Creation of the virus free nursery plantings of the genus prunus l. by the phytosanitary selection

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Abstract. Diagnostic of 12 viral pathogens of stone fruit crops, including new to the certification scheme (*Carnation ringspot tombusvirus* (CRSV), *Myrobalan latent ringspot nepovirus* (MLRSV), *Petunia asteroid mosaic tombusvirus* (PeAMV), *Tobacco ringspot nepovirus* (TRSV)) by immune-enzyme analysis was carried out in the Institute for fruit growing in 2020 – 2021. Virus-free clones of varieties and rootstocks forms of stone fruit crops have been identified to create Nuclear stock collection, including: 10 varieties of plums, 3 - cherry plums, 7 - sour cherries, 14 - sweet cherries, 5 - apricots, 4 - peaches, 10 forms of rootstocks of stone crops. Varieties of peach and apricot, as well as rootstocks of these crops, for the creation of Nuclear stock collection were generated in 2021 in Belarus for the first time.

1 Introduction

Establishment and preservation of healthy repositories in countries with an actively developing fruit growing industry is an important component of the international program of agricultural plant variety improvement. Obviously, the traditional movement of germplasm of perennial vegetatively propagated crops, entails high risks of spreading pathogens with host plants.

Especially, this applies to the pathogens with a long latent period, as well as asymptomatic ones, such as viruses and phytoplasmas, which are in a special risk group. Phytosanitary control of plant diseases from the EPPO A1 and A2 List of pests recommended for regulation as quarantine pests, is the main reason for establishing nuclear stock collections in all countries with high horticulture and developing documents on the maintenance of such collections, their replication, retesting.

The basis and the most interesting part of the Nuclear stock collection in specific horticultural areas are commercial, old local varieties, as well as perspective hybrids and forms. Increased attention is currently paid to the preservation of old local cultivars of plants (landrace), which have acquired their distinctive features as a result of many years of cultivation and selection in a particular area, as well as varieties of their own breeding. These genotypes are only grown in certain areas and require special conservation measures. Before plants are included in the basic collections, they are subjected to a number of tests:

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phytosanitary (absence of viruses, phytoplasmas, bacteria) and pomological (visual and genetic identification).

In 2018, the total area of seedlings of stone fruit crops in Belarus is 0.89 ha, including: cherry plum, plum, apricot - 0.58 ha, cherry, cherry - 0.31 ha. Planting material produced less than 150,000 pieces. Almost no Virus free seedlings are produced.

The most common and economically significant viruses of stone fruit crops are *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot ilarvirus* (PNRSV), causing yield reduction of up to 100% in some commercial varieties, especially with complex infection [1, 2].

In Romania, ACLSV, ApMV, CLRV, PDV, PNRSV and PPV (3 M, D and SwC strains), *Peach latent mosaic* (PLMVd) and *Hop stunt* (HSVd) viruses have been identified. ACLSV and ApMV viruses are rare. PPV virus has been reported in 38%, with 3 strains: M, D, and SwC, and the PPV-SwC strain has only been detected in trees older than 25 years. PDV and PNRSV were reported in 8-10%, while CLRV ranged from 3% (in collection orchards) to 8.5% in orchards older than 20 years. PLMVd was 34% in peach trees [3].

In Bulgaria in 2019, infection with PDV of cherries and sweet cherry trees averaged 14.4%. 92.4% of trees were diagnosed with PDV alone, while mixed infection with PNRSV was noted for 7.6%. Only 56.2 % of the trees were visually symptomatic [4].

In Czech Republic, 10 viruses (out of 19 tested) were found in cherry and sweet cherry trees: *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Cherry green ring mottle virus* (CGRMV), *Cherry leaf roll virus* (CLRV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA), *Little cherry virus 1* (LChV-1), *Little cherry virus 2* (LChV-2), *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV). ApMV and LChV-2 were diagnosed for the first time. CGRMV and CNRMV were isolated only from imported plants in collection plantations [5, 6].

