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The Presence of GVA and GVB Viruses in the Most Widespread Grape Varieties in Kosovo

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ABSTRACT: Viral diseases appear in vineyards with a variety of signs, depending on the cultivar, the virus strain, the specific environmental conditions or the combination of the rootstock. The study was conducted in Kosovo during 2017 in the Rahovec, Suhareka and Prizeren regions. 300 samples were taken from these regions and mainly in the varieties Vranac, Smederevka, Prokupa, Afuzali, Muskat Hamburg, Game and Italian Rizling which were analyzed for the GVA and GVB viruses. These vine samples were tested with ELISA test following the protocol of Protein (A-DAS ELISA)and Direct binding ELISA. The ELISA technique showed that the GVA virus was present in all grape varieties and in the three regions of Kosovo, while the GVB virus was present only in the Procupa variety and only in the Rahovec region. In these samples, GVA infection resulted in 14%, while GVB infection resulted 1% in the Procupa variety in the Rahovec region, while other vine varieties were found to be uninfected by this virus.
KEYWORDS: ELISA- test, GVA, GVB, RT-PCR

I. INTRODUCTION

Rahovec has very favorable conditions for a quality grape production and grape products. Today, vineyards and wine production have become one of the most strategic sectors for Kosovo. Historically, Kosovo has been a wine exporter and today not only meets its domestic needs, but also successfully exports to the markets of the region and around the world [3]. In the territory of Kosovo are estimated to be 4,965 vine-growers who cultivate grapes on a surface of 3217.45 hectares, where varieties for wine production are cultivated on an area of 2538 hectares, table grapes on a surface of 677 hectares and grapes for drying in an area of 2.45 hectares [8].

Viral diseases appear in vineyards with a variety of signs, depending on the cultivar, the virus strain, the specific environmental conditions or the combination of the rootstock [6] Grapevine virus A (GVA) is the type species of the genus Vitivirus in the family Flexiviridae [9]; GVA has a filamentous flexuous particle, and is 800 nm in length, and 11–12 nm in diameter [2]. It is associated with rugose wood (corky bark) symptoms in grapevine. The production and use of certified virus tested propagative material reduce the inoculum potential, mainly in areas where vectors are present [9]. GVB is transmitted from grape to grape by several species of the pseudococcid mealybugs genera Pseudococcus and [7]. It can be inoculated from grapevines to Nicotiana species mechanically or by insect vectors [1] and may be acquired through Cuscuta spp. (dodder) [5].

II. MATERIALS AND METHODS

In order to pursue the research objectives and to improve the organization of the future work, collecting information on the following topics appeared necessary:

- Geographical distribution of grapevine in the country
- Varieties cultivated, their characteristics and importance
- Origin of material (local or imported)
- Principal disease problems of the crop

To establish the number of grapevine samples to be collected for each variety and area of cultivation, the following information was taken into consideration: distribution of the crop in the country, surface and economic importance of the different varieties in each region, their types and origin, etc.

The study was carried out in Kosovo during 2017 in the Rahovec, Suhareka and Prizeren regions, the two regions where most of the vineyards are located. Varieties Vranac, Smederevka, Procupa, Afuzali, Muskat Hamburg, Game and Italian Rizling were analyzed for GVA and GVB viruses. A 300 samples were collected in total and divided 180 in Rahovec, 70 in Suhareke and 50 in Prizeren with suspicious symptoms, which were

brought to the laboratory to analyze and make the final identification. These samples were tested with ELISA test.

Grapevine samples were collected during December 2017 from commercial vineyards. Each sample consisted of 3-4 cuttings, 30-40 cm in length. Samples were stored at 4°C till tested.

Two different ELISA procedures were used in this study:

- Protein-A DAS ELISA
- Direct binding-ELISA

All collected samples were analyzed by ELISA to check for the presence of the following viruses:

Vitiviruses: *Grapevine virus A* (GVA)
Grapevine virus B (GVB)

ELISA test

Protein (A-DAS ELISA)

For GVA analysis, a pre-sensibilization of the plate with protein A, which has an high affinity to the (Fc) fraction of the IgGs, is necessary before coating of antibodies. This induces the orientation of IgGs with active site, optimizing antigen binding [1] The normal DAS-ELISA procedure is followed after that.

Direct binding ELISA

For GVB analysis the grapevine sample is loaded directly in the well, without a preventive sensibilization with antibodies. The procedure continues as a normal TAS-ELISA test, by adding monoclonal antibodies in PBS and antimouse enzyme-linked antibodies.

III. RESULTS AND DISCUSSION

Collected samples with suspicious signs for viruses GVA and GVB underwent serological laboratory tests, ELISA test where polyclonal antibodies were used and resulted that GVA virus is present in all varieties obtained: Vranac, Smederevka, Procupa, Afuzali, Muskat Hamburg, Game and the Italian Riezling, which were taken in all three of the above mentioned regions. Of the 300 samples analyzed, 42 samples were infected with GVA (14%), while GVB virus is present only in the Procupa variety, which resulted infected with this virus, while other varieties were negative or uninfected. Of all the samples analyzed for this virus, the infection resulted 1% with GVB, and mainly for Rahovec region, from where samples were taken.

Nr. of samples	**Varieties**	**Viruses**	
		14% GVA	**1% GVB**
60	Vranac	4	0
60	Smederevka	11	0
60	Prokupa	7	3
60	Afuzali	8	0
20	Muskat hamburg	6	0
20	Game	2	0
20	Italian riesling	4	0

In total, 300 samples were analyzed, out of which 42 samples (14%) were infected with the GVA virus, while only 3 samples (1%) were infected with the GVB virus.

The Rahovec, Suhareka and Prizeren communities resulted in the presence of GVA virus in varieties Vranac, Smederevka, Prokupa, Afuzali, Muskat hamburg, Simple Game and Rizling Italian.

The municipality of Rahovec resulted in the presence of the GVB virus in the varieties Procupa, while varieties Vranac, Smedereka, Afuzali, Muskat, Hamburg, the simplest Game and Risling Italians are free from this virus.

The GVA virus is most common in Kosovo and was found to be present in all three municipalities and in the 7 varieties we studied. The GVB virus is not very widespread, as it resulted only in Rahovec / Orahovac municipality, in Procupe cultivar.

Starting from these viral diseases affecting the vineyards, we think that to increase their lifespan, a series of control measures will be undertaken which will increase the plant's virulence security against viruses:

- Certification of planting material to produce free material from viral diseases.
- Control of vectors.
- Reduce the number of infected blocks
- Control the new blocks on the presence of the nematodeX.index vector, since when this vector is not detected, no blockhead investment should be made.

- Continuous monitoring in vineyard blocks will make it possible to limit viral diseases

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