Am. J. Pot. Res., **79**(3), 155–165.

[5] Milošević D. (1992) Occurrence of the necrotic strain of potato virus Y (PVY) in some localities in Serbia, Plant Protection, **43**(3), 201, 197–202.

[6] Gudžić S. (2006) Laboratory Manual in Phytopathology, University of Priština, Faculty of Agriculture, Lešak, Serbia (in Serbian).

[7] Vojinović M. (2006) The hosts and distribution of *Erwinia amylovora* in Nišava district, Thesis, University of Ptiština, Faculty of Agriculture, Lešak, Serbia (in Serbian).

[8] Zindović J. (2011) Presence and distribution of economically important potato viruses in Montenegro, Pestic. Phytomed., **26**(2), 117–127.

[9] Chrazanowska M. (1995) The reaction of Polish potato cultivars to PVY^{NTN}, Potato Res., **38**, 434–435.

[10] Ohshima K., K. Sako, C. Hiraishi, A. Nakagawa, K. Matsuo et al. (2000) Potato tuber necrotic ringspot disease occurring in Japan: its association with Potato Virus Y necrotic strain, Plant Dis., **84**, 1109–1115.

[11] Crosslin J. M., P. B. Hamm, K. C. Eastwell, R. E. Thornton, C. R. Brown et al. (2002) First report of the necrotic strain PVY (PVY^{N}) on potatoes in the northwestern United States, Plant Dis., **86**(10), 1177.

[12] Baldauf P. M., S. M. Gray, K. L. Perry (2006) Biological and serological properties of Potato Virus Y isolates in northeastern United States potato, Plant Dis., **90**(5), 559–566.

[13] Asia Batool M., M. A. Khan, J. Farooq, S. M. Mughal, Y. Iftikhar (2011) ELISA-based screening of potato germplasm against potato leaf roll virus, J. Agric. Res., **49**(1), 57–63.

[14] Milošević D. (1998) Potato diseases with the basics of the seed production, Publishing house "Draganić" and the Institute for Agricultural Research "Serbia", Belgrade, 1–273 (in Serbian).

Faculty of Agriculture
University of Priština
Kopaonička bb
38219 Lešak, Serbia
e-mail: slavica.ciric@mts.rs

Agricultural Advisory and Expert Service
Leskovačka 4
18000 Niš, Serbia