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Assessment of resistance of plants of Nicotiana tabacum L. and Nicotiana rustica L. to necrotic and ordinary strains of Y-virus of potato

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The purpose. To study resistance of grades of tobacco *Nicotiana tabacum* and makhorka *Nicotiana rustica* in conditions of artificial becoming infected with necrotic and ordinary isolates of *Potato virus* Y (PVY). **Methods.** Molecular-and-biological properties of isolates of *Potato virus* Y were determined using methods of transmission electronic microscopy, multiplex inverse polymerase chain reaction and ELISA. Biological properties of isolates were studied using method of plant-indicators. Resistance of plants of cultivars of tobacco and makhorka against isolates of PVY were studied using artificial inoculation with subsequent visual detection of symptoms of becoming infected and detection of viruses by method of transmission electronic microscopy and ELISA. **Results.** Probes of excreted from plants of potato isolates of *Potato virus* Y had shown that they belonged to different strain groups as to response to their infection contamination which explicated at plant-indicators. Multiplex OT-PCR had confirmed presence of *Potato virus* Y. and absence of other viruses infecting potato. They had detected different response of plants of tobacco of grades Samsun 155 and Sobalchskyi 34/40 against ordinary and necrotic isolates. With methods of transmission electronic microscopy and ELISA they had confirmed accumulation of this virus. **Conclusions.** Plants of grades Samsun 155 and Sobalchskyi 34/40 in comparison to ordinary isolate had manifested response of tolerance. These grades were non-resistant against necrotic isolate. Absence of symptoms at plants of makhorka and tobacco of grade Sobalchskyi 193 could testify to tolerance response and availability of resistance against these isolates.

Key words: *tobacco, resistance, Y-virus of potato, necrotic isolate, ordinary isolate.*

Potato virus Y (PVY) — the member of the genus *Potyvirus* family *Potyviridae*, that can infect potato, tomato, tobacco, pepper and other plants of the Solanaceae family. The strains can be determined by the symptoms that occur in host plants, potatoes and tobacco, as well as by serological reactions of selected isolates.

Currently, there are about nine groups of detected strains, but the most basic and common until recently considered are three groups of strains, namely: PVY-N, PVY-O and PVY-C [1]. Isolates belong to group of PVY-N cause severe symptoms of necrosis of veins in the tobacco plant (*Nicotiana tabacum,* L.*)*, and in the field can lead to a significant deterioration in the quality of products of this culture. At the global level, *Potato virus Y* is certainly the most damaging virus of tobacco. The latest survey of CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) on tobacco viruses confirms this situation. Losses due to necrotic strains of the virus are increasing in many countries. The influence of

this virus on Burley and Virginia tobacco are reduction of size and weight of leaves, plant height, and yield. The earlier the infections occur, the greater the impact on the tobacco crop. In Chile and New Zealand, heavily infected crops have experienced yield reductions exceeding 70%. The chemical quality of harvested tobacco is reduced also in the presence of PVY. An increase of nicotine, nornicotine, total nitrogen, nitrogen-insoluble acid and nitrate content can be observed [2, 3].

To meet the modern needs of breeding, the works on to identifying the variety groups with stable resistance under fierce natural and artificial infectious background should be done in respect to get the expected effect of the source resistance. Great importance is raising new varieties of group stability. For successful breeding in this area required starting material with resistance group to defeat pathogens that would fully meet the increasing demands on breeding performance and high level of adaptability to adverse environmental factors. Therefore, the study of varieties with a view to providing new sources of resistance to major pathogens remains relevant and necessary [4].

Moreover, the research of PVY strain has important issues regarding the interaction of virus and host plant. A plant's reaction on the stress, caused by infection, leads not only to ensure its survival, but causes changes in virus populations that have long-term consequences. The emergence of new variants of the pathogen influenced by intracellular factors of host often occurs in the presence extensive quantity of ordinary members of the population. This does not enable initially find these new variants. But in the future the situation may change. In view that the rate of mutation of RNA viruses evaluated in order one mutation per genome replication, can assume that most of the viral particles in the infected host may represent a unique genotypes [5]. Thus, these studies help to estimate the dynamics of populations and identify new isolates of PVY that have new characteristics and genomic structures [6,7].

