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THE PHYTOVIRAL STATUS OF SOME NEW ESTABLISHED SWEET CHERRY ORCHARDS IN MOLDOVA REGION, ROMANIA

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Abstract

Ten new established sweet cherry orchards in Moldova region were surveyed in the summer of 2021 to assess the phytoviral status. Nine of these were established with abroad propagated material from Italy, Belgium, Czech Republic and Netherlands, and the other one with material produced in Romania. Two blocks of 200 trees from each orchard were visually monitored for virus-like symptoms and then ten samples per orchard were randomly collected for laboratory testing. Sampling trees were tested for the presence of the following viral pathogens: Prune dwarf virus (PDV), Prunus necrotic ring spot virus (PNRSV), Apple mosaic virus (ApMV), Apple chlorotic leaf spot virus (ACLSV), Plum pox virus (PPV), Arabis mosaic virus (ArMV), Cherry leaf roll virus (CLRV), Raspberry ringspot virus (RpRSV), Strawberry latent ringspot virus (SLRSV) and Tomato black ring virus (TBRV) by DAS-ELISA using BIOREBA antiserum kits. The presence of Little cherry virus-1(LChV-1) on three samples which expressed symptoms of potentially infections was checked by RT-PCR. One out of ten surveyed sweet cherry young orchards found to be infected by one virus. PNRSV was detected in one orchard with an occurrence of 10%. The average of infection with PNRSV in the surveyed orchards from Moldova was 1%. No other infection was found in the surveyed orchards. The newly established orchards in Moldova region have generally a very good phytoviral status which is an important prerequisite for their success. However, there are some cases with virus infected old orchards in the proximity of the newly established orchards which might represent potential source of infection.

Key words*: propagation material, serological and molecular assays, survey, sweet cherry, virus.*

INTRODUCTION

Sweet cherry is the third fruit species cultivated in Romania with a harvesting area of 3,200 ha, after plum and apple crops, and ranks the fourth place in both fruit production and yield, with 37,640 tonnes, and 11.76 t/ha, respectively (FAOSTAT, 2020).

The sweet cherry (*Prunus avium* L.), member of *Rosaceae* family, genus *Prunus*, is often affected besides fungal and bacterial diseases by many viruses, some of them with economic importance regarding quality of fruits and also productivity of crops (Nemeth, 1986; Hadidi & Barba, 2011). The viruses are dangerous intracellular pathogens that can affect yield and fruit growth (Pavliuk et al., 2019). A suitable management of viral diseases targets limiting the losses of fruits by using prevention measures, because once infected by viruses trees cannot be treated in orchard. The using of the healthy planting material, setting up the new orchards far away from sources of infection, chemical treatments applied against virus vectors, using of resistant cultivars (if there are), represent useful preventive measures that contribute to limit the viral diseases in pome and stone fruits orchards. Because sometimes viral infections do not express symptoms, rigorous monitoring and removal of infected trees are required, followed by their replacement with virus free trees. Surveys and regular testing of the orchards are recommended especially for viruses that are pollen and seed transmitted (Caglayan et al., 2011).

In Romania, in the last years were established many sweet cherry orchards with planting material produced both in our country and in different European countries.

The aim of this study was to get information about the initial phytoviral status of planting material by assessing the occurrence of the viruses in some new sweet cherry orchards in Moldova region, and to assess their potential success depending on the viral status.

MATERIALS AND METHODS

Field surveys

Ten newly established sweet cherry orchards (1-6 years old) from five counties (Bacău, Galați, Iași, Vaslui and Vrancea) in Moldova region were surveyed in the summer of 2021 to assess the phytoviral status. Nine of these were established with abroad propagated material, produced in Italy, Belgium, Czech Republic and Netherlands, and the other one with material produced in Romania. The orchards surveyed were mainly established with valuable cultivars, such as Regina, Kordia, Tamara, Bigarreau Burlat and Carmen. Two blocks with a total of 200 trees (100 trees per block) from each orchard were individually monitored by visual observation for potential viruses-like symptoms. Then, randomly were collected ten samples per orchard for laboratory analyses. Each sample consisted in 5-10 leaves. Additionally, when symptomatic leaves were observed, samples from these trees were analyzed in laboratory by serological or molecular assays. Also, when was the case of presence of old sweet cherry trees or orchards in the proximity of the newly established orchards, those were surveyed in order to check the potential viral external inoculum sources. When virus-like symptoms were observed, a few symptomatic trees were sampled and laboratory tested.

Serological and molecular assays

Sampled trees were serological tested for the presence of the following viral pathogens: *Prune dwarf virus* (PDV), *Prunus necrotic ring spot virus* (PNRSV), *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Plum pox virus* (PPV), *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Raspberry ringspot virus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV) and *Tomato black ring virus* (TBRV). Serological assays were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark & Adams, 1977) using commercial polyclonal antiserum against to all viruses mention above, according to the manufacturer's instructions (Bioreba, Switzerland). Absorbance values were measured at 405nm after 1h substrate hydrolysis. Samples were considered positive if their absorbance values were more than twice those of the negative control. Then, the occurrence was established for each virus.