In recent years, the spread of Cherry detrimental canker has been established in Northern Bavaria. Trees of all cherry cultivars grafted on *Prunus avium* rootstocks were affected. *Petunia asteroid mosaic virus* (PAMV) was detected by serological testing in diseased trees [7]. The virus appears on cherry plants as necrosis of shoot tips and necrotic spots on leaf veins and fruits. Earlier, this disease was described in other countries as Cherry detrimental canker. *Petunia asteroid mosaic tombusvirus* (PAMV) – tombusviruses have isometric particles about 30 nm in diameter, the RNA genome of the virus is single-stranded, 180 protein subunits, and a molecular weight of about 40,000. Cells infected with tombusviruses contain characteristic multivesicular bodies in their cytoplasm [8,9].

In 1987, another virus, *Carnation Italian Ringworm Virus* (CIRV), was found on cherry plants with similar symptoms. This was the first report of this virus in woody plants. The virus causes the same symptoms as PAMV, but the effect on the tree is less severe. The distribution of PAMV and CIRV virus particles has been found to be highly irregular. Using ELISA, viruses could be reliably detected only in symptomatic plant tissue samples. Therefore, indexing latent PAMV or CIRV infections in cherry trees is currently not possible. Since virus detection correlates significantly with the occurrence of symptoms, PAMV and CIRV are considered to be the causative agent of viral twig necrosis [9].

In Turkey (Trakai region), the presence of *Plum pox virus* (PPV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV) in wild stands of *Prunus* L. (PPV - 24.1 %, ACLSV - 11.1 %, ApMV - 22.2 %) was determined by DAS-ELISA, which shows the presence of uncontrolled reserve of virus infection in *Prunus spinosa* L. [10].

In 1990, Bud blight disease (BBD) was first reported on cherry in Japan. Symptoms of the disease include browning and death of buds, irregular brown spots and chlorotic mottling on leaves, and bark necrosis [11]. Significant yield reduction is the main outcome of the disease. The use of RT-PCR diagnosis made it possible to identify a complex of 5

viruses in the affected plants: *Little cherry virus-1* (LChV-1), *Little cherry virus-2* (LChV-2), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA) and *Cherry green ring mottle virus* (CGRMV) [12 -14]. A large proportion of cherry trees in Japan has been found to be infected with at least one of these viruses [15]. *Prune dwarf virus* (PDV) and/or *Apple chlorotic leaf spot virus* (ACLSV) can also cause Bud blight disease [16].

Studies on the spread of viral pathogens aim to analyze the phytosanitary situation in plantations and to create pathogen-free plants for further replication.

The aim of the research is to create stone fruit cultivars and rootstocks for the creation nuclear stock collection in accordance with EPPO recommendations and "Unified phytosanitary quarantine requirements to quarantinable products and quarantinable items at the customs border and customs territory of the Eurasian Economic Union".

2 Materials and methods

Research objects: mother plants of stone fruit crops (cherry, sweet cherry, plum, cherry plum, apricot, peach) in open and protected ground and in vitro cultures.

Viruses: *Apple chlorotic leaf spot trichovirus (*ACLSV), *Apple mosaic ilarvirus (*ApMV), *Carnation ringspot tombusvirus*r (CRV, CRSV), *Cherry leaf roll nepovirus (*CLRV), *Myrobalan latent ringspot nepovirus* (MLRSV), *Petunia asteroid mosaic tombusvirus* (PAMV, PeAMV), *Plum pox potyvirus* (PPV), *Prune dwarf ilarvirus (*PDV), *Prunus necrotic ringspot ilarvirus (*PNRSV), *Raspberry ringspot nepovirus (*RpRSV), *Tobacco ringspot nepovirus (*TRSV), *Tomato ringspot nepovirus (*TomRSV).

ELISA testing of viruses was used to diagnose pathogens [17].

3 Results and discussion

Allocation of initial plants of promising cultivars and rootstocks for further testing was carried out in the departments of biotechnology, nursery and fruit crops breeding of the Institute for fruit growing.

Visually healthy plants with typical cultivars features, without symptoms of viral and virus-like diseases were selected based on the results of plantation inspection in the spring period. The selected plants in previous years were characterized by good indicators of winter hardiness, general condition of plants, productivity, resistance to fungal diseases.

