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THE SPREAD OF GRAPEVINE VIRUSES ON THE SOUTH UKRAINE

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Screening investigations of 726 grapevine samples for the presence of grapevine leafroll-associated viruses (GLRaV-1 and GLRaV-3) grapevine fleck virus (GFkV), grapevine fanleaf virus (GFLV), grapevine virus A (GVA), grapevine virus B (GVB), Rupestris stem pitting associated virus (RSPaV) have been conducted. The grapevine material selected from vineyards of the southern regions of Ukraine was tested by enzyme-linked immunosorbent assay (ELISA-test) and reverse transcription - polymerase chain reaction (RT-PCR). Using both these methods data about the spread of latent viral infections on the vineyards were obtained. The protocol of polymerase chain reaction was optimized during the investigation. GLRaV 1, GLRaV 3, GFLV and GFkV were revealed. Our data indicate the necessity of grapevine planting material certification in Ukraine.

Introduction. Viral diseases of grapevines in Ukraine were firstly investigated and described in 1971 [11]. Since then, more viruses have been found: grapevine leafroll associated virus 3 (GLRaV-3), GLRaV-1, grapevine fleck virus (GFkV), grapevine fanleaf virus (GFLV), grapevine vein mosaic virus, grapevine vein necrosis virus, grapevine stem pitting virus.

Grapevine fanleaf virus, grapevine leafroll associated viruses 1-7, grapevine fleck virus, grapevine virus A, grapevine virus B, Rupestris stem pitting associated virus are the most harmful grapevine pathogenes. They are included by European Community to the list of viral infections that should be tested in certified grapevine planting material production [13]. Unfortunately, propagation planting material (especially regular material) may contain the pathogenic viruses.

Viral infections cause great losses to grapegrowing. Suppression of growth of root system, shoots, leafs, berries and pollination process may occur. Also viral diseases negatively affect the metabolizm and cause the pigmentation of different plant organs (Fig. 1). Viral infections often proceed in latent form [3].

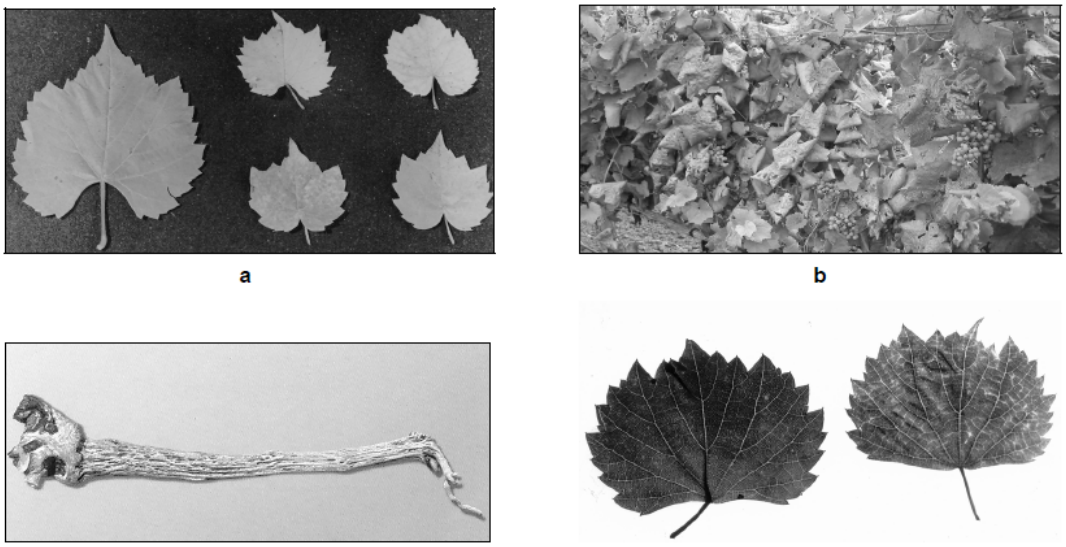

Fig. 1. Various symptoms of viral diseases of grapevine. a: Healthy grape leaf (left) and yellowed stunted leaves of *V.riparia x V.rupestris* 101-14 indicator (right) infected with grapevine fanleaf virus. b: Typical symptoms of grapevine leafroll. c: Typical symptoms of grapevine stem pitting on *V.rupestris* du Lo indicator. d: Stunted growth and vein chlorosis (right) on the leaf of *V.rupestris* du Lo indicator caused by grapevine fleck virus.

