

## DETECTION OF GRAPEVINE VIRUSES IN THE SOUTH UKRAINE USING ELISA AND RT-PCR

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Grapevine leafroll-associated virus 1 and 3 (GLRaV-1,3), grapevine fanleaf virus (GFLV), grapevine fleck virus (GFkV), grapevine virus A (GVA) and grapevine virus B (GVB) are widespread in the Ukrainian vineyards, resulting in major reductions in grape quality.

779 grapevine samples have been tested for the presence of latent viral infections. Grapevine samples were collected from South Ukrainian vineyards and from introduced imported planted material.

Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction with reverse transcription (RT-PCR) have been used for harmful grapevine viruses detection in the southern region of the Ukraine and in the planting material from Republic Moldova. These investigations allowed us to reveal and identify the next viruses: GFLV (2,3 – 18,9%), GFkV (16,9 – 30,2%), GLRaV-1 (3,3 – 43,4%), GLRaV-3 (3,3 – 62,3%), GVA (69,7%), GVB (81,8%).

Investigations of different grapevine cultivars for latent viruses presence revealed a high level of virus diseases infection (2,4 – 100%) due to low quality of grapevine planted material.

We revealed that both regular planted material (4,2 – 40,0%) and clones (0,75 – 13,8%) produced in the Ukraine and imported from the foreign countries were infected with virus diseases agents.

We have modified and optimized RT-PCR protocols to increase their sensitivity and specificity in order to provide a robust and cost-effective method to screen vineyards. Different concentration of magnesium ions were applied for decreasing of unspecific amplification products. Annealing temperatures ( $T_{an}$ ) also were investigated to improve the amplification results. The optimal  $T_{an}$  were established (53 °C for CPV/CPC primers; 56 °C – C547/H229, C995/H587, C410/H28; 60 °C – 13/14; 61 °C - oligoV1/oligoC1; 62 °C - RD1/RD2). Optimal  $Mg^{++}$  concentrations in reaction mixes (1,3 mM for diagnostics of GFLV and GFkV, 1,5 mM for diagnostics of the rest investigated viruses) were found out.

Vineyards free from grapevine viruses were indentified and recommended for grapevine planting material production.