Viruses of sour and sweet cherry in Belarus

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Introduction

The cherry fruit and nursery industries are important sectors of agriculture in Belarus. Virus diseases cause serious problems in commercial *Prunus* orchards decreasing plant productivity drastically [1]. More than 30 viruses infect cherry trees worldwide. *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Cherry leaf roll virus* (CLRV) and *Raspberry ringspot virus* (RpRSV) are the most common pathogens of stone fruit trees and they often occur in mixed infections [2,3,4]. Epidemiological control of virus diseases is necessary for creation of stone fruit nurseries and production of fruits with stable high yields.

The aim of the study was to investigate the occurrence of sweet and sour cherry viruses in the orchards of the Institute for Fruit Growing (Samochvalovichi, Belarus) based on the results of ELISA test and to estimate the most common cherry viruses.

Materials and methods

Orchard surveys. Commercial cherry orchard and variety collection of the Institute for Fruit Growing (Belarus) were inspected for symptoms of virus infection in 2012-2014 to assess the sanitary status of cherry trees.

ELISA. Leaves of 105 trees of 24 sour cherry and 17 sweet cherry cultivars were collected and tested by DAS-ELISA technique for the presence of *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Cherry leaf roll virus* (CLRV) and *Raspberry ringspot virus* (RpRSV).

Absorbance values two times or more than those given by healthy controls were considered to indicate infection. Antiserum for testing was from commercial ELISA kit (Sediag).

Results and discussion

The sanitary status of sweet and sour cherry trees in the orchards of the Institute for Fruit Growing (Samochvalovichi, Belarus) was preliminary evaluated, showing high rate of virus infection. Sour cherry samples were more infected by viruses than sweet cherry samples (infection rate is 41.7% and 36.2% respectively). The presence of 4 viruses (PNRSV, PDV, ACLSV and RpRSV) was confirmed. The most important and quarantine PPV virus was not detected in the collected samples.

The most frequent virus of cherry trees was PNRSV detected in 36.2% and 14.3% of sour and sweet cherry samples correspondently. The incidence of other tested viruses was lower. PDV was detected in 9.1% and 5.7% of sour and sweet cherry trees respectively. The incidence of ACLSV varied from 6.7% in sour cherry trees to 4.4% in sweet cherry trees. RpRSV was detected only in sour cherry samples (5%). ApMV and CLRV viruses were not detected.

Both sour and sweet cherry samples were singly infected by one of four detected viruses. Mixed infection of PDV and RpRSV was found in one sample collected from sour cherry tree cultivar Vjanok.

The low sanitary status of cherry trees calls for the implementation of certification programs in the production of propagation material in Belarus in order to prevent further spread of the viruses. ELISA test could be used in combination with RT-PCR technique for the identification of genetically distant virus isolates and for detection of viruses with a low concentration in plant tissues. Further molecular characterization of cherry viruses and continue field studies are required.

