

Research of necrotic and ordinary isolates of Y-virus of potato

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The purpose. To determine biological characteristics of 2 isolates of Y-virus of potato, belonging to different groups of strains. One of isolates caused necrotic symptoms on plants of potato, another — of soft mosaic. **Methods.** Bioassays on plant-indicators. Direct methods of research of viruses: electronic microscopy with negative contrasting, DAS-ELISA for research and identification of the revealed isolates. **Results.** There were revealed 2 isolates of Y-virus of potato, one of which caused striped mosaic, another — light mosaic. Necrotic isolate which caused light mosaic on host-plant — potato, caused symptoms of system necrosis on display plants of *N. tabacum* (cultivar Samsun). Usual isolate which induced light mosaic on plants of potato, on plant-indicators caused mosaic. **Conclusions.** Biological characteristics of 2 isolates of Y-virus of potato are investigated. It is determined that according to phenotypical features of infection of isolates on plant-indicators these isolates belong to different strain groups — necrotic and usual strains. The further molecular research of phylogenetic relationship of the revealed isolates is necessary.

Key words: *potato, tobacco, Y-virus of potato, necrotic strain, display plants, immunoenzymatic analysis.*

Support for agrobiodiversity is not possible without the use of a large variety of cultivar varieties. Due to the wide varieties of cultures, the need for assortment of agricultural products is met, sustainable yields are provided, and the bases of biosecurity of the state are created. An important issue is the receipt of seed material of various varieties, which must meet the requirements of high condition and be free from pathogens. Potato seed certification programs aimed at getting large seeds of seeds have faced increasing problems in the last decades in the visual detection of plants with viral infections.

Detecting infections of viruses that are economically important due to their high vulnerability to potato culture is especially needed for seed certification programs. In particular, it is Y-potato virus (Potato virus Y, PVY), which has become widespread in potato culture and can reduce the yield of tubers by 30-70%. His strains can induce serious symptoms not only on the leaves of plants, but also on the tubers, causing the disease of necrotic ring spots of potato tubers. Detection of these strains is extremely important for the development of effective strategies for controlling the viral infection [1,2]. The variability of the symptoms of a viral infection can be caused both by the properties of the variety, and by the action of abiotic factors and creating obstacles to the detection of infected plants. Awareness of the fact that PVY is becoming a top-priority threat to potato seed certification programs, prompts virologists to thoroughly investigate all the factors that may lead to this.

The classification of isolates PVY has historically developed on the basis of detecting the supersensitivity reaction in potato plants with the *Nytr* or *Nc* resistance genes, as well as in the study of induction of necrosis in tobacco plants *N. tabacum*. From the now known five PVY strain groups, only isolates of PVYN PVYNTN groups can cause necrosis in tobacco plants [3].

In addition, new strains developed as recombinant variants of PVY (PVY N: O, PVY N-Wi and PVYNTN) are often associated with moderate symptoms on the leaves. These symptoms may be temporary in many popular potato varieties or the infection may be asymptomatic [4, 5]. The absence of symptoms on plants in breeding areas is often considered a manifestation of resistance to a virus, when in fact it may indicate asymptomatic infection in plants of tolerant varieties. These varieties sometimes contain the content of the virus, similar or exceeding this figure in varieties with clear symptoms [6]. In plants of these asymptomatic or

temporarily symptomatic varieties it is extremely difficult to visually detect in the field conditions of plants with a virus infection. This leads to an underestimation of the infestation of PVY plantations and causes high infection with the virus of subsequent generations of potato seeds. Therefore, when creating new potato varieties and for the effective cultivation of existing varieties, it is necessary to investigate not only the variety of infections that infect the culture, but also the diversity of their strains.

The purpose of the research was to establish the biological characteristics of two isolates PVY, causing various symptoms on potato plants to clarify their strain affiliation.

