ABSTRACT

Pavliuk L.V. **Scientific basis of improvement of virus-free clones selection in the system of certification of sweet and sour cherry planting material. — Qualification scientific work as the manuscript.**

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The thesis analyzes the viral diseases of sour and sweet cherry. The occurrence of pathogens in the territory of Ukraine has been established and the molecularly biological peculiarities of the certain isolates have been investigated.

In Ukraine, the plantings of sour and sweet cherry occupy 49.5 % of the stone fruit crops plantations total area. Therefore, there is a necessity for quality planting material. Viral pathogens are the limiting factor in obtaining both planting material and stable and high yields. The compatibility of variety-rootstock combinations under the influence of viruses has not yet been clarified. Therefore, it was relevant to study the effect of pathogens on the growth and development of nursery transplants.

In accordance with the European and Mediterranean Plant Protection Organization (EPPO) recommendations and their PM (4) 29 (1) «Certification scheme for cherry» standard, it is necessary to test the planting material for 15 viral pathogens. As only five viruses have been identified in the sour cherry and sweet cherry orchards at the time of our research planning, it is important to carry out phytovirological monitoring for the expanded panel of viruses. The research results made it possible to find out the presence and distribution of pathogens in plantations, and the study of the molecular and genetic characteristics of the detected isolates made it possible to determine their variability, origin and influence on the plant. So, the research carried out is very timeli.

The novelty of the research lies in the identification of a pathogens panel of sour cherry, sweet cherry and their root-stocks in accordance with the PM(4)29(1) standard. For the first time in Ukraine, monitoring of plantings of sour cherry, sweet cherry and their rootstock was carried out for 10 viruses from the PM(4)29(1) standard, as well as PPV, one of the most harmful viral stone fruit pathogens. It was established that the infection rate of sour cherry plantings is 33 %, sweet cherries – 30,6 %, root-stock — 13 %. For the first time, a number of pathogens have been detected in the plantations of sour cherry, sweet cherry and their rootstock: petunia asteroid mosaic tombusvirus (PeAMV), tomato black ring nepovirus (TBRV), apple mosaic ilarvirus (ApMV), arabis mosaic nepovirus (ArMV), raspberry ringspot nepovirus (RpRSV) and strawberry latent ringspot virus (SLRSV).

A phylogenetic analysis of such isolates as prunus necrotic ringspot ilarvirus (PNRSV), prune dwarf ilarvirus (PDV) selected from sour cherry and sweet cherry plants, as well as of plum pox virus (PPV) isolated from mahaleb cherry was carried out. It was revealed that PNRV isolates belong to different groups, which, for their part, affect the plant both in moderate and strongly negative way.

The influence of viral pathogens on variety-rootstock combinations and the functional state of sour cherry and sweet cherry has been studied.

In order to study the phyto-virological state of the plantings of sour cherry, sweet cherry and their rootstock, the plant material was tested for 10 viral pathogens identified by EPPO, as well as PPV, since this virus is dangerous for all the stone fruit crops. The main testing method was serologic diagnosis, since it allows to establish reliably the plants viral status and is designed to analyze a large amount of material.

Over the years of research, 291 samples have been tested, including 134 sweet cherries, 103 sour cherries, and 54 root-stock ones. The plant material was selected in ten regions of Ukraine in farms of different ownership forms. During the research, all viral pathogens for which testing was carried out were detected. Serologic diagnosis of the samples showed a high infection level of the material — 28,6 %. The largest share

of infected samples was recorded in the sour cherry material — 33 %, sweet cherry — 30,6 %, and the smallest - in the tested root-stock samples — 13 %. Among the viruses detected, PNRV prevailed, namely, in sour cherry samples — 14 %. The cultures under study within 5,5—9,7 % are infected with two or more viruses.

It was found that the infection rate of the tested sweet cherry varieties ranges from 23 to 45,5 %, depending on the cultivar; sour cherries — from 7,1 to 42,9 %, and root-stock — 4,2 % (VSL-2) and 33,3 % *(Prunus mahaleb).* Despite the significant percentage of cultivars infection, we have succeeded in selecting pure clones for further reproduction and establishment of industrial plantings. A total of 209 samples were selected, including 95 sweet cherry plants, 67 sour cherry plants, and 47 rootstock.

For true serologic diagnosis of PNRV and PDV, the optimal timing and types of plant material tissues have been selected for effective pathogens detection. The highest viral titers have been reported in leaf material in spring (April-May), while testing in October gives false negative results.

For phylogenetic analysis, PNRV and PDV isolates recovered from sour cherry and sweet cherry plants have been selected, as well as PPV isolates from the seed rootstock of mahaleb cherry. In the course of the investigation, the sequenced isolates were deposited into the global GenBank with the assignment of the corresponding numbers.

Based on the analysis of the PNRV nucleotide sequence, it was found that the recovered isolates belong to different groups: PV-96 (MT828889) and PV-32 (MT892676), circulating in the Kyiv and Zaporizhzhia regions. Symptoms differ depending on the pathogen strain, which explains the fact that some infected plants do not show symptoms of the disease. Clustering of isolates confirmed the presence of three groups independent of the host plant and geographic origin.

Phylogenetic analysis of PDV established that domestic isolates MT828888 and MT828887 have a high identity — 99,6 %. The closest to them was the Slovak sweet cherry isolate, its identity with the domestic one was 94,9—95,3 %. During clustering,

two groups were formed with division into two subgroups. In our case, the second group included only the sweet cherry isolates, which may indicate the existence of a separate cluster formed on the basis of the host plant.

