UDC 632.3.01/.08

Detection of virus, bacterial and phytoplasmic diseases on vineyards of Odesa oblast

Konup A., Muliukina N., Konup L.
NSC «V.Ye.Tairov Institute of viticulture and winemaking» of NAAS, 40-richcha Peremohy Str., 27, Odesa, 65496, Ukraine; e-mail: lkmicrobiol@ukr.net

The purpose. To determine presence of virus, bacterial and phytoplasmic diseases of grape on grape plantings of Odesa oblast. **Methods.** Field, laboratory. Fytosanitory diagnostic study of vineyards in grape-cultivation farms (Ovidiopol and Tatarbunar regions of Odesa oblast). They identified causal organisms of diseases of grape plants using serological (immunoenzymatic — ELISA) and molecular-biological (polymerase chain reaction (PCR) with electrophoretic detection) methods. **Results.** At visual diagnostic study of grape plantings in Ovidiopol region they detected plants with symptoms of leaf curl and on occasion — with symptoms similar to that caused by fanleaf virus and virus of a complex striation of wood of a grape. Grape plants with symptoms of bacterial cancer and phytoplasmic diseases are detected on vineyards of Ovidiopol and Tatarbunar regions. Presence of causal organisms of virus (fanleaf of grapevine, leaf curls, and viruses of a complex striation of wood of a grape), phytoplasmic diseases and bacterial cancer is confirmed by methods of ELISA and PCR. High scale of defeat is registered by the causal organism of bacterial cancer on scrubs of variety Cabernet Sovinion, by the causal organism of phytoplasmic disease of wood blackening — on variety Shardone. **Conclusions.** The greatest percent of plants of grape struck by virus and phytoplasmic diseases, has appeared in Ovidiopol region, by the causal organism of bacterial cancer — in Ovidiopol and Tatarbunar regions of Odesa oblast. It is established that approximately 10% of the determined diseases of grape are virus ones, nearby 25 are bacterial cancer, and 65% are phytoplasmic diseases.

Key words: *viruses of grape, wood blackening, IFA, PCR, bacterial cancer of grape, grape.*
DOI: https://doi.org/10.31073/agrovisnyk201904-04

Virus, phytoplasma diseases of grapevine and crown gall of grapes cause significant damage to viticulture around the world [1, 2, 3]. Recently, due to the significant decrease in the production of own domestic planting material of grapes and the decline of nurseries, the vast majority of seedlings are imported from abroad, which, despite the presence of a certificate confirming their belonging to the biological category "certified", often latently infected with pathogens of viral, bacterial and phytoplasma diseases. Thus, a large amount of planting material of grapes affected by pathogens of crown gall and phytoplasma diseases — bois noire — was brought to the territory of southern Ukraine from France, Italy, Germany, Austria Visually, with the exception of crown gall, phytoplasma diseases and sometimes grapevine leafroll, so for their control it is necessary to conduct laboratory testing.

The harmful viruses of vine plants included in the system of sanitary certification of planting material include: the grapevine leafroll virus from the 1st to the 9th serotype (with priority attention to the detection of the first and third serotypes) - grapevine leafroll-associated viruses (1-9 (GLRaV1-9), grapevine fanleaf virus (GFLV), grapevine virus grapevine (GFkV), grapevine virus A (GVA) and grapevine virus B (GVB) grapevine virus of the Rugose Wood Complex (RWC) [4].

The causative agent of crown gall of grapes is the gram-negative bacterium *Rhizobium vitis* (*Agrobacterium tumefaciens*), which has the Ti-plasmid, which determines the tumor properties [5]. The causative agents of phytoplasma disease of the grapes are phytoplasma - unicellular chemoheterotrophs with complex nutritional needs, without a cell wall. The phytoplasma cells are surrounded by a plasma membrane only about 10 nm thick, which determines their plasticity and variety of cellular outlines [6].

Among the phytoplasma diseases of grapes, the most common occurrence is the bois noire; flavescence doree, which is a quarantine object, is rarely encountered.