Materials and methods. The identification of the viruses was performed using ELISA (double sandwich variant, DAS-ELISA), using commercial test systems (LOEWE, Germany). The results are recorded at 405/630 nm wavelengths at the Thermo Labsystems Opsi MR (US) spectrophotometer with software [7]. The data processing of optical density of samples was performed by means of determining the mean value of the data and the standard deviation. The threshold of optical density, which distinguishes the positive results of the enzymatic reaction from the background value, was determined for each plate individually.

The morphology of viral particles in a drop of potato bch was examined using transmissive electron microscopy (JEM 1230 microscope (JEOL, Japan) using negative contrast agents: 2% solution of phosphorus tungstic acid and 2% uracil acetate solution for 2 minutes [8]. For biological testing, *N.tabacum* plants, which were inoculated with homogenate from the leaves of potato plants at the age of 4-6 leaves, were used. After inoculation, tobacco plants were cultivated under laboratory conditions. Potato plants of the *S.tuberosum* varieties of Slovyanka and Lady Rosetta would grow shafts in a cultivating room of tubers, selected in the field conditions of the previous season. Results and discussion. In potato plants cultivated in cultivating rooms, the young leaves revealed symptoms of necrotic stroke on the veins of leaves on the young leaves, which are typical signs of striped mosaic. We isolate the virus from these plants as 19Pot. Another isolate - 6Pot, infected potato plants asymptomatic or the manifestation of infection was in the form of a mild mosaic (Fig. 1).

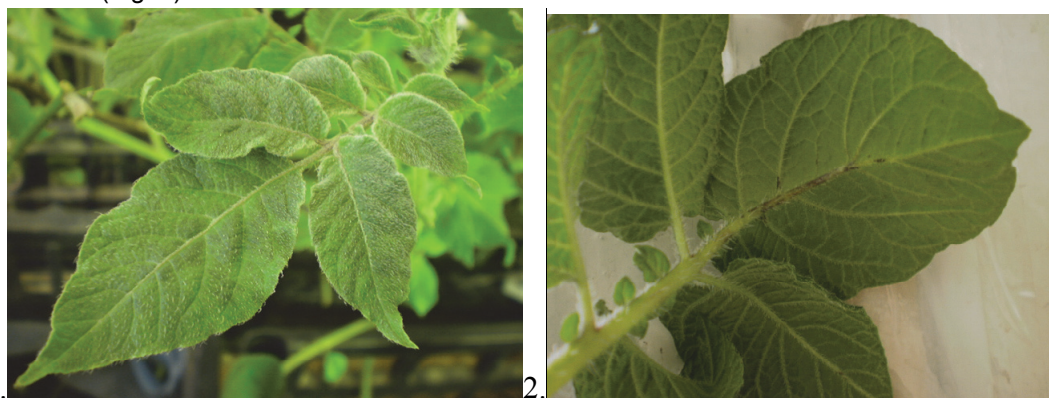


Fig.1 Symptoms on *S.tuberosum* potato plants infected with PVY insulators: 1. Isolate 6Pot; 2. Isolate 19Pot

The DAS-ELISA method confirmed the presence in the potato plants infected with 6Pot and 19Pot isolates, PVY antigens (Table 1).

Table 1. The content of PVY antigens in potato plants *S.tuberosum* infected with 6Pot and 19Pot isolates.

Isolat	PVY	PVM	PLRV
19Pot	2,918±0,012	0,054±0,008	0,047±0,005
6Pot	0,815±0,012	0,063±0,012	0,045±0,004
Negative control	0,042±0,005	0,048±0,009	0,035±0,008
Positive control	3,005±0,009	0,735±0,021	0,428±0,015

High indexes of optical density of samples indicate active accumulation of the virus in potato plants when infected with these isolates.

At electron microscopic examination of untreated preparations from potato leaves *S.tuberosum* found in juice of potato plants infected with 19Pot isolate, filamentous viral particles in the size of 710x11 nm, and in a preparation from a plant infected with 6Pot - filamentous virions in the size of 670 x 12 nm - (Fig.2).

