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Elisa Test for Determination of Grapevine Viral Infection in Rahovec, Kosovo

[1]Lumta Dida,[2]Dhurata Shehu,[3]Thanas Ruci
[1] *University Of Prishtina, Faculty Of Agriculture, Kosovo*
[2] *Plant Protection Department, Faculty Of Agriculture And Environment Kodër- Kamëz, Tiranë*
[3]*Plant Protection Department, Faculty Of Agriculture And Environment Kodër- Kamëz, Tiranë*

Abstract: Vineyard in Kosovo is estimated to have a great economic potential. There are thousands of hectares of vineyards that contribute to the economic potential of Rahovec by expanding the cultivation area year by year. The vines are affected by a number of viral diseases or pathologies similar to them, which significantly have an impact against the plant life and their production. Therefore, this study was conducted in several farms in Rahovec to determine whether there is a presence of viral infection in the vines. Application of Das-Elisa, Protein A-DAS and Antigen Direct Binding - DASI verified the final identification of viral infection in the collected material. The yellow colour reaction shown on the plate showed the positive result of the Elisa assay for viruses GFLV, ArMV, GLRaV-1, GLRaV-2, GLRaV-3, GVA and GVB in varieties Vranac, Smederevka, Prokup, Afuzali, Grocaka, Demir Kapi, Plovdina, Melika, Zhillavka. The use of specific antibodies will enable the examination of viral diseases in plant materials collected from vineyards and will be oriented to their phytosanitary status.

Keywords: Antigen Direct Binding specific antibody, Das-Elisa test, Protein A-DAS, grapevine, viruses

I. INTRODUCTION

Vineyard in Kosovo is considered to be a great economic potential for the country. Vineyards are related to the culture and civilization of the population of this area. There are thousands of hectares of vineyards that have the potential for Rahovec area to expand its surface [1]. The citizens of Rahovec had their own vineyard husbandry. Vineyards show a high level of emancipation and civilization of this country that seems to have inherited the nobility of an Illyrian tradition. Ernest Hemingway had said that the level of development of viticulture and wine growing is the greatest signs of the civilization of a country's life and society (K. Rahovec Harvesting grape festival). The investment for establishing the new vineyards is a technical activity which is set to emerge by designing of the project and its implementation. The creation of new blocks within vineyards and the planting of a vineyard plot bring forth a lot of technical and organizational problems which can be solved only after the specialist maps the technical project. Proper determination of all the technical components in the vineyard construction project facilitates the work on its erection and guarantees the success of the production of grapes and Rakia wines, etc. with the greatest fruitfulness [1]. From numerous studies on viticulture, it results that the vine plant in the vineyards is affected by some viral diseases, for which their determination is made through the biological test in the indicative plants as well as by the Elisa method. More than 70 infectious agents among viruses, viroids and phytoplasmas have been recorded from grapevine [2]. Viral diseases appear in vineyards with a variety of signs, depending on the cultivar, the virus strain, the specific environmental conditions or the combinations on the subcutaneous [3]. In Kosovo the phytosanitary situation of vineyards looks similar to that in Albania. A recent study has shown the presence of the following viruses: GLRaV 1, GLRaV 3, GFkV, GFLV, GVA, while there are no studies on the presence of phytoplasma [4]. The productive life of the vineyards can be shortened and the quantity and quality of the crop badly affected. [5] Prevailing disease agents are viruses transmitted by nematodes (nepoviruses and Strawberry latent ringspot virus), pseudococcid mealybugs and soft-scale insects (closteroviruses and vitiviruses [5].

Nepoviruses and closteroviruses are commonly found on grapevine and they may cause considerable damages, e.g. crop losses, lower fruit quality, deterioration of must quality, progressive decline or death of diseased plants [6].

