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Occurrence of Rugose Wood and Leafroll Virus Disease Complexes their Causative Agents and Vectors in Albanian Grapevine Varieties

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Abstract

In Albania the virus diseases of Leafroll and Rugose Wood caused by Grapevine Leafroll-associated Virus complex (GLRaV1-7) and the Grapevine viruses A-F (GVA, B, D, E, F), respectively, were reported to have the highest incidence among other virus diseases tested. The viruses pertaining to these complexes are transmitted by grafting, insect vectors or both. This research aimed at understanding their current status and potential by analyzing the incidence of the causative agents of leafroll and rugose wood diseases by ELISA and identifying their known Pseudococcidae vectors based on morphological keys. Results showed that leafroll disease complex is predominant with the causal agent GLRaV-3 being the most prevalent virus within the complex, followed by GLRaV-7, 1 and 2 in decreasing order. In the rugose wood disease complex, the causal agent GVA had the highest incidence, while GVB was found sporadically. Out of all viruses tested GVA had the highest incidence with 47.7% of positive samples. Within the known potential vectors of leafroll and rugose wood complex *Planococcus ficus* and *Pseudococcus maritimus* were identified. The results showed that both virus complexes are present together with some of their potential vectors which presume that epidemiological conditions are met to satisfy spread of these viral diseases in native Albanian grapevines varieties and nurseries.

Keywords: Leafroll complex, rugose wood complex, mealybugs

1. Introduction

Grapevine farming is an important activity at both household and industrial level in Albania. After collapse of the vineyard industry in early 1990s as a result of country transformation from a closed communist regime to an open democratic society progress has been made by the private sector initiative in recovering the vineyards. Based on FAOSTAT estimate of 2013 a total area of 8600 Ha was reported [2]. According to official report from the Ministry of Agriculture in Albania, among imported table and wine varieties a great majority of the planted area is grown to native varieties, mainly used for the wine industry [8]. It was assumed that native wine varieties that were most likely to dominate the area are Shesh i Bardhe, Shesh i Zi, Kallmet and Vlosh. However it was highlighted that planting stock for such varieties were produced locally out of regular certification schemes for virus-free planting material, a condition which persists to date. Earlier studies have reported the incidence of Grapevine Fanleaf Virus (GFLV), Grapevine Leaf Roll-associated Virus complex (GLRaV-complex), Grapevine Fleck Virus (GFkV) and Roogose Wood complex (GVA, B, D, E, F) in native and imported varieties [1, 5, 7]. Based on recent years visual observations in different vineyards in Albania it is was suggested that leafroll and rugose wood complex are the most prevalent viruses in native and imported varieties (Merkuri, *personal communication*). The present study was undertaken to evaluate the significance of leafroll and rugose wood complexes in key native grapevine varieties based on ELISA detection of the involved viruses and determination of their potential vectors.

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2. Materials and Methods

2.1 Plant material and sampling procedure

Four Albanian native varieties with significance in the local wine industry, namely, Shesh i Bardhe, Shesh i Zi, Kallmet and Vlosh, were sampled randomly in defoliated canes during Autumn-Winter 2014-2015 in the districts of Durres, Shkodra and Vlora. Farmers' plots were sampled in a diagonal pattern and for each sampled row three neighbor plants were selected randomly according to Golino and coworkers [3] and four cane pieces per each plant, two from each left and right side vines, were pooled to represent one biological sample. A total of 331 samples were collected, stored cool at 4C° and extracted ELISA analysis. The tested viruses in the leafroll complex were GLRaV-1,2,3,7, while in the rugose wood complex GVA and GVA were tested. Incidence was used a parameter of disease occurrence and was calculated as a percentage of infected out of the total analyzed samples for a given virus.

2.2 ELISA

The kits for testing grapevine viruses were received from Agritest (Agritest Srl, Valencano, Italy) and the procedure followed manufacturer instructions. For GVA a TAS-ELISA protocol was employed with amplification by Protein A. GVB was tested through an indirect ELISA protocol. All tested members of GLRaV complex were assayed with DAS-ELISA. Biological positives and negatives were employed for each virus in addition to those provided with the test kits. Samples were tested in duplicates.