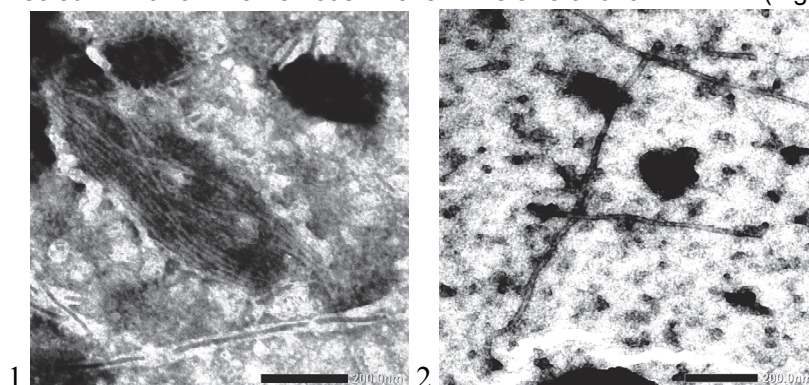


Fig.2 An electron of the viral particles found in the leaves of S.tuberosum potatoes: 1 - 6Pot isolate (contrast 2% of FVC) 2 - 19Pot isolate (contrasting 2% of uracil acetate)

It is known that the appearance of symptoms and severity of the course of the disease caused by PVY depends on the type of potato, the PVY strain and the environment, as well as whether the type of infection is primary (the infection is transmitted in the current season) or secondary (infection is transmitted through the tuber generation) [9]. We assumed that the isolated isolates belonged to different strain groups of the virus, therefore, research was conducted using indicator plants.

The first signs of infection of the virus on tobacco plants *N.tabacum* were detected on day 9 after inoculation with 6Pot isolate in the form of clarification of the veins of young leaves. The upper leaf, in some cases, has deformed in the form of an arc downwards across the central vein. Later on the leaves of infected plants revealed necrosis of veins, which captured the central vein and spread to the veins of the first and second order. Distribution of necrosis began with the basal part of the leaf plate (Fig. 3).

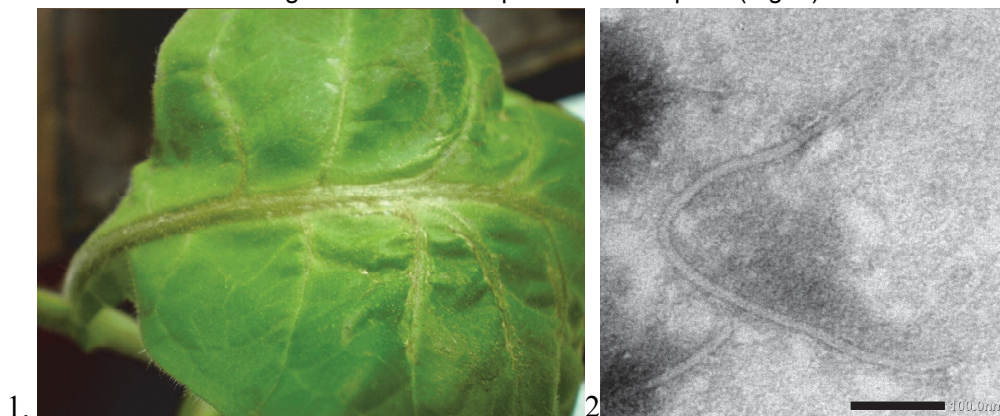


Fig. 3 Inoculation by isolate 6Pot of tobacco plants N.tabacum: 1) symptoms of viral infection on the leaves; 2) viral particles found in N.tabacum plants

In contrast to the development of the virus infection by inoculation with 6Pot isolate, 19Pot isolation of tobacco plants did not result in visible symptoms. In general, the infection caused only mild mosaic and some deformation of the tissue of the sheet (Fig. 4). Subsequently, the mosaic symptoms disappeared.