During the pomological evaluation of cultivars, the following features were taken into account: crown (shape, density); shoot (length, thickness, growth character, pubescence, length of internodes); leaf blade (size, shape, base, tip, serration, pubescence); fruit (size, shape, surface, skin color, color and consistency of pulp). During pomological characterization of the rootstock, the following characters were taken into account: mother bush (shape, growth strength); shoot (bark color, thickness, curvature, pubescence); bud (base, size, bud position relative to shoot); leaf blade (apex, base, leaf position relative to shoot, serration); petiole length; leaf stalk shape; anthocyanin color of shoot tip; internodes length.

Pomological evaluation of original plants of cultivars and commercial rootstocks of 6 stone fruit crops was conducted, including: plum (Vengerka Belaruskaya, Blufry, Stanley, Kubanskaya Rannyaya, Chachan Lepotitsa, Kroman, Volat, Dalikatnaya); cherry plum (Vetraz-2, Panna, Lodva); cherries (Turgenevka, Uyfehertoy Furtosh, Confitur, Livenskaya, Nesvizhskaya, Pamyat Yenikeeva, Rovesnitsa); cherries (Regina, Syubarovskaya, Krasavitca, Valery Chkalov, Annushka, Iput, Burlat, Lapins, Silvia); apricot (Lyavon, Pamiat Govorukhina, Kameya, Debut); peach (Pamiat Loiko, Donetsk bely, Iskra, Alex, Zolotoy Jubilee); Cherry and cherry tree rootstock (VSL-2, RVL-9, Izmailovsky, Gisela-5,

FIL-6); plum, cherry plum rootstock (VPK-1, Mirobalan 29C, VVA-1); peach rootstock (Vesennee Plamya); apricot rootstock (Speaker).

After pomological evaluation of original plants, plant samples were selected for virus diagnosis. Plants of stone fruit crops cultivars were isolated in the open ground. Source plants of rootstocks for diagnosis were isolated in the open ground and in the previously established collection in vitro and ex vitro (Table1). After testing, all forms of rootstocks were introduced in vitro for rapid multiplication.

Table 1. Plum, cherry, cherry, apricot, peach clonal rootstock used to create a repository

Fruit crop	Location of initial plants			Forms, N
	in vitro	*ex vitro* (in pots)	open ground	
Plum rootstocks	VPK-1, Mirobalan 29C,	Mirobalan 29C	VPK-1, VVA-1	3
Cherry rootstocks	VSL-2, RVL-9, Izmailovsky	Izmailovsky	VSL-2, RVL-9, Izmailovsky, Gisela-5, FIL-6	5
Peach rootstocks	Vesennee Plamya	-	Vesennee Plamya	1
Apricot rootstocks	Speaker	-	Speaker	1

Cultivars and rootstocks of plums and cherry plums have been tested for viruses: ACLSV, ApMV, PPV, PNRSV, PDV, TomRSV, TRSV, CRSV, PeAMV, MLRV, PRMV. All tested plants are free from the listed viruses. For Nuclear stock collection have been determined virus free clones of the 10 cultivars of plums (Vengerka Belaruskaya, Blufry, Stanley, Kuban early, Chachan lepotitsa, Kroman, Volat, Dalikatnaya); 3 cultivars of cherry plums (Vetraz-2, Panna, Lodva) and 3 forms of rootstocks (VPK-1, Mirobalan 29C, VVA-1).

Cultivars and rootstocks of sour and sweet cherries have been tested for viruses: ACLSV, ApMV, PPV, PNRSV, PDV, TomRSV, TRSV, CRSV, PeAMV, CLRV, RRV, MLRV. The presence of *Prunus necrotic ringspot ilarvirus* has been established in the original plantation (Etika, Sylvia (2), Burlat, Confitur (3)); ApMV (Confitur (3)). For Nuclear stock collection have been determined virus free clones of the sour cherries (Turgenevka, Uyfehertoy furtosh, Confitur, Livenskaya, Nesvizhskaya, Pamyat Yenikeeva, Rovesnitsa); sweet cherries (Krasavitca, Valery Chkalov, Annushka, Iput, Regina, Syubarovskaya, Burlat, Lapins, Silvia, 10/98, Pobeda, Mariaya, Germa, Lubava Kievskaya); sour and sweet cherries rootstocks (VSL-2, RVL-9, Izmailovsky, Gisela-5, FIL-6).