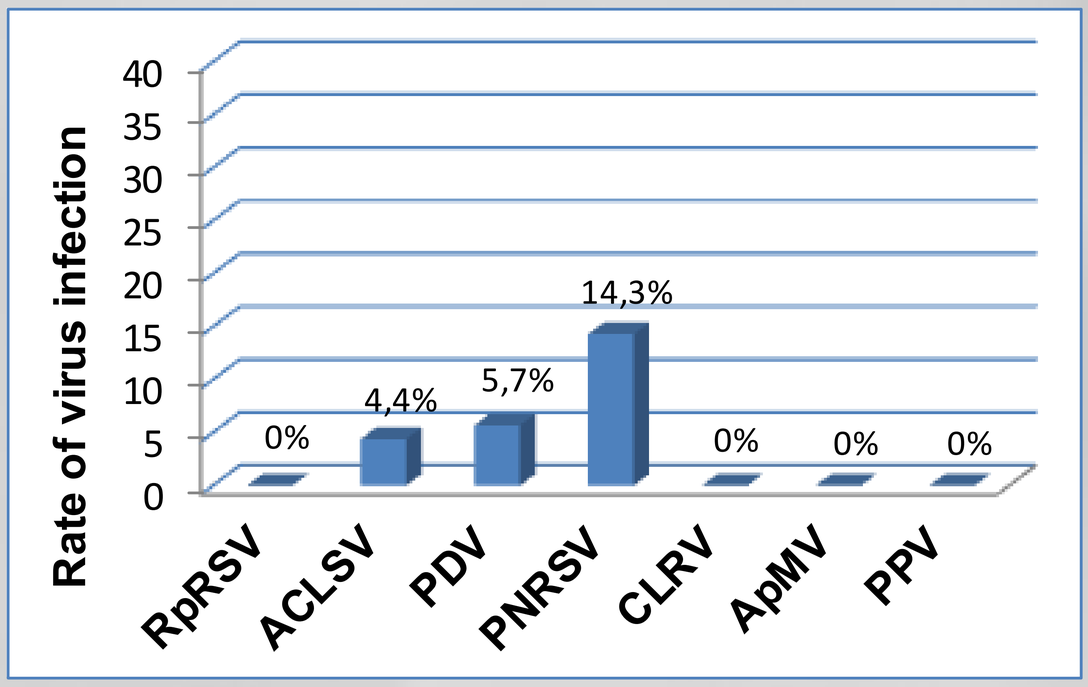

Fig. 1. Viruses detected in sweet cherry samples

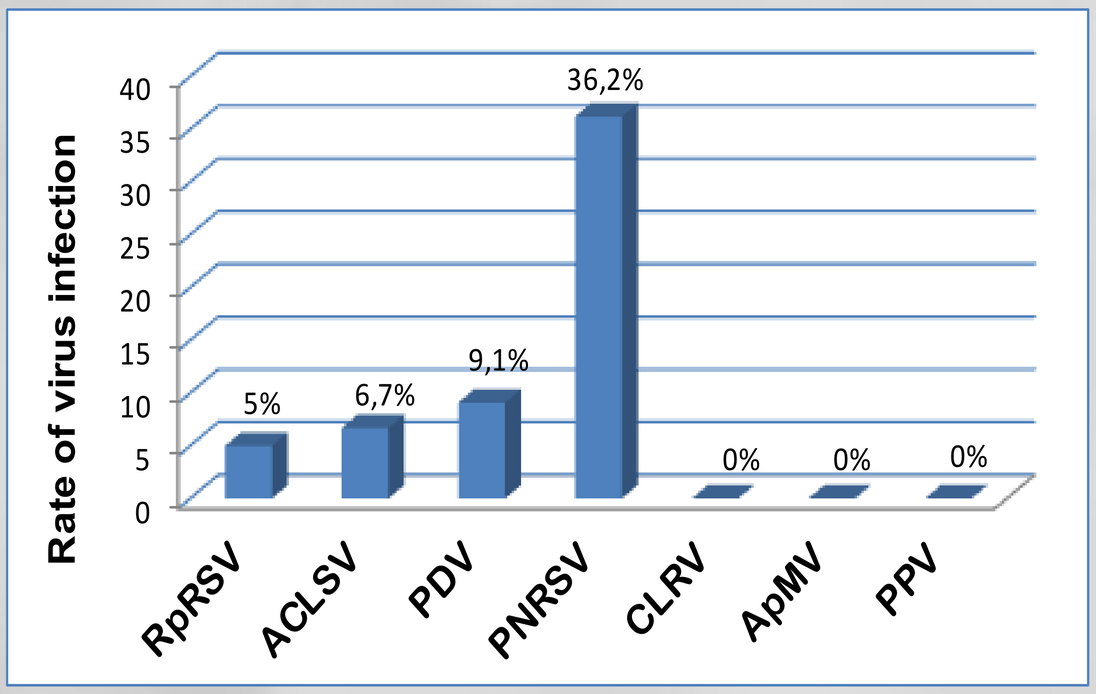

Fig. 2. Viruses detected in sour cherry samples

References: 1) Andersone et al., 2002. Effect on infection by viruses on vegetative and reproductive growths of sweet cherry on Damil and Inmil rootstocks. Hort. Sci. (Prague), 29: 99-104; 2) Mandic et al., 2007. Viruses of sweet and sour cherry in Serbia. J. Plant Pathol., 89: 103-108; 3) Everett, Milne and Forster, 1993. Sap-transmissible viruses in flowering cherry in New Zealand. New Zealand J. Crop and Hort. Sci., 21: 311-316; 4) Meziani et al., 2010. Assessment of the main stone fruit viruses and viroids in Algeria. Julius-Kuhn-Archiv, 427: 289-292.