Materials and Methods. In our studies we were evaluated the resistance of plants *N.tabacum* varieties: Samsun 155, Sobalchsky 34/40, Sobalchsky 193 and *N.rustica* varieties: Matsui Field and Actec to necrotic and common isolates of PVY. Isolates were selected in the field of potato plants that cultivated in the Kyiv region, Ukraine.

Belonging of the isolates to a group of PVY strains were determined by biological tests on plants *N.bentamiana* and *N.tabacum*, var. Samsun.

Indicator plants and experimental plants *N.tabacum* and *N. rustica* grown in a greenhouse at a temperature of 25 °C and 16-hour light period. We used tobacco plants to test at the age of 4-6 leaves. Were inoculated five plants per variety with sap of infected plants in the solution 1:2 in the phosphate buffer saline (PBS, pH 7,4). Mock-infected plants were treated with PBS (pH 7,4).

The plants investigated by means DAS-ELISA, RT-PCR and transmission electron microscopy. The isolates were tested serologically using polyclonal antibodies for PVY, *Potato virus M* (PVM) and *Potato leaf roll virus* (PLRV) (LOEWE® Biochemica GmbH, Germany). Total RNA was isolated from 5 g of whole leaf tissue using the procedure as described by Boom et al. [8]. Multiplex RT-PCR, which included the definition of PVY, PVM, PVS, PVX, PLRV and *Potato spindle tuber viroid*, carried out as described [9]. We used commercial kits for RT-PCR of production AmpliSens, (Russian Federation).

For determine the viruses by DAS-ELISA using polyclonal serum to the complex strains of PVY, which includes antibodies PVY^{O} and PVY^{N}, as well as diagnostic tools for determining *Potato virus M* (PVM) and *Potato leaf roll virus* (PLRV) (LOEWE Biochemica GmbH, Germany). Results were recorded at wavelengths of 405/630 nm using a reader Termo Labsystems Opsis MR (USA) and software Dynex Revelation Quicklink [10]. Data processing optical density of the samples was performed using descriptive statistics, to determine the average value and standard deviation. Optical density threshold that distinguishes the positive results of the enzymatic reaction on the value of the background, was determined for each board individually, as recommended [11].

Morphology of viral particles in plants potatoes and tobacco were investigated by transmission electron microscopy using a microscope JEM 1230 (JEOL, Japan). We used negative contrasting of preparations potato plants and tobacco 2% solution of phosphotungstic acid or 2% solution of uracil acetate for 2 minutes [12].

Results. PVY^N isolate caused systemic necrosis symptoms on plants indicators. Necrosis developed on leaf tissue between the veins as a light brown spots, which eventually increased to small light brown necrotic spots. At stems also appeared necrotic light brown stripes. The leaves with necrosis after 11-12 days of inoculation wilted, wilting begins with the basal part of the leaves and the edge of the leaf blade. The leaves are withered, in some cases the plants died. These symptoms are characteristic to infection of necrotic strains of PVY [13].

In indicator plants that inoculated isolate PVY^O have not found out noticeable symptoms of infection. In general, the infection caused only mild mosaic and some deformation of the tissue between the veins. Finally, symptoms of mosaic disappeared. This reaction of plants to PVY infection are typical for common isolates of virus [5]. DAS-ELISA found antigens of PVY in the samples of indicator plants, other viruses are not detected.

Products amplification of cDNA of PVY capsid gene were found by multiplexed RT-PCR in samples of indicator plants *N.benthamiana*, which were inoculated PVY^N and PVY^O isolates from potato plants (Fig. 1).