The purpose of the research is to conduct a phytosanitary examination of grape plantings for the presence of symptoms of virus, phytoplasma and bacterial diseases of grape plants in the Ovidiopol and Tatarbunar districts of the Odessa region and to identify the causative agents of these diseases by laboratory methods with determining the extent of damage by virus, bacterial and phytoplasma diseases of grapes.

Materials and research methods. The material for the study were grape plants of industrial plantations and mother plantations of the south of Ukraine. To carry out this work, a phytosanitary examination was used; to identify the causative agents of grape diseases, plant samples with typical symptoms were selected according to ISO 16578: 2013 [7]. Identification of grape viruses was performed by ELISA [8]. In the study used commercial test systems *Agritest*, Italy. Diagnostic sets LOEWE, Germany were used to identify of crown gall. Classical PCR was used to identify pathogens of phytoplasma diseases of grapes. PCR amplification was carried out with a universal pair of primers for different parts of the genome specific for phytoplasma fU5 / rU3 [9, 10]. The synthesis of primers was carried out according to our order by *Fermentas*, Lithuania. Composition of the reaction mixture (40 μl): 4 μl 10x buffer for PCR; 1.2 μl 1.6 mM $MgCl_2$; 5 μl 2.5 mM dNTPs; 2 μl 5 μM primer fU5; 2 μl 5 μM primer rU3; 0.4 μl 5U / μl Taq DNA polymerase (*AmpliSense*, Russia); 22.8 μl deionized water and 2 μl undiluted isolated phytoplasma DNA. The amplification was carried out in the programmable DNA amplifier “Tercyk TP4-PCR-01” (NPO DNA-Technology, Russia), which included 35 cycles: 95 ° C/3 min. - denaturation, 55 ° C/1 min. - annealed and 72 ° C/6 min. 30 sec - elongation. After the first amplification, the mixture was diluted in a ratio of 1:50 and 2 μl was added to the reaction mixture for a second amplification with the same primer pair - FU5: 5'CGG CAA TGG AGG AAACT-3 '; rU3 5'-TTC AGC TAC TCT TTG TAA CA-3 '. To control the purity of the reaction, deionized water was used. The detection of amplifications was performed by electrophoresis in 1.5% agarose gel (TBE buffer, ethidium bromide) for 40 minutes. at an electric current of 60 mA. Used marker length of DNA fragments 50-1000 bp. (*Termo Scientific O'RangeRuler 50 bp DNA Ladder*). The PCR product had a molecular weight of 800 bp. The gel was visualized and photographed using the video system "*Mintron*" in ultraviolet radiation (wavelength 312 nm). To determine the species composition phytoplasma used two pairs of primers R16F2n / R16R2 and P1 / P7 [9, 10]. R16F2n: 5'-ACG ACT GCT AAG ACT GG-3 '; R16R2: 5'-TGA CGG GCG GTG TGT ACA AAC CCC G-3 '; P1: 5'AAG AGT TTG ATC CTG GCT CAG GAT T-3 '; P7: 5'-CGT CCT TCA TCG GCT CTT-3 '. The first amplification was carried out with a pair of primers P1 / P7, the second - with a pair of R16F2n / R16R2.

Research results. As a result of the phytosanitary survey of industrial grape plantations in the beginning of the summer, in the Odessa region, Chardonnay, Sukholimanskij White, Merlot, Riesling, Cabernet Sauvignon varieties, with symptoms grapevine leafroll disease were discovered, which, as it turned out, was later caused by phytoplasma, rarely – of the grapevine leaf roll -associated virus of grapes. When a virus was infected by leaf roll, as a rule, leaf blade rolling was moderate, with the presence of tissue sections without discoloration along the veins of the first to third orders (Fig. 1). The characteristic symptoms of phytoplasmic diseases are leaf curling, as a result of which the leaves take the shape of a triangle and, depending on the variety, change color from green to yellow to white-berry grape varieties and from green to red to dark-grape varieties (Fig. 2). In this case, the sugar in the berries decreased, the bushes differed slow development. The number of bushes with symptoms in individual varieties was significant. Manifestations of grape leaf roll varied depending on the nature of the disease (phytoplasma or virus), the affected variety, the survey period and the weather conditions of the current growing season.