Preliminary results of the development of a PCR-based marker linked to resistance to cherry leaf spot (Blumeriella jaapii [Rehm] Arx.)

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INTRODUCTION

Cherry leaf spot is a widely-spread disease in most sour and sweet cherry growing areas all over the world caused by a fungus called *Blumeriella jaapii* (Rehm) Arx. Under humid climate, the disease causes severe leaf defoliation in the second half of the season. The early leaf defoliation during summer weakens the tree and increases frost susceptibility of the fruit-bearing parts of the tree [1]. Some *Prunus* species, like *Prunus serrulata*, *P. sachalinensis* and *P. maackii* are often used in breeding programs as a source of monogenic and polygenic resistance to cherry leaf spot disease. However, there is no information about a localization of resistance genes to cherry leaf spot, as well as molecular markers linked to the disease resistance haven't been developed. The aim of the study was the development of RAPD marker linked to monogenic resistance to cherry leaf spot derived from *P. maackii*, and its conversion to a PCR-based marker.

MATERIALS AND METHODS

Plant material. A collection of cherry germplasm of the Institute for Fruit Growing (Samochvalovichi, Belarus) was used to form three groups of cherry cultivars: a group of susceptible cultivars, a group cultivars with monogenic resistance to cherry leaf spot inherited from *Prunus maackii* Rupr., and a group of cultivars with resistance inherited from any other *Prunus* species.

DNA extraction. Total DNA was extracted from plant leaves using DNeasy Plant Mini kit (Qiagen).

RAPD-analysis. 40 short (10 nucleotides long) primers with an arbitrary sequence were used for identification of genetic polymorphism among screened genotypes which might related to resistance to cherry leaf spot.

RAPD-fragment which might be linked to cherry leaf spot resistance were cut from the agarose gel, purified by JenGet Gel Purification kit (Fermentas) and send for sequencing. Sequence specific primer pair was designed on the base of the obtained nucleotide sequence.

RESULTS AND DISCUSSION

One out of forty tested primers provided a RAPD fragment which might be closely linked to monogenic resistance to cherry leaf spot introgressed from *P. maackii*. The PCR product of approximately 1200 bp was amplified only in resistant individuals ancestors of *P. maackii,* but not in the susceptible individuals or individuals resistant to the disease but not an ancestors of *P. maackii.* Since RAPD analysis has a low reproducibility [2], the amplified RAPD fragment was sequenced and a pair of sequence specific primers was designed for amplification 400 bp product only in resistant from *P. maackii* individuals.

Further verification of the primer pair for their suitability to identify resistant genotypes is required. The use of molecular markers-based screening assay will be of significant practical value in the identification of resistant sour cherry selections to leaf spot, as it will provide a rapid, reliable, means of screening large numbers of potentially resistant seedlings.

Table 1. *Prunus* germplasm screened by RAPD-analysis

Germplasm with monogenic resistance to cherry leaf spot inherited from *Prunus maackii* Rupr.	Germplasm with resistance to cherry leaf spot inherited not from *Prunus maackii* Rupr.	Germplasm susceptible to cherry leaf spot
Prunus maackii	Damil	Kazdangskaya
Almaz	Gisela 5	Molodeznaya,
OVP-2	VSL-2	Shokoladnica,
Ismailovski	Assol	Oblachinskaya,
V-5-172	Lasuha	Zarya Povolzhya,
P3 Moskovia	Gurtjevka	Mcenskaya,
LC-52	Bulatnikovskaja	Mestnaya
11-59-2	Zarya Tatarii	Vjanok
VP-1	Pamjat Enikeeva	Novodvorskaya
Rubin	Griot Seridko	Rovesnitsa
C-8-111	Turgenevka	Bistrinka
B-2-180	Nochka	Veteranka
B-2-230	Ksenia	Doneckij Velikan
Novella	Nord Star	Zhagarskaya
Dolgozhdannaya	Igrushka	Zvesdochka
		Konkurentka
		Rastorguevka
		Skromnitsa
		Stojkaya
		Lutovka
		Shumadinka
		Volochaevka

