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Some Of The Viruses In Grape Cultivars In Albania

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***Abstract*— The grapevine is affected by a number of viral diseases, or similar pathologies, which significantly affect the lifespan of the plants and their production. Viral diseases appear in vineyards with a variety of signs depending on the cultivar, the strain of the virus, the specific environmental conditions or the combinations on and the rootstock. In this study random samples were taken to determine the of GFLV, GLRV, GFkV, GRWV, GVA, GVB in the cultivars Kallmet, Shesh i Zi, Shesh i Bardhë, Afuzali, Merlot, Kardinal, Muskat d' Ada with the ELISA test in several districts such as Durres, Elbasan, Lushnje, Vlore, Shkoder, etc. The sampled plants were extracted in the laboratory and analyzed by ELISA test. The results showed the presence of GFLV, GLRV, GFkV, GRWV, GVA and GVB viruses in the analyzed plants, according to the DAS-ELISA protocol. Taking some indirect measures will limit the viral diseases that we have found in our vineyards. This will make possible a safe perspective for the development of viticulture in our country.**

Keywords—Das Elisa protocol, viral disease, cultivar, viticulture

I. INTRODUCTION

Vine, this culture of ancient as humanity itself, is hard not to find present in all the rural dwellings of the entire surface of the country. Grapes have an important nutritional and dietary function, while wine with the relevant phenols in its composition affects the fight against cancer and reduces coronary attacks by 40%, so present in today's heart diseases [4]. The large quality production of grapes is created in vineyards and pergolas. The vine constantly asks you to stay close and monitor it in a systematic way. The vine is a venerable plant at all times, which gives grapes life-giving and to many processings, where wine and brandy stand out, along with jams, juices, which so much grace celebrations, rituals, health [2]. The grapevine is affected by a number of viral diseases, or similar pathologies, which significantly affect the lifespan of the plants and their production. Viral diseases appear in vineyards with a variety of signs depending on the cultivar, the strain of the virus, the specific environmental conditions or the combinations on and the rootstock [5]. Being that the vine is a plant with special economic values for our country, the protection of the vine from diseases and pests is one of the most important segments of the annual work that the vine itself requires [8]. More than 70 viruses infect grapevines world- wide. Grapevine virus diseases can ruin crops and inflict great costs to winegrape producers because of the detrimental impact on vine health, productivity, the quantity and quality of berries, and the quality of the finished wines [3]. Vine culture is affected by a series of diseases that, according to the type of the cause, are classified into parasitic diseases and physiological diseases. From an economic point of view, vine diseases have different importance, which actually depends on the frequency of disease occurrence within years and between them, on the intensity of the impact and the degree of damage they cause in production, and on the other hand, on the cost and difficulty of implementation of control measures [9].

II. MATERIALS AND METHODS

A. Place of study

The study was conducted during the year 2020-2021 in the vineyards planted with vines in the areas of Durres, Elbasan, Lushnje, Vlore. Cultivars were present in these vineyards; Kallmet, Shesh i Bardhe, Shesh i Zi, Afuzali, Merlot, Cardinal, Muskat d'Ada,

B. Symptom monitoring and sampling.

We can say that the same plant should be observed at different times of the year for the appearance of different diseases. For the inspection of the vine, we must look in the field in the spring-early summer for the inspection of diseases caused by nepoviruses (short knots or infectious degeneration of the vine) and in the fall for those caused by closteroviruses or ampeloviruses (twisting of the leaves of the vine). The presence of symptoms usually coincides with the maximum concentration of the pathogen in the plant tissues, and for this reason this is the most suitable moment for taking samples for laboratory analysis [1].

With the detection of suspicious symptoms, samples were immediately taken and put in plastic bags which were brought to the laboratory where they were stored in a refrigerator at a temperature of 4°C until further examination with the ELISA method.

ELISA – procedure (Enzyme-linked Immunosorbent Assay)

This technique is one of the most used techniques, which is at the same time the most used diagnostic technique in plant virology, since the serological reaction is evidenced through an enzymatic reaction.

These viruses were tested by DAS-ELISA using a covering antibody (immunoglobulin G) and an antibody conjugated to the enzyme alkaline phosphatase. Plates were read in an ELISA reader at a wavelength of 405 nm. A sample was considered positive when the average value of absorbance of 405 nm radiation in the ELISA reader of both replicates exceeded three times that of the average of the negatives of the kit

1. Sensitization of the plate

Dissolve the first antibody in Coating buffer at a ratio of 1 : 200. For each well of the plate, 100 µl are added.

The plate is incubated at 37 ºC for 4 hours

Washing after incubation 3 times for 3 minutes with washing buffer.

2. Preparation and distribution of samples

The material is homogenized in the presence of extract buffer. For each well, 100 µl is discarded.

Incubate at 4 ºC for 16 hours.

Washing after incubation 3 times for 3 minutes with washing buffer.

3. Preparation and distribution of the conjugate

The second antibody is dissolved in conjugate buffer in the ratio 1:200.

100 µl are discarded for each well.

The plate is incubated at 37 ºC for 4 hours

Washing after incubation 3 times for 3 minutes with washing buffer.

4. Preparation and distribution of the substrate

Dissolve the substrate pNPP (penitrophenylphosphate) in buffer substrate at a ratio of 1 mg to 1 ml.

100 µl are discarded for each well.

The Elisa plate is incubated at room temperature and a yellow color reaction is expected to appear in the case of a positive result, when the sample is viral. The result of the analysis is expected in 30-60 minutes.

Read at 405 nm with (ELISA Reader)

III. Results and Discussion

For the diagnosis of these viruses, the serological method was used, which is based on the use of specific antibodies that are able to identify a certain virus. The obtained samples were sent to the laboratory undergoing the DAS-ELISA test and using a universal set of antibodies. The Das-Elisa test for these viruses shows a positive serological reaction and the concentration of the virus in plant samples. The contents of the wells were read at 405 nm using Elisa Reader. Microplate reading was done after 30-60 minutes of standing at room temperature. The examinations carried out showed the presence of GFLV (Grapevine fanleaf virus), GLRV (Grapevine leafroll virus), GFkV (grapevine flack virus), GRWV (Grapevine rugose wood virus) GVA (Grapevine virus A), GVB (Grapevine virus B) in the analyzed samples and proved that the plant samples are infected. The test results showed the concentration of viral antigens in these plant samples which revealed the presence of the virus, the cause of viral diseases.

Table 1 Spread of vine viruses in these grape cultivars

No	Varietes	GFLV	GLRV	GFkV	GRWV	GVA	GVB
1	Afuzali	+			+		
2	Shesh i zi	+	+				
3	Shesh i bardhe	+					
4	Merlot				+		
5	Kallmet		+	+	+	+	+
6	Kardinal				+		
7	Muskat d'Ada						
8	Italia				+		

IV. Conclusion

For the control of viral diseases in the vineyard, we do not have direct measures, but they have to do with labeling the affected plants in the field and excluding them from further multiplication.

Control of Xiphinema *index* nematode vectors.

Planting resistant cultivars

The certification of the planting material, going through several stages until receiving the pure planting material, such as:

Clonal and health selection in vineyards

The use of thermotherapy and the culture of the tip meristem, etc.

Taking these measures will limit the viral diseases that we have found in our vineyards. This will make it possible to produce seedlings with a safe perspective for the development of viticulture.

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