The most effective method for viral diseases prevention is a production of virus free grapevine material that can be used for the planting of new vineyards. So early diagnostics of viral diseases allows to define planting material quality quickly. The most sensitive and specific diagnostic methods are enzyme-linked immunosorbent assay and reverse transcription - polymerase chain reaction [2, 12].

Certification of grapevine planting material in Ukraine is actual now [5].

The aim of our work was to investigate the spread of grapevine viruses on the South Ukraine.

Materials and methods. The investigated vineyards are located in the southern regions of Ukraine. During the investigations the certified and regular grapevine planting material of different scion and rootstock cultivars (Pinot noir, Cabernet Sauvignon, Merlot rouge, Chardonnay, Riesling, Aligote, Sauvignon vert, Muscat tzitronny, Verdelho, Bastardo magaratchsky, Ranny Magaratcha, Perlinka, Ay-Petry, Pervenetz Magaratcha, Antey Magaratcha, Haydamack, Kafa, Muscat Livadia, Tcheurnaya apiana, Tzitronny Magaratcha, Chabache, Strashensky, Vostorg, *V.berlandieri* x *V.riparia* Kober 5BB, *V.riparia* x *V.rupestris* 101-14) were tested by enzyme-linked immunosorbent assay and polymerase chain reaction with reverse transcription.

Infected plants kindly donated from University of Bary (Italy) were used as positive controls.

Viral antigens detection was conducted by ELISA using test-systems "Agritest" (Italy). Viral RNA was detected by RT-PCR. Cambial scraping samples, leaves, and petioles were used. Templates and reaction mixtures for RT-PCR were prepared according Rowhani et al. [9]. The following pairs of primers were used: CPV and CPC for GLRaV-1 detection [8], oligo C1 and oligo V1 for GFLV detection [10], C547 and H229 for GLRaV-3 detection [7], RD1 and RD2 for GFkV detection [1], C410 and H28 for GVB detection [7], and C995 and H587 for GVA detection [6, 7] were used.The concentrations of Mg^{++} 1,3 mM for GFkV and GFLV detection and 1,5 mM for GLRaV-1, GLRaV-3, GVA, GVB detection were used. All the reagents were supplied

from "AmpliSens", Russia, except reverse transcriptase from "Fermentas" (Latvia). Lithuania. RT-PCR was performed in termocycler "Tercik" ("DNA-Technology", Russia). Reverse transcription was carried out during one hour at 42ºC followed by 35 cycles of PCR according to Rowhani et al. [9]. The annealing temperature for the primers to genome sequences of GLRaV-3, GLRaV-1, GVA, GVB viruses was 56 ºC [4]. For the primers to GFkV genome sequences, the annealing temperature was 62ºC, and for GFLV the annealing temperature was 61ºC.

Amplicons were analysed in 1,5% agarose gels. The marker 800-200 bp ("AmpliSens", Russia) was used. The buffer for electrophoresis ("AmpliSens", Russia) contained ethidium bromide. Gels were vizualised by UV transilluminator and photographed using a "Samsung" video system.

The obtained data were statistically analyzed by applied program "Statistica 6.0".

Results and Discussion. The results of viral disease diagnostics in vineyards of Odesa region showed that 24,0 % of plant samples were latently infected by grapevine fleck virus (Fig. 2).

