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Diagnostic of *Grapevine fanleaf virus* (GFLV) on Durres district, Albania

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***Abstract*—*Grapevine fanleaf virus* (GFLV) is the viral disease of the most known and widespread in the world. *Grapevine fanleaf virus* (GFLV) is a plant pathogenic virus of the family Secoviridae. It infects grapevines, causing chlorosis of the leaves and lowering the fruit quality. The study was conducted during 2018 in some areas of the Durres district, in the Rrashbull, Synej, Arapaj, Ramanat area. The cultivares taken in the study are: Cardinal, Sheshi i bardhe, Shesh i zi, Moscato D' Amburgo, Regina, Tajke e kuqe, Merlot, Trebbiano toskano, Italia, Afuzali, Perlet which are widespread in this area. The samples were brought to the lab and tested with the Das Elisa test and showed that samples taken in the Rrashbull and Arapaj areas are infected with the GFLV (*Grapevine fanleaf virus*). By testing the varieties of the grapevine resulted that Sheshi i bardhe, Sheshi i zi and Afuzali varieties were infected with GFLV. While other varieties as Cardinal, Muckat D' Ambourg, Regina, Tajke e kuqe, Merlot, Trebbiano toskano, Italia, Perlet were clear of this virus.**

***Keywords*—*Grapevine fanleaf virus*, cultivares, Das-Elisa test**

I. INTRODUCTION (*Heading 1*)

Grapevine fanleaf virus (GFLV) is one of the most known and widespread viral disease in the world. It is caused by the GFLV virus belonging to the nepovirus group and consists of isometric particles with diameter 28-30nm [5].

The nepovirus group is one of the most rapidly expanding taxonomic groups of plant viruses [6]. Several of the European nepoviruses (GFLV, ArMV, TBRV, GCMV) have distorting and chromogenic strains that induce malformation of leaves and canes or chrome yellow discoloration of the foliage [7]. This is one of the most widespread viruses in the vineyard cultivation sites that causes a lot of damage as it results in the degeneration of the vine. Other viruses belonging to the nepovirus group such as Arabian mosaic virus (ArMV), Strawberry latent ringspot virus (SLRV), Rasberry ringspot virus (RRSV), Tomato black ring virus (TBRV), have a limited spread and their damage in the vineyard is still not confirmed as in the case of the *Grapevine fanleaf virus* (GFLV), which causes infectious degeneration to grapevine [4]. In addition to the bad form, the leaves are not symmetrical, with irregular ribs and pronounced yellowing. The slabs have the closest nodes and have rickety growth. Production and quality of grapes are always on the decrease. Healing from this virus of these vines is almost impossible, in addition to helping for a better growth through abundant fertilizers, scarce soil work, good protection from parasites [1]. The virus has many stems and causes various symptoms with high intensity [5]. *Grapevine fanleaf virus* (GFLV) is a plant pathogenic virus of the family Secoviridae. It infects grapevines, causing chlorosis of the leaves and lowering the fruit quality. Because of its effect on grape yield, GFLV is a pathogen of commercial importance. It is transmitted via a nematode vector, Xiphinema index. This nematode acquires the virus through feeding on the root of an infected plant, and passes it on the same way [3]. In the first syndrome, infectious malformations, the vines may be stunted or show reduced vigor. There are two distinct syndromes, or sets of symptoms, depending on the virus strain and host response to infection. Leaves are severely distorted, asymmetrical, cupped and puckered, and exhibit acute dentations [8].

II. MATERIALS AND METHODS

A. Place of study

The study was conducted during 2018 in some villages of the Durres district which are Rrashbull, Synej, Arapaj and Ramanat. The vineyards taken in the study are 5-15 years old. Varieties monitored in Rrashbull are: Cardinal, Sheshi i bardhe, Sheshi i zi, Muscat D' Ambourg, Regina, Tajke e kuqe, Merlot and Trebbiano toskano. In Synej it was monitored only Italia variety. Varieties monitored in Ramanat are Sheshi i zi and Sheshi i bardhe, while in Arapaj are Afuzali, Perlet and Muscat D' Ambourg.

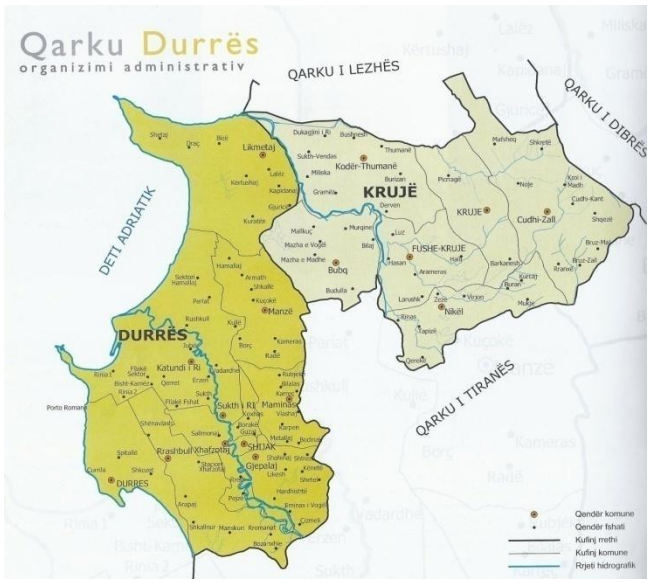


Fig. 1. *Map of Durres region*

B. Taking samples

The samples were taken in the period May-June to observe the disease caused by nepoviruses such as *Grapevine leafroll virus* (GLRV) and were brought to the laboratory where they were stored in the refrigerator room in 4°C until conducting laboratory tests for the final diagnosis of the viral pathogens with Das Elisa test.