II. MATERIALS AND METHODS

The study was carried out during 2015 in the Rahovec region, which is famous for the vineyards in Kosovo. Some vineyards of this region with different grape cultivars such as: Vranac, Smederevka, Prokup, Afuzali, Grocaka, Demir Kapi, Plovdina, Melika, Zhillavka have been monitored. Successive and consistent observations have been made mainly for viruses: GFLV, ArMV, GLRaV-1, GLRaV-2, GLRaV-3, GVA and GVB. During these monitoring, which were carried out in spring for nepovirus and in autumn for closaroviruses

or ampeloviruses, suspicious signs were detected compared to the normal condition. The changes included colour, shape, size of the tissue, and so forth. Below we provide pictures from the study site:

Fig.1. View from the vineyard

Fig.2. Samples in the laboratory

Samples of vineyards were placed in the plastic bags and brought to the laboratory for the final identification of viral pathogens through the Elisa test.

2.1. Elisa Test

The serology is an excellent tool for virus detection. It is based on the use of virus-specific antibodies. For its reliability and low cost, especially in large-scale survey, the most useful routine serological technique is ELISA [7].

2.2 Preparation of plant samples

Grapevine phloem were grinded and put in eppendorf (4 eppendorfs for 1 sample, 150 g/each), they were putted in liquid nitrogen and milling, after that, extracted in presence of extraction buffer 1500 µl until obtaining a homogeneous solution. The homogenate samples was centrifuged at 2000 rpm for 10 min and stored in refrigerator at -4 ° C.

2.3 Preparation of buffer solutions

Buffers and Solutions for ELISA

Table1.

PBS buffer (1X) (pH 7.4/ for 1l) dissolved in distilled water		
	NaCl	8.0 g
	KH_2PO_4	0.2 g
	Na_2HPO_4	1.15 g
	KCl	0.2 g

	NaN_3	0.2 g
Coating buffer (pH 9.6/ for 1l) dissolved in distilled water		
	Na_2CO_3	1.59 g
	$NaHCO_3$	2.93 g
Washing buffer (pH 7.4/ for 1l) dissolved in PBS (1X)		
	NaCl	8.00 g
	KH_2PO_4	0.20 g
	Na_2HPO_4	1.15 g
	KCl	0.20 g
	Tween 20	0.5 ml
Extraction buffer (pH 7.4/ for 1l) dissolved in PBS (1x)		
	Polyvinylpyrrolidone, MW 24000	20.0 g
	Tween 20	0.5 ml
	TRIS	2.40 g
	NaCl	8.00 g
	KCl	0.20 g
Conjugate buffer (pH 7.4/ for 1l) dissolved in PBS (1x)		
	Polyvinylpyrrolidone, MW 24000	20.0 g
	TRIS	2.40 g
	NaCl	8.00 g
	Tween 20	0.5 ml
	KCl	0.20 g
	$MgCl_2.\ 6\ H_2O$	0.20 g
	Bovine serum albumin (BSA)	2.0 g
Substrate buffer (pH 9.8/ for 1l) dissolved in distilled water (pH adjusted with HCl)	Diethanolamine	97.00 ml

Three different ELISA procedures were applied in this study:

- Double antibody sandwich (DAS-ELISA)
- Protein-A DAS ELISA
- Direct binding-ELISA

All collected samples (260) were analyzed by ELISA to verify the presence of the following viruses:

Nepoviruses: Grapevine fanleaf virus (GFLV)
Arabis mosaic virus (ArMV)
Closterovirus: Grapevine leafroll associated virus-2 (GLRaV-2)
Ampeloviruses: Grapevine leafroll associated virus-1 (GLRaV-1)
Grapevine leafroll associated virus-3 (GLRaV-3)
Vitiviruses: Grapevine virus A (GVA)
Grapevine virus B (GVB)

2.4 Double antibody sandwich ELISA (DAS ELISA)

This assay was applied to detect GLRaV-1, GLRaV-2, GLRaV-3, GFLV, ArMV and GFKV. The standard procedure was adopted. Polystyrene plates were coated with polyclonal antisera diluted in coating buffer and incubated at 37°C for 2 hours. After washing three times with washing buffer, 100 µl of extracted samples were loaded to each well, and the plates incubated at 4°C, overnight. After new washing of the plates, 100 µl of alkaline phosphatase conjugated antibodies were added to each well, and the plates incubated at 37°C for 2 h. After washing, freshly prepared p-nitrophenil phosphate in substrate buffer (1mg/ml) was loaded to each well. The plates were incubated at room temperature and photometric absorbance was read at 405 nm after 1-2 h with an ELISA reader.