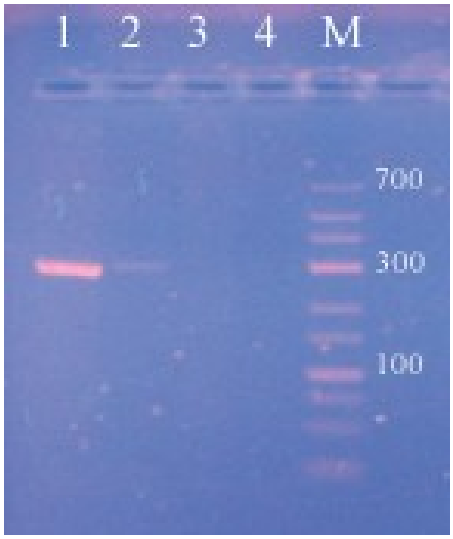


Fig.1. The results determine of the cDNA to RNA segments of PVY, PVM, PVS, PVX, PLRV and Potato spindle tuber viroid in samples N.benthamiana, infected PVY^O and PVY^N. Tracks 1-2: amplification products PVY (365 bp), PVS (213 bp), PVX (411 bp). Tracks 3-4: amplification products PVM (276 bp), PLRV (300 bp), PSTV (150 bp); M - marker. The samples of isolate PVY^O are on tracks 1 and 3. The samples of isolate PVY^N are on tracks 2 and 4

Thus, used isolates of PVY, belonging to different groups of strains and didn't contain other viruses. Research of response of plants *N. tabac*um and *N. rustica* to inoculation of selected isolates revealed the following.

When inoculated tobacco plants varieties Samsun 155 and Sobalchskyy 34/40 with the isolate of PVY^N appeared symptoms of vein necrosis in the basal part of the leaf blade. Further necrosis spread to the petioles, sometimes on stalks of plants. The first indication of virus infection on plants of var. Samsun 155 found in 9 day after inoculation as a clarification of the veins of young leaves (Fig.2 B).