Since the PPV isolates selected are 100 % similar to each other, only one, MW055900, was deposited with GenBank. It was found that this isolate belongs to strain D and is not a typical sour cherry host. This indicates the adaptation of the virus to new host plants, which means it is a threat to a wider range of crops.

To improve the certification scheme, it was proposed to amend the existing documents, namely: national standard DSTU 4791:2007 «Rootstocks of fruit crops. Quality Determination Methods» and DSTU 4792: 2007 «Saplings of fruit crops. Quality Determination Methods». Clarification of the pathogens panel reflected in their appendices D is required. Due to the composition of the pathogens identified on the territory of Ukraine, it is necessary to determine such a list for testing plant material: apple mosaic ilarvirus (ApMV), prunus necrotic ringspot ilarvirus (PNRSV), prune dwarf ilarvirus (PDV), arabis mosaic nepovirus (ArMV), cherry leaf roll nepovirus (CLRV), tomato black ring nepovirus (TBRV), raspberry ringspot nepovirus (RpRSV), strawberry latent ringspot virus (SLRSV), apple chlorotic leaf spot virus (ACLSV), petunia asteroid mosaic tombusvirus (PeAMV), plum pox virus (PPV).

In the process of studying variety-rootstock combinations, the response of sensitive rootstocks to grafting with infected oculants was investigated. Within the rootstock of VSL-2 and LC-52, the gummosis was observed at the site of inoculation, and subsequently a change in the color of the leaves to an anthocyanin coloration. The color intensity was different - intense red was observed on VSL-2, while on LC-52 it was less bright. Also in the spring, 100% of attacks were observed in the second field of the nursery on these rootstocks. Unlike previous rootstocks, no such reactions were recorded at VC-13. The survival rate of infected oculants was 50 % with the Nizhnist` cultivar and 76,6 % with the Bohuslavka and Kseniia varieties, while in uninfected control plants this indicator was equal 90 %.

During the observation of the studied plants growth dynamics, it was noted that some infected buds remained dormant, while healthy ones began to grow back.

The influence of pathogens on the functional state of sour cherry and sweet cherry plants was studied on infected and uninfected plants in the second field of the nursery-garden. The negative effect of the virus on the state of the plant's photosynthetic apparatus was revealed. In general, there was a decrease in chlorophyll *a* from 20,9 to 38 %, chlorophyll *b* from 31 to 43,9 %, depending on the cultivar and the virus that infected the plant. Applying the method of leaves chlorophyll fluorescence induction, it was found that in seedlings with a viral infection, when compared with virus-free ones, chlorophyll fluorescence increases over the entire time range of its induction changes registration. It was also found that prunus necrotic ringspot and prune dwarf viruses have a negative effect on the frost resistance of the studied varieties reproductive buds. It was determined that the viral load on the plant entailed damage to the bourgeons of infected of Kseniia varieties (PNRV) amounting to 3,5—4,5 points, and Bohuslavka (PDV) — 4,5. At the same time, in healthy (control) plants, the level damage was from 1,7 to 3,5 points, depending on temperature, pathogen and variety. When studying the effect of a viral infection on the quality of one-year-old seedlings, a decrease in the area of the leaf blade by 7,6—31 % was recorded, depending on the pathogen and plant variety. The specific surface density of the infected cultivars Bohuslavka (PDV) and Nizhnist (PDV + PNRV) was 5,5 and 39,9 % higher, respectively, while in cultivar Kseniia (PNRV) this indicator was less than the control by 27,4 %. An increase in the specific surface density of leaves (SSDL) in some variants influenced an increase in the water content in the cells: by 1,4 % in the Bohuslavka samples, 5,7 % in Kseniia ones, with a complex infection of the variety, while with a complex infection of the Nizhnist` variety, a decrease in hydration by 20,2 % was recorded.

When analyzing the parameters of the grown seedlings, there is a tendency to a decrease in the average diameter of the stem under the influence of viral infection. In

sweet cherry saplings of the Nizhnist`variety, the diameter of the bole decreases by 18,8 %, in the Bohuslavka sour cherry variety — by 13,3 %. Sour cherry plants of the Kseniia variety became an exception, where the difference between the infected plants and the control was not recorded. At the same time, the average height of seedlings decreases by 3,1 and 13 % in the Bohuslavka and Kseniia sour cherry varieties, respectively, and in the Nizhnist` sweet cherry — by 36,3 %.

As the research progressed, we have calculated that using infected material for growing seedlings reduces their yield when compared to using virus-free material. In total, only 8,88 thousand units per ha of the Bohuslavka variety plants were received, 11,1 and 26,64 thousand pcs per ha of cultivars Nizhnist and Ksenia, respectively. At the same time, in the control variants this figure was 49,95 thousand pcs per ha.

Calculations of the economic efficiency of growing seedlings indicate an increase in profitability when using planting material tested for viruses.

Consequently, the studies carried out provide an assessment of the phyto-virological state of sour cherry, sweet cherry and their rootstocks plantings in Ukraine. Planting monitoring allows you to neutralize the focus of infection and prevent further spread of viruses. Isolation of pure clones, which can later be used as planting material, is of a great practical importance.

Key words: viruses, ELISA, RT-PCR, phylogenetic analysis, chlorophyll, growth dynamics, seedlings.