2.5. 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 (Boscia et al., 1990). The normal DAS-ELISA procedure is followed after that.

2.6. 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

During the monitoring carried out in the vineyard, some plants with suspicious symptoms were observed and these plants were labelled, whereas the samples were taken for analysis in the laboratory. The application of the Elisa test to the plant protection laboratory determined the final identification of viral infection for GFLV, ArMV, GLRaV-1, GLRaV-2, GLRaV-3, GVA and GVB viruses. From the examinations carried out, the samples analyzed were viral infection. The yellow colour reaction that appeared on the plate showed the positive result of the Elisa test for these viruses that we analyzed. This result shows the concentration of viral antigens in plant samples for the Rahovec region in the above cultures.

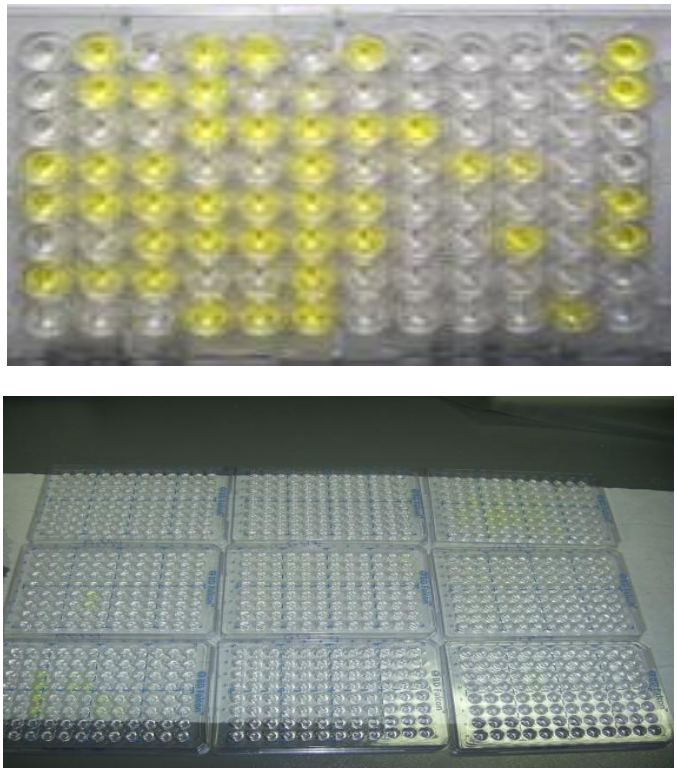

Fig 3. Elisa plates with results

During this study, the application of the Elisa test enabled the possibility to identify the viruses GFLV, ArMV, GLRaV-1, GLRaV-2, GLRaV-3, GVA and GVB in some vineyards of the Rahovec region. These viruses were found in the cultivars of the Rahovec region and iclude: Vranac, Smederevka, Prokup, Afuzali, Grocaka, Demir Kapi, Plovdina, Melika, Zhillavka. We find that these viruses do not have a massive spread, so measures should be taken to keep the vines under control in order for them to be healthy. This is achieved by carrying out continuous and careful monitoring so that infected plants are removed from the vineyard by implementing the necessary measures for their elimination. The certification of planting material is highly recommended. Furthermore, before the creation of the new blocks the presence of the nematode X.index vector should be verified. Also the application of thermotherapy as well as the culture of the meristemes is very important in order for the plants to be virus-free.

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