C. Materials and reagents used for the Elisa test

Elisa's sponge, 8x12-dimensional plastic material with 96 pieces.
Micropipeta with 1, 4, 8 channels.
Scheme to record the data obtained in the Elisa Spit pipettes, cylinders, erlenmeyer flasks, beakers, etc.

D. Buffer solution

1. Physiological buffer
in PBS (1x) -pH 7.4 per 100 ml of distilled water
2. Washing buffer
3. Buffer solution for sensitization of plates in coating buffer - pH 9.6 per 100 ml of distilled water
4. Solution to titrate the plant material extraction buffer pH 7.4 per 1000 ml
5. The substance-related solvate (p-nitrophenyl phosphate), on which the enzyme acts and gives the reaction
Substrate buffer pH 9.8 per 1000 ml of distilled water
6. Solution blocking. At the end of the test, when necessary, block the hydrolysis of enzyme-catalyzed sustrat.

NaCl	18g
KH_2PO_4	0.2g
Na_2PO_4	1.15g
KCl	0.2g
NaN_3	0.2g
Tween 20	0.5ml
NaN_3	0.2g
Na_2CO_3	1.59g
$NaHCO_3$	2.93g
Polyvinilpyrolidone (floeme)	
Tween 20 0.5ml ne PBS (1 x)	
BSA (Bovin serum albumin)	2g
Diethanolamine	97ml
NaN_3	0.2g
NaOH	3M

E. Technique used

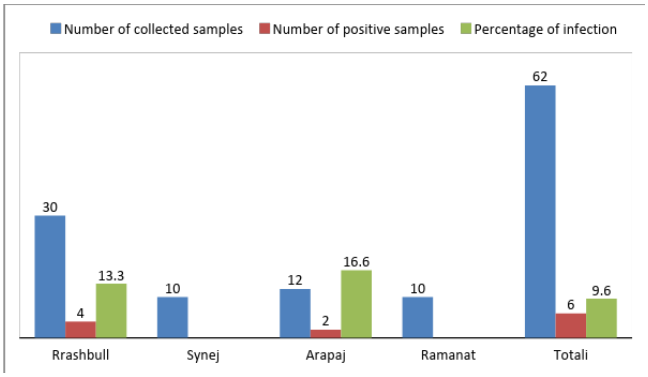
The procedure Das Elisa test passes through these stages:
1. Polyclonal antibodies are placed on Elisa plate, which are polystyrene and block antibodies during the diagnostic procedure.
2. Placement antigen
3. Placement of the polyclonal antibody associated with the enzyme
4. Placement of the substrate, p-nitrophenyl phosphate, which in the presence of the enzyme develops a colorimetric reaction
5. Reading of colorimetric reaction values

III. Result and Discussions

Samples taken in family courtyards and gardens near the houses were tested for GFLV virus. The yellow spots at the end of the test for the samples of Rrashbull and Arapaj showed a positive serological reaction, and proved that these plant samples are infected with GFLV (*Grapevine fanleaf virus*).

TABLE I.

Regions where samples were taken	Number of collected samples	Number of tested samples	No. of positive samples (infected)	Percentage of infection (%)
Rrashbull	30	30	4	13.3
Synej	10	10	-	-
Arapaj	12	12	2	16.6
Ramanat	10	10	-	-
TOTALI	82	82	6	9.6



From tests done on the varieties of the vine resulted that Shesh i bardhe, Shesh i zi and Afuzali varieties were infected with GFLV. While other varieties such as Cardinal, Muscat D'Ambourg,

Regina, Tajke e kuqe, Merlot, Trebbiano toskano, Italia, Perlet resulted to be free of this virus. This result is also presented through the following table.

TABLE II.

	Varieties of grapevine	Virus testing *Grapevine leafroll virus*	Test results infected + uninfected -
1	Cardinal	GFLV	-uninfected
2	Shesh i bardhe	GFLV	+infected
3	Shesh i zi	GFLV	+infected
4	Muscat D'Ambourg	GFLV	-uninfected
5	Regina	GFLV	-uninfected
6	Tajke e kuqe	GFLV	-uninfected
7	Merlot	GFLV	-uninfected
8	Trebbiano toskano	GFLV	-uninfected
9	Italia	GFLV	-uninfected
10	Afuzali	GFLV	+infected
11	Perlet	GFLV	-uninfected

IV. CONCLUSIONS

The discovery of infection was achieved through monitoring and appropriate measures were taken to eradicate and limit the spread of the disease.

Application of Elisa test has enabled the final identification of the GFLV viral pathogen.

From testing the varieties of the grapevine resulted that Sheshi i bardhe, Sheshi i zi and Afuzali varieties were infected with GFLV. While other varieties as Cardinal, Muckat D' Ambourg, Regina, Tajke e kuqe, Merlot, Trebbiano toskano, Italia, Perlet were clear of infection.

The existence of this viral disease makes it possible to take all necessary measures to limit its spread, as this disease is considered high risk for grape vines.

Successful implementation of all preventive measures will make it possible to limit the infection and allow safe development of viticulture.

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