

VIRUS AND PHYTOPLASMA DISEASES OF GRAPES. DIAGNOSTICS

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Testing of grapes in the laboratory is very important, because it allows you to avoid the spread of disease to new vineyards. In recent years, detection of virus diseases of grapes accompanied by labor-intensive and slow maturity biological tests. Today most dangerous viral diseases of grapes can detect rapid laboratory tests. In some cases, the disease manifest typical symptoms of grapes that can be easily identified in the field, but the most common symptoms that can be caused by several reasons, including physiological rejection.

In most viral diseases for characteristic symptoms occur only in determining the seasons, so in viral disease leaf rolling - reddening leaves of red varieties appears in late summer or autumn.

Most harmful and widespread among the diseases caused by Phytoplasma in Europe is golden yellowing vine and blackening wood vine. The two illness symptoms are very similar, their identification can be carried out only by laboratory molecular diagnostic methods.

Recently, the vineyards of Ukraine demonstrated a very dangerous disease caused by Phytoplasma. This disease is also common in Italy, France, Yugoslavia, Germany, and in the CIS up to 2004 are not recorded. Losses from this disease is very significant.

In 2004, she was found in the Odessa region on the sort of Chardonnay. This is the most sensitive variety to fitoplazmovoyi pathogen infection. Was the identification of the infection, and found that the disease is blackening wood and belongs to the trunk.

Diagnosis of dangerous diseases is an important task to prevent their spread. Our research has focused on the development of rapid and reliable methods for diagnosis of these diseases.

The importance of addressing these issues and led to the relevance of these studies.

The aim of this work was to study some grape varieties imported origin for virus and phytoplasma diseases of grapes.

The material for the study were seedlings Cabernet Sauvignon and Chardonnay varieties, producing Slovenia, Germany and France and Moldova.

Detection of viral antigens studied by enzyme-linked immunosorbent assay (ELISA) using test kits firms "Agritest" and by polymerase chain reaction with reverse transcription (RT-PCR). Virus isolation was performed in lignified shoots. Samples for PCR were prepared according Rowhani. The reverse transcription was carried out in a thermostat at 52 ° C for 30 minutes. Amplification consisted of 35 cycles (94 ° C - 30 sec, 56 ° C - 45 sec, 72 ° C - 60 sec), and elongation at the time of the last cycle reached 7 minutes. For GLRaV-1 in the study, annealing temperature was 52 ° C, and for GFLV - 60 ° C.

The reaction was carried out in a programmable thermostat "Tertsyk" Company "DNA - technologies". Electrophoresis was performed in 1,5% agarose gels. Bromide bromide to visualize the PCR products was part of the tris-borate buffer for electrophoresis ("AmplifySens").

To diagnose infection fitoplazmovoyi DNA isolation from woody shank performed by the method of N. Habili. To increase the yield of the product of PCR amplification was performed two because after the first visually PCR products was not observed.

The amplification with these primers consisted of 35 cycles of 95 ° C 3 min. - denaturation, 55 ° C 1 min. - annealing and 72 ° C hv.30 6 sec. - Elongation in a programmable thermostat "Tertsyk" Company "DNA - Technology". To control the purity of the reaction using deionized water. PCR products (5 ml) were subjected to electrophoresis on 1,5% agarose gel in Tris-Borate-EDTA buffer (TBE).

The study was set percentage of the bushes with latent infection among clones from vineyards Odessa area. In plantations Cabernet Sauvignon vineyards in the Odessa region as a

result of the studies found some samples infected by virus serotype 1 twisting on the plantations of the variety Chardonnay was identified phytoplasma disease - blackening wood.

This method has been audited a large part of the material promising clones that were recommended for further multiplication latent virus lesions twisting vine leaves . All tested bushes were free of the virus twisting vine leaves.

As a result of investigations by ELISA and PCR revealed that latent viruses affected leaves curl and GFLV is planting material production Moldova and Slovenia and identified blackening wood to a variety Chardonnay, made in France , Germany and Italy.

Thus, using modern diagnostic methods can prevent the importation of diseased plant material and its distribution.

Therefore,

- found that the most affected by the latent form of the virus twisting of leaves and grapes are GFLV planting material production Moldova;
- the use of modern diagnostic methods, such as ELISA and PCR allows a short time to detect and identify virus diseases of grapes and thus prevent their spread;
- research grape varieties Chardonnay plants revealed that he struck phytoplasma infection, such as blackening wood grape production in France, Italy and Germany.