Due to the similarity of symptoms for the differentiation of virus and phytoplasma diseases, it is necessary to conduct laboratory tests (Figs. 1, 2). On the bushes of the Cabernet Sauvignon variety,

symptoms of manifestation of short grapes in the form of shortening of internodes, suppression of growth, and almost total lack of harvest were found. Symptoms of the grape bois noire were observed on Chardonnay bushes in the form of uneven ripening of wood, cracking, the harvest was significantly reduced, the berries on the hand differed pea and reduced sugar content. Symptoms of virus diseases are the first step for diagnosis, but it should be borne in mind that they do not always correspond to the presence of the virus in these plants. Symptoms of crown gall of grapes in the form of tumors were found on the trunk of grape bushes, varieties Cabernet Sauvignon and Merlot (Fig. 3). From the bushes of grapes, had symptoms of virus, phytoplasma diseases and crown gall, plant material was selected for the diagnosis and identification of pathogens.

Fig. 1. A bush of grapes with symptoms of leaf roll, a variety of Sukholimansky white (Odesa region, 2017)

Note: similar symptoms were also observed on Cabernet Sauvignon, Chardonnay, Sukholimansky white.

Fig. 2. Grape bush with symptoms of phytoplasmic disease, Chardonnay variety (Odessa region, 2017)

Note: Similar symptoms were also observed on Cabernet Sauvignon.

Fig. 3. Grape bush affected by the causative agent of crown gall, Cabernet Sauvignon variety (Odessa region, 2016)

In Fig. 3 shows a Cabernet Sauvignon shrub affected by crown gall with a tumor on the trunk.

As a result of carrying out ELISA with samples of grape plants of varieties Cabernet Sauvignon, Merlot, Chardonnay, viruses were identified that cause disease, grapevine leaf roll-associated virus (1st and 3rd serotypes), grapevine fanleaf virus and rugose wood complex (grapevine virus B). Phytoplasma disease – bois noire- was identified on the Chardonnay variety (Fig. 4).

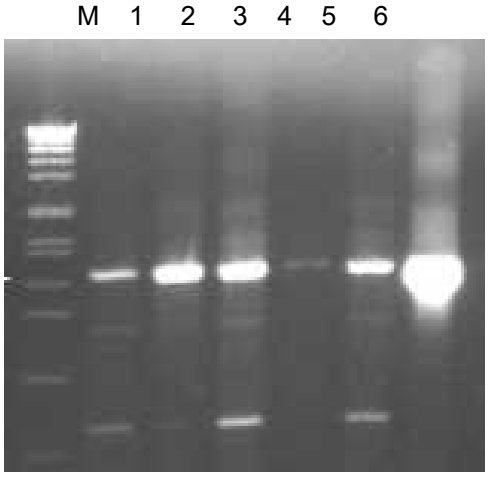

Fig.4. Electrophoregram of PCR amplification products of phytoplasma DNA: 1-6 positive samples of the Chardonnay variety, molecular weight marker of DNA fragments 50-1000 bp (Termo Scientific O'RangeRuler 50 bp DNA Ladder)

As a result of phytosanitary examination and identification of grapes pathogens, it was found that the highest percentage of disease incidence falls on the vineyards of the Ovidiopol district. The degree of infestation of vineyards by the causative agent of crown gall is higher in the Tatarbunary region, which may be related to the source of infected planting material, the varietal composition of the plantings and, to a certain extent, the climatic conditions of the regions.

Conclusions

As a result of a phytosanitary examination of vineyards in the Odessa region, symptoms of viral and phytoplasmic diseases as well as manifestations of crown gall were detected. Identification of pathogens

of virus iseases by ELISA showed that they are caused by viruses: grapevine leaf roll -associated virus (1st and 3rd serotypes), grapevine fanleaf virus and grapevine virus B of the Rugose Wood Complex. PCR with gel electrophoretic detection identified the causative agent of phytoplasmac disease – bois noire - on Chardonnay variety. The greatest degree of infestation by the causative agent of crown gall is found on the Cabernet Sauvignon variety. The largest percentage of vine plants affected by viral and phytoplasma diseases were found in vineyards of Ovidiopolsky district, a crown gall, both in Ovidiopol and in the Tatarbunary districts of the Odessa region. It has been established that about 10 percent of the grapevine diseases detected are viral diseases, about 25 - on crown gall and 65 percent - on phytoplasma diseases.