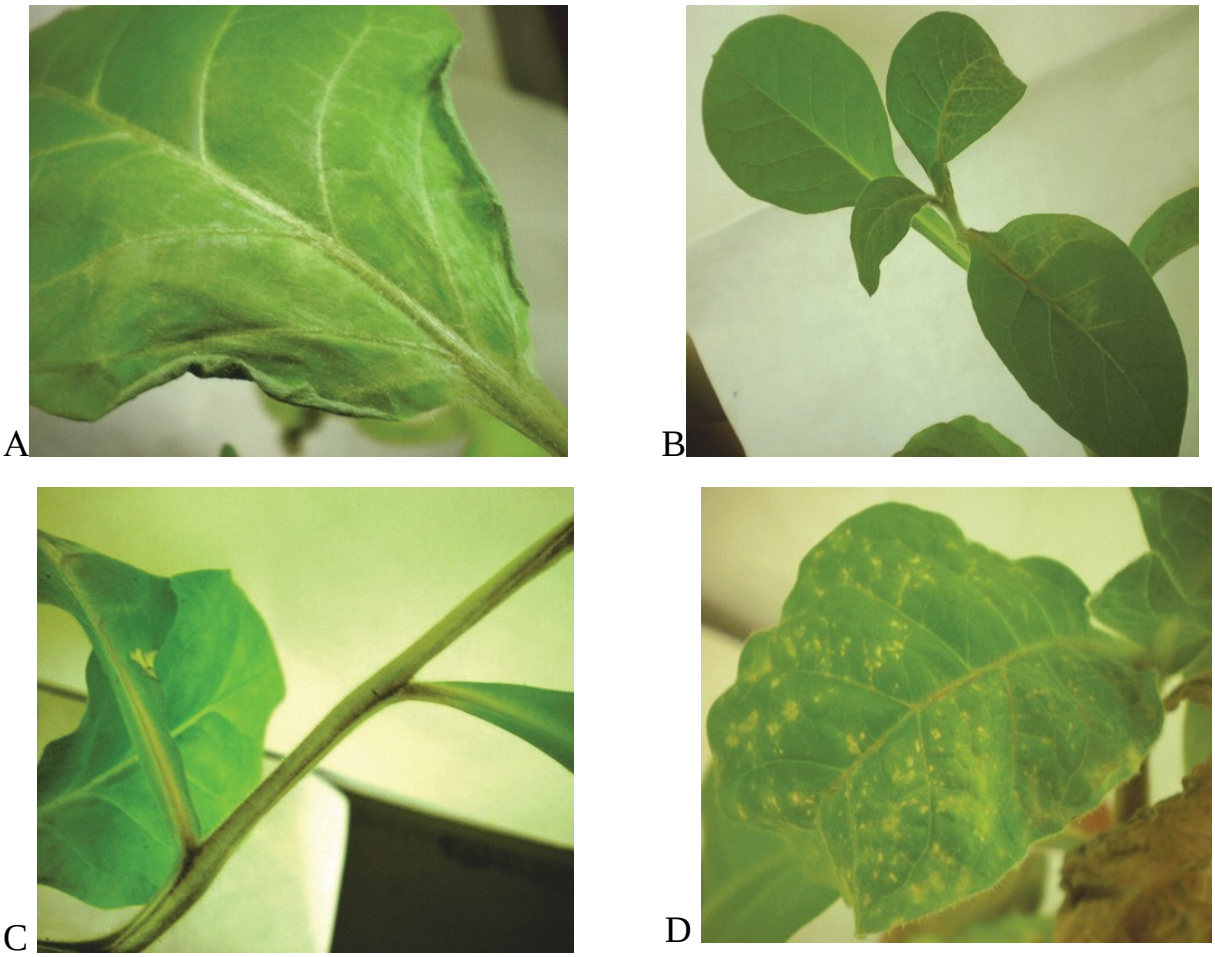


Fig.2. Symptoms of tobacco plants with infection of isolate of PVY^{N}: A — The necrosis veins and wilting leaf plate in tobacco plants var. Samsun 155; B — The systemic necrosis and clarification veins, plant var. Samsun 155; C — The necrosis of the stems and petioles of leaves, plant var. Sobalchskyy 34/40; D — The point necrosis and necrosis of the central vein, plant var. Sobalchskyy 34/40

Systemic necrosis quickly spread by leaf veins, causing wilting and drying of leaf blade (Figure 2 A). Part of plants of this variety died 30 days after inoculation. Inoculation of tobacco *N. tabacum* var. Sobalchskyy 34/40 plants with isolate PVY^{N} also led to the emergence of necrosis of veins, but infection didn't not lead to death of the plant. Plants infected with necrotic virus's isolate, were developing for a long time, appeared symptoms of necrosis on the young leaves was the evidence of systemic infection.

On the other hand, the plants, inoculated with PVY^{O} isolate did not show any symptoms or had only slight mosaic symptoms (fig.3).

Fig 3. Plants N.tabacum after inoculation isolate PYV^{O} (19-day): a - leaf of plants tobacco variety Samsun 155; b - leaf plants tobacco variety Sobalchskyy 34/40; c — mock-infected plant

DAS-ELISA established the presence of antigens of PVY in plants both varieties, other viruses were not detected (Tab. 1).

Table 1. The content of antigens of viruses in the plants *N.tabacum*, which were inoculated of isolates of PVY

Variety	Isolate	PVY	PVM	PLRV
Sobalchskyy 34/40	PVY^N	1,517±0,012	0,071±0,010	0,073±0,016
Sobalchskyy 34/40	PVY^O	1,205±0,011	0,045±0,012	0,055±0,011
Samsun 155	PVY^N	1,015±0,009	0,053±0,010	0,059±0,014
Samsun 155	PVY^O	0,915±0,018	0,059±0,015	0,067±0,013
Control positive		1,013±0,015	0,617±0,09	0,425±0,011
Negative control		0,045±0,010	0,045±0,010	0,045±0,09

In the leaves of tobacco plants varieties Samsun 155 and Sobalchskyy 34/40 by transmission electron microscopy discovered filamentous virus particles that are morphologically identical PVY. The particle size were 670-710x11-12 nm (fig.4).