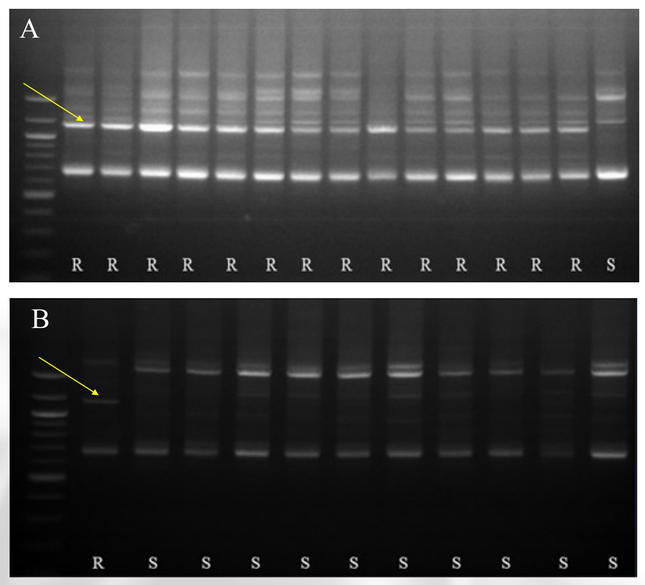

Fig. 1. The results of RAPD-analysis with primer linked to the resistance to cherry leaf spot using resistant (R) and susceptible (S) individual.
Germplasm used: *from left to right A) P. maackii,* Almaz, OVP-2, VP-1, Rubin, C-8-111, B-2-180, B-2-230, Novella, 11-59-2, V-5-172, LC-52, Ismailovski, P3 Moskovia, Kazdangskaya.
B) P. maackii, Molodeznaya, Shokoladnica, Oblachinskaya, Zarya Povolzhya, Mcenskaya, Mestnaya, Vjanok, Novodvorskaya, Rovesnitsa, Kazdangskaya.

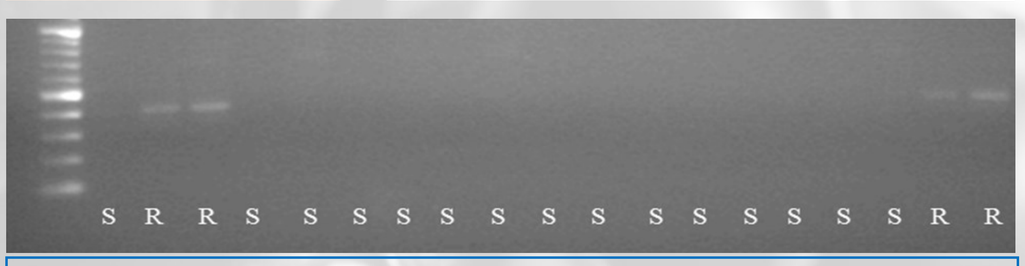

Fig. 2. PCR products obtained using the sequence specific primers in *Prunus* germplasm. A band (400 bp) is observed in resistant (R) individuals, with no amplification in susceptible (S) individuals. Germplasm used: *from left to right* Kazdangskaya, *P. maackii,* Almaz, Molodeznaya, Shokoladnica, Oblachinskaya, Zarya Povolzhya, Mcenskaya, Mestnaya, Vjanok, Novodvorskaya, Rovesnitsa, Bistrinka, Veteranka, Zhagarskaya, Konkurentka, Zvesdochka, B-2-180, B-2-230.

REFERENCES: 1) Holb, 2009. Some biological features of cherry leaf spot (*Blumeriella jaapii*) with special reference to cultivar susceptibility. International J. Hort. Sci., 15: 91–93; 2) Penner et al., 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. Genome Res., 2:341-345.