2.3 Identification of Pseudococidae vectors

The *Pseudococcidae* vectors were collected during May 2015 in the same plots sampled for ELISA tests. Identification was based on morphological keys [4] on the female individuals of the sampled populations.

3. Results and Discussion

Based on several visual observations of symptoms of leafroll and rugose wood virus diseases in Albanian vineyards it was hypothesized that in the last two decades pertaining to the transition of Albanian agriculture sector from a centralized one to a private, open market-oriented economy the incidence of these two virus disease complexes were high. In the present study we confirmed, based on random sampling of defoliated vineyards that leafroll and rugose wood are widely spread in native cultivars.

3.1. Incidence of leafroll and rugose wood

Based on ELISA it was found that 48.3% of the tested samples were positive for the rugose wood-associated viruses GVA and GVB and similarly 47.4% of the tested samples were positive for GLRaV-associated viruses (Table 1). This result confirms earlier reports of high incidence of these viruses in Albania [5, 7]. Therefore, it seems that due to unchanged practice of propagation with planting stock from traditional nurseries the situation has not changed in the last three decades in Albania.

3.2. Identification of Pseudococcidae vectors

It has been assumed that the incidence is influenced more from the planting of uncertified nursery stock and that vectors played a minor role in epidemiology. In this study we confirmed the presence of two economically important mealybug pests of grapevine which vector leafroll- and rugose wood-associated viruses, namely the grape mealybugs *Planococcus ficus* Signoret and *Pseudococcus maritimus* Ehrhorn belonging to the family *Pseudococcidae*. *P. maritimus* and *P. ficus* vector some of the viruses associated with leafroll complex, GLRaV-1,3,4 [9]. In the present study the incidence of GLRaV-3 reached 41.7%, therefore a vector-virus association is expected to influence epidemiology in infested vineyards. Rugose wood-associated viruses are transmitted by mealybugs belonging to the genus *Planococcus, Pseudococcus, Heliococcus, Phenacoccus* and *Neopulvinaria* [6]. Both GVA and GVB that were subject to this study are each transmitted by the hitherto identified mealybug vectors *P. ficus* and *P. martimus*. The highest incidence identified by ELISA corresponded with GVA (47.7%) also establishing that virus inoculum in the field favors vector-mediated epidemiology.

Table 1. Incidence of GVA, GVB and GLRaV-1,2,3,7 as revealed by ELISA on *cv.* Kallmet, Shesh i Zi, Shesh i Bardhe and Vlosh in the districts of Shkodra, Durres and Vlora.

District	Variety	No. tested samples and incidence	Tested viruses by ELISA					
			GVA	GVB	GLRaV1	GLRaV2	GLRaV3	GLRaV7
Shkoder	Kallmet	131	70	2	1	1	50	3
		Incidence	53.4	1.5	0.8	0.8	38.2	2.3
Durres	Shesh i Bardhe	50	18	0	0	0	29	3
		Incidence	36.0	0.0	0.0	1.0	58.0	6.0
	Shesh i Zi	55	28	0	0	0	11	2
		Incidence	50.9	1.0	1.0	0.0	20.0	3.6
Vlore	Vlosh	95	42	0	1	0	48	8
		Incidence	44.2	0.0	1.1	0.0	50.5	8.4
Total		**331**	**158**	**2**	**2**	**1**	**138**	**16**
		Incidence	**47.7**	**0.6**	**0.6**	**0.3**	**41.7**	**4.8**

4. Conclusions

It may be suggested that mealybug vectors may play a modest role in the field spread of the virus complex in contrast to dissemination through propagation material. Certification is by far the most important method of disease management and in plots with high population of mealybugs the use of chemical control mya provide the most satisfactory methods to manage the spread of leafroll and rugose wood in Alanian grapevine varieties. A survey on local nurseries will be needed to evaluate potential incidence coming with the planting stock.

5. References

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