INVESTIGATION OF PHYSICAL AND CHEMICAL PROPERTIES OF POTATO LEAFROLL VIRUS ISOLATES IN CULTURES OF MAMMALIAN CELLS

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Potato leaf rolling virus (PLRV) is among the most economically important pathogenic viruses. Yield losses due to infection by PLRV, depending on potato variety, climate and soil conditions, range from 20% to 90% [1; 2]. PLRV is common in all countries, where potato is grown. Potato leaf rolling virus affects a wide range of plants (today, this list comprises about 40 species) belonging to different families: *Amaranthaceae*, *Cucurbitaceae*, *Chenopodiaceae*, *Brassicaceae*, *Asteraceae*, *Lamiaceae*, *Portulacaceae*, *Nolanaceae*, *Solanaceae*, but the main virus host is potato [3; 4]. Different types of crops: tobacco, tomatoes, strawberry tomato and others are also susceptible to virus. Potato leaf rolling virus is found in weed and ornamental plants which, due to their susceptibility may be reserves of the virus in agroecosystems.

Great harm of PLRV is explained first of all by rapid virus spread by insect vectors and transmission vegetatively through tubers that wholly or partly lose their germination ability.

Three hazard zones were distinguished in the area of PLRV distribution [5]. In the zone of strong harmfulness the prevalence of disease on potatoes is more than 40%, there are frequent epiphytotic. In the area of average harmfulness epiphytotic occur at the intervals of 8-10 years and in the zone of low harmfulness the disease does not exceed 10%. The zone of strong harmfulness brings together the countries of Central Asia, the Caucasus, Kazakhstan, the North Caucasus, the western regions of Ukraine. Average harmfulness is characteristic for Belarus, Moldova, Central Black Earth area, Primorsky Krai, the Baltic countries, Sakhalin, Altai, Nizhny Novgorod, Ivanovo and Novgorod regions. On the rest of potato cultivation territory disease development does not exceed 10%.

But the issue of harmfulness and prevalence of the virus in different climatic zones of Ukraine remains uncertain. In 1989-1992 and 2008-2009 the prevalence of potato

viral diseases was studied on the basis of Ustymivsk Experimental Station [6]. It was shown that severe viral diseases, described earlier as gothic, are complex diseases caused by the compatible action of PLRV with mosaic virus group. Thus, virus leaf rolling is the most common and harmful disease in the conditions of southern Woodlands of Ukraine.

Incidence and prevalence of potato varieties and hybrids by PLRV is studied in the countries of Europe, the Americas, Africa, Asia, Australia and New Zealand. There is an opinion about the cyclicity of epiphytotic caused by PLRV and their relationship to solar activity and droughts [7; 8].

PLRV has such features as extremely low concentration in plant extracts, determined by virus localization in the phloem, inability of mechanical transmission and small size of virions, which complicates the study of virus isolates and diagnosis of diseases that are caused by it.

To study the properties of the isolates and strains of PLRV potato plants or plants-indicators are used, to which the virus is transferred from infected plants by grafting or aphids. Aphids are previously fed on infected plants or through membranes or virus preparation is injected through micro-injections [9]. Sometimes, for PLRV reproduction, protoplasts of tobacco or potato mesophyll and poly-L-ornithine are involved, which forms complex with viral particles and facilitates protoplasts infection [10].

In 2009 we described the phenomenon of PLRV productive infection in mammalian cells cultures [11; 12]. According to the results of further research neutralization and identification reaction in cell culture was suggested to indicate PLRV in plant material [13].

In this regard, the objective of our work was to separate potato leaf rolling virus isolates into a pure culture from vegetating plants, tubers and aphids and explore their physicochemical properties.

Materials and methods. To separate potato leaf rolling virus isolates the primary

seed growing plot of CJSC “Chernihivelitkartoplia” was examined. 18 samples of potato leaves with potato leaf rolling virus symptoms and aphids were selected.

The separation of PLRV isolates was conducted in cell cultures of finite line of pig embryonic kidney (PEK) and finite line of newborn hamster kidney (BHK-21), which was grown in test tubes. Before the virus introduction culture medium was removed from test tubes, the monolayer was washed twice with 0.15M NaCl or Hanks' solution. Investigated samples of 0.1cm³, broken by chloroform, were added and kept in an incubator at 37°C for a contact within 1 hour. Then the monolayer was washed with Hanks' solution and supportive medium was poured. Inoculated tubes with cells culture were incubated in an incubator at 37°C until the signs of virus cytopathic effect (CPE) appeared. 3-5 blind passages were conducted in cell cultures [11].

Virus thermoresistance was studied by heating the samples on a water bath over 1 hour at 50°C in the presence of 1M MgCl₂ and without it. Cold virus samples and heated in the presence of divalent ions of magnesium and without them were titrated [14].

The stability of viruses at different pH of the medium was studied in PEKV cell culture. For that, viruses were kept in the medium with pH 2.0; 3.0; 4.0; 7.2; 10.0; 11.0 at room temperature for 10 minutes. Then pH value was adjusted to 7.2 in all samples. Virus sensitivity to acidic and alkaline pH was determined by the difference of virus titres compared with control [14].

Temperature effect on the performance of PLRV – animal cells system was studied in BHK-21 cell culture. Cell culture monolayer was inoculated with Sednivskyi strain of potato leaf rolling virus at 1 TCD₅₀. The incubation was carried out at 2, 10, 24 and 37 °C. Degenerative changes of the monolayer, time of their occurrence and infectious virus activity were recorded [11].

So, four isolates of potato leaf rolling virus were identified in mammalian cell cultures that occurred to be resistant to lipid dissolving agents, cultural media with pH from 4.0 to 10.0 and were thermoresistant at 50°C in the absence of divalent ions of magnesium, TIP is within the range of 60-65°C. Optimum temperature for PLRV reproduction is +24°C and above.