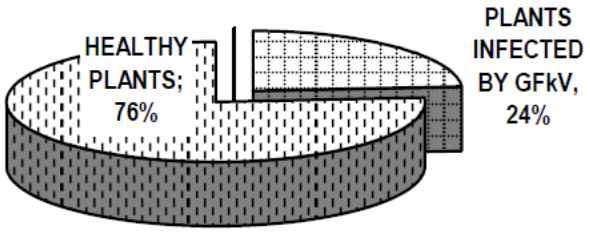

Fig. 2. Results of detection of grapevine viruses in some Odesa region vineyards.

We also revealed four latent viral infections in grapevine planting material from Crimea. The highest level of infection was registered for the grapevine leafroll associated virus: 1 – 24,6 %. Grapevine fleck virus was identified in 16,9 % of tested samples. Grapevine fanleaf virus and grapevine leafroll associated virus 3 were revealed in 2,3 % and 3,3 % correspondingly (Fig. 3).

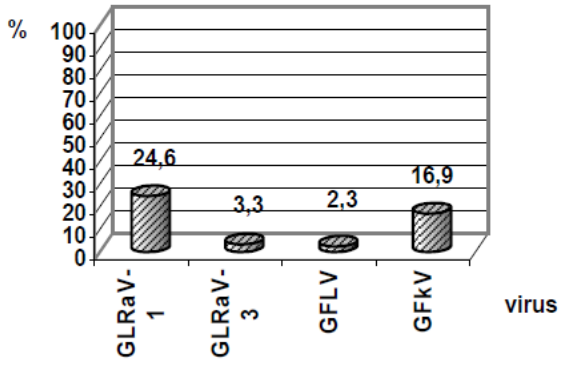

Fig. 3. Results of detection of grapevine viruses in some Crimea vineyards.

Grapevine plantations in Kherson region also were tested for the presence of grapevine viruses. During the investigations we revealed grapevine leafroll and grapevine fanleaf diseases. Grapevine leafroll associated virus 3 was identified more often than other viruses (6,7 % of tested grapevine samples were infected by this virus). Grapevine plants were infected by grapevine fanleaf virus and grapevine leafroll associated virus 1 in 3,3 % cases (Fig. 4).

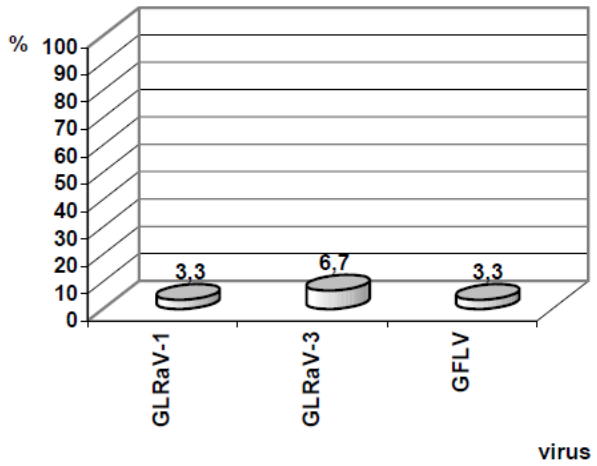

Fig. 4. Results of detection of grapevine viruses in some Kherson region vineyards.

Thus, we determined different levels of grapevine viruses contamination on the south Ukraine vineyards. In some vineyards viruses are widely spread. Such high levels of contamination are explained by non-satisfied quality of planting material.The obtained data suggest the necessity of viral diseases control and introduction of cultivar certification system in our country.

Conclusions. 726 grapevine samples from south Ukraine vineyards were tested for the presence of latent viral infections.

Enzyme-linked immunosorbent assay and polymerase chain reaction with reverse transcription have been used for harmful grapevine viruses detection. Our investigations allowed us to reveal and identify the next viruses: grapevine fanleaf virus (2,3 – 3,3 % of tested plants), grapevine fleck virus (16,9 – 24,0 %), grapevine leafroll associated virus-1 (3,3 – 24,6 %), grapevine leafroll associated virus-3 (3,3 – 6,7 %).

For the first time in Ukraine the harmful grapevine viruses were detected by PCR based technique.

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

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