Cultivars and rootstocks of apricot have been tested for viruses: ACLSV, ApMV, PPV, PNRSV, PRMV, PDV, TomRSV, TRSV, CRSV, PeAMV, MLRV. Presumptive presence of ApMV and TRSV in the some individual plants of apricot (cultivar Kameya) determined. However, the results of ELISA do not allow us to reliably confirm the presence of the virus, the optical density of the samples exceeds the value of the optical density of the negative control by 1.5 - 1.7 times, while a reliably positive result is considered to be twice the excess. All tested plants of rootstock (Speaker) are free of the listed viruses. For Nuclear stock collection have been determined virus free clones from the cultivars: (Lyavon, Pamiat Govorukhina, Kameya, Debut) and rootstock for apricot - Speaker.

Cultivars and rootstocks of peaches have been tested for viruses (ACLSV, ApMV, PPV, PNRSV, PRMV, PDV, TomRSV, TRSV, CRSV, PeAMV, MLRV) in Belarus for the first time. Presence of ApMV in the some individual plants of peaches (cultivars Pamiat Loiko, Donetsk bely) determined. Presumptive presence of PRMV *(Peach rosette mosaic virus)* in the some individual plants of peaches (cultivars Pamiat Loiko (3 plants) Iskra (2 plants), Donetsk bely (1) Alex (1), Zolotoy Jubilee (1)) determined. A detailed analysis of plants

presumably affected by PRMV showed an excess of optical density for samples isolated only from lignified shoots. Samples from leaves and annual shoots are reliably free of the virus. Due to the ambiguity of the results, only clones with an optical density of about to negative control were distinguished as Nuclear stock. For Nuclear stock collection have been determined virus free clones from the cultivars: (Donetsk bely, Iskra, Alex, Zolotoy Jubilee) and rootstock for peach - Vesennee Plamya.

Virus-free graft material is stored on rootstocks (1660 pcs.) for laying a new rotation of the repository of stone fruit crops.

4 Conclusion

Diagnosis of viral pathogens (PeAMV, CRSV, MLRSV, PRV, ToRSV) new to the certification scheme in Belarus was carried out for the first time. Reliable infection with viruses of initial plants of stone fruit cultivars and rootstocks was not established by ELISA method.

Virus-free clones of cultivars and rootstock of stone fruit crops have been identified to create Nuclear stock collection in accordance with EPPO recommendations and "Unified phytosanitary quarantine requirements to quarantinable products and quarantinable items at the customs border and customs territory of the Eurasian Economic Union", including:

- cultivars of plums (Vengerka Belaruskaya, Blufry, Stanley, Kubanskaya Rannyaya, Chachan lepotitsa, Kroman, Volat, Dalikatnaya), cherry plums (Vetraz-2, Panna, Lodva) and rootstocks (VPK-1, Mirobalan 29C, VVA-1), free from ACLSV, ApMV, PPV, PNRSV, PDV, TomRSV, TRSV, CRSV, PeAMV, MLRV, PRMV;
- cultivars of sour cherries (Turgenevka, Uyfehertoy furtosh, Confitur, Livenskaya, Nesvizhskaya, Pamyat Yenikeeva, Rovesnitsa), sweet cherries (Krasavitca, Valery Chkalov, Annushka, Iput, Regina, Syubarovskaya, Burlat, Lapins, Silvia, 10/98, Pobeda, Mariaya, Germa, Lubava kievskaya) and rootstocks (VSL-2, RVL-9, Izmailovsky, Gisela-5, FIL-6), free from ACLSV, ApMV, PPV, PNRSV, PDV, TomRSV, TRSV, CRSV, PeAMV, CLRV, RRV, MLRV;
- cultivars of apricots (Lyavon, Pamiat Govorukhina, Kameya, Debut) and rootstock Speaker, free from ACLSV, ApMV, PPV, PNRSV, PRMV, PDV, TomRSV, TRSV, CRSV, PeAMV, MLRV;
- cultivars peaches (Donetsk bely, Iskra, Alex, Zolotoy Jubilee) and rootstock Vesennee Plamya, free from ACLSV, ApMV, PPV, PNRSV, PRMV, PDV, TomRSV, TRSV, CRSV, PeAMV, MLRV.

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