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Virus's diseases of grapes in the South Ukraine

A.I. Konup

National Scientific Centre
"Institute of Viticulture and Winemaking n.a. V.E. Tairov"

Viral diseases of grapes lead to large losses in all regions with developed viticulture, including - south Ukraine.

Phytosanitary selection - one of the ways to reduce the spread of viruses. In the European Economic Community bush vine clones tested for the following viruses: grapevine fanleaf virus (GFLV), grapevine leafroll associated virus (GLRaV 1-7), grapevine fleck virus (GFkV), grapevine virus A, grapevine virus B and lack of affinity, pitted wood LN-33. We know that the wine world each year loses about 10% of the crop of lesions viral diseases.

Visual phytosanitary control can not detect bushes with latent infection and prevent them from billet vines vegetative propagation of plants. Years virus diagnosis was based on the grafted variety indicator. However, this method requires several years of research. Appears necessary diagnostics using modern rapid serological and molecular - genetic analysis methods. PCR allows rapid in terms define infection vines phytopathogenic viruses [5, 6, 9, 11]. For detection of viral infections grapes used effectively ELISA method, which is also highly sensitive and specific method of diagnosis.

The aim of this work was to study some grapes for the presence of viral diseases.

During the period from 2010 to 2012 were conducted screening study 440 samples clonal material and ordinary grapes of different varieties for the presence of viruses following: grapevine fanleaf virus (GFLV); grapevine fleck virus (GFkV). Samples were taken in the vineyards of southern Ukraine.

To test the bushes grape clones in August - September selected upper leaves of plants. With the onset of cold weather virus isolation were carried out in the lignified shoots. Samples were transported to the laboratory and examined on the same day or stored at - 20 ° C for several months.

To test the grapes for viruses using ELISA diagnostic kits from the company "Agritest" and PCR analysis of different pairs of primers for detection of GLRaV 1- CPV / CPC; GLRaV-3 - C547 / H229; GVA - C995 / H587; GVB - C547 / H229; RSPaV -13/14; GFLV - oligoC1 / oligo V1; GFkV - RD1 / RD2.

The reaction mixture for reverse transcription and polymerase chain reaction (RT-PCR) volume 25 ml water containingu, 10 × PCR buffer (KCl 500 m, 100 m Tris-NCl, pH 9, O), sucrose (20%) and red krezolov 2 mM dezoksinukleozydtryfosfat (dNTP), 0,1 M ditiotryyetol (DTT), 10 pmol of each primer, 1,25 U Taq-polymerase ("AmpliSens", Russia), 8 U revertazy ("Amplisense", Russia), 1, 5 mM MgSO4 (for GLRaV-1 and GLRaV-3), 1.3 mM MgSO4 (for GFLV). In the reaction mixture was brought to 2 ml prepared sample.

Reverse transcription was performed at 52 ° C in an incubator for 30 minutes. Amplification consisted of 35 cycles (94 ° C - 30 seconds, 56 ° C - 45 seconds, 72 ° C - 60 seconds) and elongation time in the last cycle reached 7 minutes (Rowhani A., personal communication). For GLRaV-1 annealing temperature was lowered to 53 ° C, and for GFLV increased to 61 ° C.

The reaction is carried out in a programmable thermostat "Tertsyk" Company "DNA - Technology" (Russia). Electrophoresis was performed in 1.5 % agarose gels. Etidiy bromide for visualization of PCR products was part trysborat buffer for electrophoresis ("Amplisense", Russia). The gel was photographed using video "Samsung" UV - radiation (wavelength 312 nm).

To estimate the molecular weight fragments amplified using marker 800 - 100 base pairs ("Amplisense", Russia). grapevine fanleaf virus (GFLV) and twisting vine leaves grapes carried diagnosed by RT-PCR-Rt in real time. We used primers oligoC1 / oligoV1 for grapevine fanleaf virus (GFLV) and CPV / CPC for grapevine leafroll associated virus GLRaV 1. Based on the

nucleotide sequences of the genes were chosen primers and probes labeled with fluorescent markers FAM, JOE, that allow detection of fluorescence in real time. Amplification was performed according to the recommendations [9] in termotsykleri Rotor Gene 6000 (Corbett, Australia).

The Cabernet Sauvignon, Rhine Riesling, Merlot Rose, Chardonnay and assayed for virus infestation grapes. It was found that the bush Rhine Riesling, Chardonnay and Merlot Rose did not contain pathogens of viral diseases of grapes. Cabernet Sauvignon was grapevine leafroll associated virus GLRaV 1.

Diagnosis grapevine fanleaf virus by RT-PCR in real time gives a highest-quality and accurate results of PCR analysis replaces product PCR detection by electrophoresis. This does not make sense to open and allows amplification products that minimizes the risk of contamination of the laboratory PCR products. Automatically record results eliminates human error in interpreting the data.

Conclusions:

- the method of virus detection GFLV by RT-PCR detection of fluorescence in real time.
- as a result of research by ELISA and PCR revealed that latent viral diseases have affected planting material both imported and domestic production.
- a molecular genetic method for detecting viral pathogens.
- PCR method allows you to test a large number of test material in a short time.