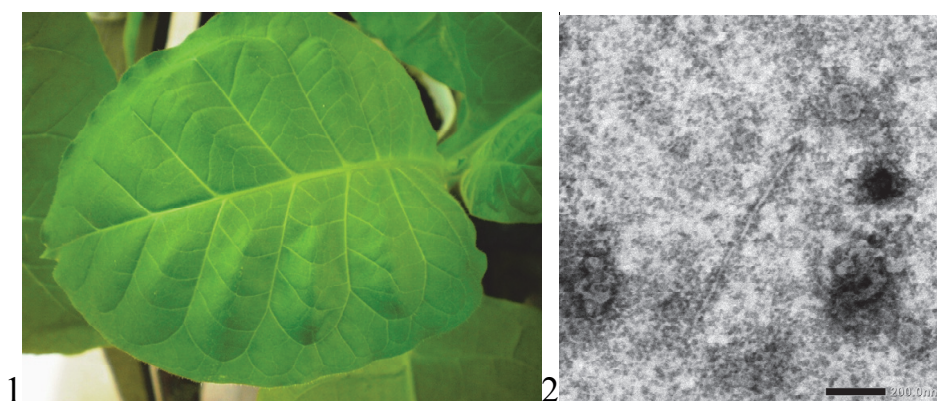


Fig. 4 Inoculation by isolation of 19Pot plants of tobacco *N.tabacum*: 1) symptoms of viral infection on the leaves; 2) viral particles found in *N.tabacum* plants

The size of the viral particles for the 6Pot isolate was 668x12 nm, and for the isolate 19Pot - 760 x 13 nm, which corresponds to the morphology of PVY particles [10]. Diagnosing DAS-ELISA Tissue Plants for Virus Maintenance confirmed that the detected particles belong to PVY. Antigens of other viruses have not been detected, which indicates that the symptoms of tobacco plant necrosis are caused by the infection of PVY (Fig. 5).

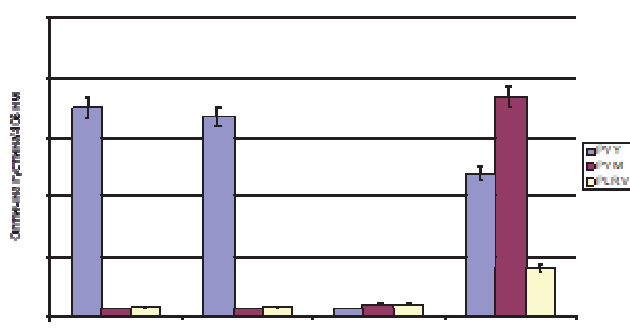


Fig. 5 The content of antigens of viruses in the juice of *N.tabacum* plants, inoculated with isolates from potato plants: 1- isolate 6Pot; 2. Isolate 19Pot; K- negative control; K+ - positive control

It should be noted that in previous studies there was a significant distribution of necrotic PVY strains on potato plants in the Polissya area of Ukraine [11]. In addition, studies of phylogeny of the Ukrainian isolate PVY [12] showed that it is a recombinant virus with possible phenotypic manifestations of both the ordinary strain O and necrotic strain N, which will depend on the specific conditions of the development of the viral infection.

These reasons lead to in-depth studies of the PVY population in Ukraine to find effective measures to control this dangerous pathogen. In studies of the interaction of the virus it is important to combine different approaches, which will help to obtain a complete understanding of the interaction of the host plant - PVY. The results of biochemical, transcriptional, proteomic, and metabolic studies will contribute to the design of the potato response model for the infection of PVY. This model will further enable prediction of the results of the virus infection, which may be useful in the selection of potatoes [13].

Conclusions

In our studies, the presence of PVY isolates belonging to biological manifestations in PVYO potatoes in different strain groups has been demonstrated: to regular strains of PVYO and to necrotic strains of PVYN. Given the high ability to recombine PVY strains and the possibility of more aggressive recombinant variants

of the virus, especially in the PVYN strain group, further studies are needed to establish the phylogenetic relationship of the PVY isolates we discovered. In addition, it is necessary to monitor the infection of different strains of PVY in potato plantings, which will allow the development of effective measures to control the dangerous strains of the virus.

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