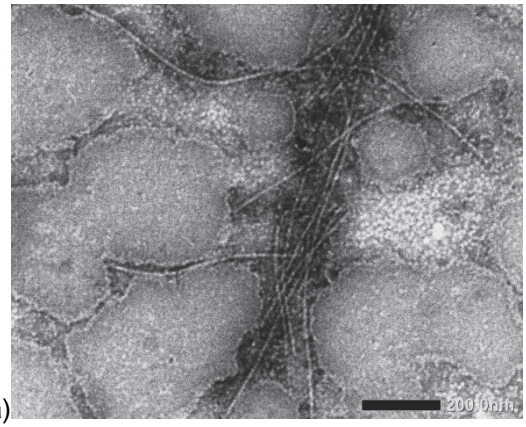
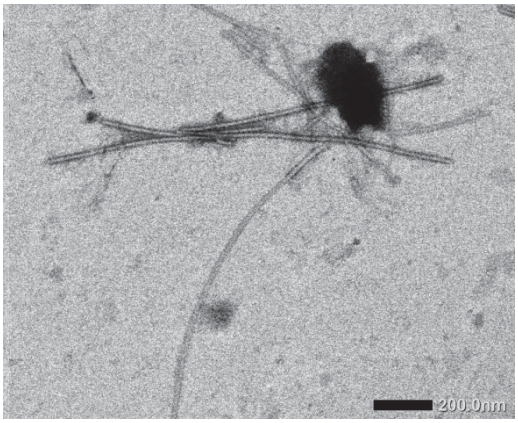
a)  b) 

Fig 4. Electronogram of purified preparations of isolates of PVY from plants tobacco variety Samsun 155, filamentous particles 670-710 nm (bar = 200 nm): a) isolate PVY^N; b) PVY^O

Thus, the inoculation of tobacco plants discovered different reactions to inoculation common and necrotic isolates of PVY. Inoculation of plants *N.rustica*, varieties Matsui Field and Actec and plants tobacco variety Sobalchskyy 193 didn't cause any visible symptoms of infection (Tab. 2).

Table 2. Results of tobacco plants inoculation by necrotic and common isolates of PVY

Plants/variety	PVY isolates	
	PVY^N	PVY^O
N.tabacum, cv. Samsun 155	NV, M	MM
N.tabacum, cv. Sobalchsky 34/40	NV, M	No symptoms
N.tabacum, cv. Sobalchsky 193	No symptoms	No symptoms
N.rustica, cv. Matsui Field	No symptoms	No symptoms
N.rustica, cv. Actec	No symptoms	No symptoms

NV — necrosis veins, M — mosaic, MM — mild mosaic

Thus, the results of laboratory tests showed that the varieties of tobacco *N.tabacum*, cv. Sobalchsky 193 and *N.rustica*, cv.Matsui Field and cv.Actec may have resistance to necrotic isolate of PVY.

Conclusions

Significant spread of PVY cultivated plants today attracts the attention of researchers, as the virus is one of the ten most damaging plant viruses. Especially dangerous is the virus strains that cause severe

symptoms in host plants. According to studies conducted in Chernihiv region, potato plantations necrotic PVY strains are common in recent years, even discovered samples of infected PVY^{NTN} strain, which is the most dangerous to the potato culture [14]. The emergence of new strains of the virus was recorded over the last decade in many countries [15]. Obviously, changes in the populations of the virus in our country meet the global trend of changes in the structure of strains of PVY. This leads to in-depth study of both the pathogen and its infection reactions in plants - hosts.

The use of genetically resistant plants is one of the most efficient, durable and commonly used strategies to combat viral infections in the field. For centuries, this method helped create plants with high economic qualities, combined with the absence of symptoms [16]. However, today the program of improvement of plants can benefit from the study of interactions between plants and viruses for the creation of resistant varieties, suitable for use in agriculture. Detection of our varieties of tobacco that are tolerant to infection severe necrotic PVY virus isolate, makes it possible to use them to create new forms of tobacco and conduct directed selection for resistance to the culture of this dangerous pathogen. Also, our studies provide an opportunity to further study and establish mechanisms of resistance identified tobacco plants that expand knowledge in this area.

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