Molecular assay was performed by RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) in order to check the presence of *Little Cherry virus*-1 (LChV-1) in some suspect trees. For total RNA extraction was used Spectrum Total Plant RNA kit. The iScript cDNA Synthesis Kit was used for reverstranscription and FastStart Taq DNA Polymerase kit for amplification, according to the manufacturer's instructions. Specific primers for detection of LChV-1 were used according to Bajet et al. (2008). The amplification products were separated by electrophoresis in 1.5% agarose gel stained with RedSafe and visualized under UV light using Gel Doc XR.

RESULTS AND DISCUSSIONS

Field surveys

More than 2,000 trees were visually inspected for the potential viral symptoms in ten new sweet cherry orchards in Moldova region. Generally, no typical virus-like symptoms were observed on inspected trees. However, chlorotic rings and necrotic spots on leaves were sporadically observed in only one orchard from Bacău County. In another orchard, from Galați County, reddish of the leaves and rolling of the leaf edges upwards were observed on some trees. In both cases, the sampled leaves were analyzed by serological or molecular assays.

DAS-ELISA test

A total of one hundred samples (ten from each orchard) were tested by DAS-ELISA. Based on the obtained results, the viruses occurring in surveyed sweet cherry orchards in Moldova region was determined (Table 1).

Virus infection was detected in one out of ten new sweet cherry orchards surveyed. More precisely, PNRSV was detected inside an orchard from Bacău County. The occurrence of PNRSV in this orchard was calculated at 10%.

No other infections were detected in the new sweet cherry orchards surveyed in Moldova region according to DAS-ELISA test.

Table 1. The occurrence of viruses based on DAS-ELISA test in some new sweet cherry orchards in Moldova region

Orchard location/ county code	Provenance of plant material	The occurrence of viruses (%) based on DAS-ELISA test									
		PPV	PDV	PNRSV	ACLSV	ApMV	ArMV	CLRV	RpRSV	SLRSV	TBRV
1. Pădureni (IS)	Netherlands	0	0	0	0	0	0	0	0	0	0
2. Podu Iloaiei (IS)	Czech Republic/ Belgium	0	0	0	0	0	0	0	0	0	0
3. Plopana (BC)	Romania	0	0	**10**	0	0	0	0	0	0	0
4. Valea Mare Ivăneşti (VS)	Italy	0	0	0	0	0	0	0	0	0	0
5. Grumezoaia (VS)	Italy	0	0	0	0	0	0	0	0	0	0
6. Crasna (VS)	Italy	0	0	0	0	0	0	0	0	0	0
7. Vârlezi (GL)	Netherlands	0	0	0	0	0	0	0	0	0	0
8. Urecheşti (VN)	Netherlands	0	0	0	0	0	0	0	0	0	0
9. Coteşti (VN)	Netherlands	0	0	0	0	0	0	0	0	0	0
10. Odobeşti (VN)	Belgium	0	0	0	0	0	0	0	0	0	0

The overall occurrence of PNRSV in Moldova regions was calculated at 1%. No infections with PDV, ApMV, ACLSV, PPV, ArMV, CLRV, RpRSV, SLRSV and TBRV were found in the surveyed sweet cherry orchards in Moldova region.

RT-PCR assay

Samples from three trees that suggested potential infections with LChV-1, tested by RT-PCR assay, revealed the absence of this virus (data not shown). Most probably, the symptoms were caused by other factors.

Inoculum source nearby

In the proximity of two newly established orchards were found old trees or orchards which expressed symptoms on leaves of possible viral diseases (chlorotic diffuse mottling, chlorotic rings or spots, chlorosis of veins). DAS-ELISA test revealed the presence of PDV in eight samples collected from the proximity of two newly sweet cherry orchards (two samples nearby of orchard no. 3, and six samples nearby of orchard no. 6). These old orchards might represent potential source of viral infection for the newly established sweet cherry orchards.

Studies about the occurrence of viruses in commercial orchards, variety collections or nurseries of pome and/or stone fruits were performed in many countries during time, such as Albania (Digiaro et al., 1994), Serbia (Mandic et al., 2007), Turkey (Ulubas, 2008; Ulubas & Ertunc, 2008), Bosnia and Herzegovina (Matic et al., 2008), India (Brakta et al., 2012), China (Ni et al., 2012), Latvia (Gospodaryk et al., 2012), Lebanon (Nassar et al., 2012), Bulgaria (Kamenova et al., 2019; Borisova et al., 2021) and different percentage of infections with viruses were found. However, our study focused on some newly sweet cherry commercial orchards (1-6 years old). Similar studies were performed in some newly plum orchards in the same region (Zagrai et al., 2021), as well as in some newly sweet cherry and plum orchards from Transylvania (Zagrai et al., 2022 - in preparation).

Corroborating these results we will be able to create an image about the phytoviral status of some new established orchards of plum and sweet cherry in these regions of Romania.

CONCLUSIONS

Newly established sweet cherry orchards in Moldova region generally revealed a very good phytoviral status. This situation represents an important prerequisite for a successful start of cherry industry in this important fruit area of Romania.

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