References

1. *Almeida R.P.P., Daane K., Bell V.* (2013). Ecology and management of grapevine leafrolldisease. *Frontiers in Microbiology*. V. 4, P. 94. doi:10.3389/fmicb.2013.00094

2. *Keldysh M. A.* (2003).Virusy, viroidy i mikoplazmy rasteniy. [Viruses, viroids and plant mycoplasmas]. Moscow: PFUR, 157 p. [in Russian].

3. *Lemanova N.B., Gatina E.Sh.* (1991). Bakterialnyye bolezni vinograda i plodovykh kultur. [Bacterial diseases of grapes and fruit crops]. Chisinau: Shtiintsa, 116 p. [in Russian].

4. *Martelli G. P.* (2012). Grapevine Virology Highlights: 2010–2012: *Proceedings of the 17th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG).* P. 13–31. October 7-14, 2012, Davis, California, USA. [in English].

5. *Vizitiu (Bălăşoiu) Diana, Dejeu L.* (2011).Crown gall (Agrobacterium spp.) and grapevine. *JOURNAL of Horticulture, Forestry and Biotechnology.* V. 15(1), P. 130- 138.

6. *Borhsenius S.N., Chernova O.A.* (1989). Mikoplazmy: molekulyarnaya i kletochnaya biologiya, patogennost, diagnostika. [Mycoplasmas: molecular and cellular biology, pathogenicity, diagnosis]. L.: Science, 156 p. [in Russian].

7. *Molecular biomarker analysis – General definitions and requirements for microarray detection of specific nucleic acid sequences European and Mediterranean Plant Protection Organization (EPPO)*. (2013). ISO 16578. http://www.eppo.org [in English].

8. *Esteban A., Engel E.A., Escobar P.F.* (2009). A diagnostic oligonucleotide microarray for simultaneous detection of grapevine viruses. *Journal of Virological Methods.* V. 2, Is. 163. P. 445–451. doi: 10.1016/j.jviromet.2009.11.009

9. *Lee I.-M., Gundersen D.E., Hammond R.W., and Davis R.E.* (1994). Use of Mycoplasmalike Organism (MLO) Group-Specific Oligonucleotide Primers for Nested-PCR Assays to Detect Mixed-MLO Infections in a Single Host Plant. *The American Phytopathological Society.* V. 84, P. 559-566. doi: 10.1094/Phyto-84-559

10. *Sahar A.Youssef, Yasmin Sayed, Osama S. Hassan, Gehan Safwat and A. A. Shalaby.* (2017). Universal and Specific 16S-23Sr RNA PCR Primers for Identification of Phytoplasma associated with sesame in Egypt. *International Journal of Advanced Research in Biological Sciences.* V. 4, Is. 7. P. 191-200. doi: 10.22192/ijarbs

11. *Avramov Z., Gillet J., Laginova M.* (2008). First Detection of Stolbur Phytoplasma in Grapevines (Vitis vinifera cv. Merlot) Affected with Grapevine Yellows in Bulgaria. *J. Phytopathology.* V. 156, P. 112 – 114. doi: 10.1111/j.1439-0434.2007.01339.x

12. *Haas Jerry H., Moore Larry W., Ream Walt, Manulis Shulamit*. (1995). Universal PCR Primers for Detection of Phytopathogenic *Agrobacterium Strains. Applied and Environmental Microbiology.* V. 61. № 8. P. 2879 – 2884.

13. *Konup L.A., Gayday A.E. , Milkus B.N.* (2001). Bakterialnyy rak vinograda v Ukraine. [Bacterial cancer of grapes in Ukraine]. *Visnik ONU. Vip. 4. T. 6. S. 181 – 183.* [in Ukrainian].