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THE VIRAL DISEASE COMPLEX OF GRAPEVINE INFECTIOUS DEGENERATION AND ITS ASSOCIATION WITH GRAPEVINE FAN LEAF VIRUS IN ALBANIA

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ABSTRACT

Recent studies have shown that major grapevine viruses for the Mediterranean region are prevalent in the native cultivars that have been grown for long in Albanian vineyards. Infectious Degeneration disease complex caused by Grapevine Fan Leaf Virus (GFLV), and other viruses, comprises a common phenotype in the field, but GFLV detection in random samplings has shown low frequency. In the present study we aimed at evaluating the association between Infectious Degeneration phenotype in the field and GFLV in the native cultivar Kallmet, by using DAS-ELISA of extracts from lignified canes of symptomatic plants. The results showed that Infectious Degeneration phenotype is widely spread but only 20% of the tested symptomatic plants were found positive for GFLV indicating that other viruses involved in the disease complex may account for the phenotype in the field. It is suggested that the full set of viral agents involved in Infectious Degeneration disease complex be tested to understand their implication in the disease phenotype.

INTRODUCTION

The first report of grapevine associated virus diseases in Albanian grapevines dates back to 1988 and highlighted the presence of several important viruses in vineyards and nurseries of propagation material (Martelli, 1988). Though the report was based on symptom evaluation it was made possible to the expert knowledge that Infectious Degeneration disease complex was present in several vineyards. The first report on one of the nowadays well known causative agents of Infectious Degeneration phenotype, the Grapevine Fan Leaf Virus (GFLV), came few years later and was based on biological, physical and molecular studies (Merkuri et al., 1993,). A recent study based on random sampling of four Albanian native cultivars showed that GFLV had only a minor incidence of 2.7% (Sadikaj et al., 2016). Since GFLV comprises one of the most important causative agents of the Infectious Degeneration disease complex in the Mediterranean area it was considered valuable to analyze its association with the disease phenotype under field conditions. It was therefore hypothesized that GFLV is associated with the Infectious Degeneration phenotype. To test this hypothesis, symptomatic plants were tested by DAS-ELISA for the incidence of GFLV.

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MATERIALS AND METHODS

Plant material and location

For the purpose of our hypothesis and since the fate of propagation material for grapevine has been the same in Albania since decades, the native cultivar Kallmet with significant importance in local wine industry and that elapses plantations established since 1970s in Albania was selected. The study was carried out in Kallmet's historical plantation area, in the district of Skadar, where around 28% of the vineyards were investigated in the present study.

Disease Evaluation

Infectious Degeneration is a complex disease characterized by three distinct types of symptoms; malformations, yellow mosaic and vein banding. Malformation is a complex syndrome and was defined by leaf distortion such as fan leaf, asymmetry, puckering, deep lobes, open petiolar sinuses, acute denticulation and shoot distortion such as double nodes, bifurcation, zig-zag growth and short internodes. Yellow mosaic was defined as relatively bright yellowing of the leaf blades either in a diffusible or discrete pattern, present in single shoots of individual vines. Vein banding was defined as yellowing of the vines and adjacent leaf blade cells in a distinct pattern, easy to be characterized. The three types of

symptoms comprising the Infectious Degeneration phenotype were searched for by end of spring, beginning of summer (May-June 2015), corresponding with the growth stage in which the phenotype of Infectious Degeneration is expressed at maximum. Plants with only one or more type of symptoms were considered positive and labeled. In total, 14 vineyards, comprising around 28% of the *cv.* Kallmet planted area in the district of Skadar were investigated.

DAS-ELISA

After leaf fall in winter the symptomatic labeled plants was sampled. For each symptomatic vine four canes, two from each side, were sampled, polled together as one biological sample and stored at 4°C degree until they were used for DAS-ELISA.

belong to malformation type of disease phenotype (Fig. 1a). Vines with yellowing of leaf blades in individual shoots were found less frequently and suspected to belong to the yellow mosaic disease phenotype (Fig. 1b). Finally, individual vines with vein banding pattern were found sporadically and were considered as possible symptoms of GFLV+GYSVd co-infection (Fig. 1c). In total 88 individual plants were labeled. Out of them, 68% were grouped in the malformation, 27% in the yellow mosaic one and only 5% in the vein banding phenotype (Table 1). The labeled vines sampled and analyzed by DAS-ELISA resulted in only 20% positive tests for GFLV (Table 1). Establishing the percentage detection of GFLV positive tests relative to the symptomatic plants in each symptom type it was figured out that malformation had the lowest association between the phenotype and GFLV detection.

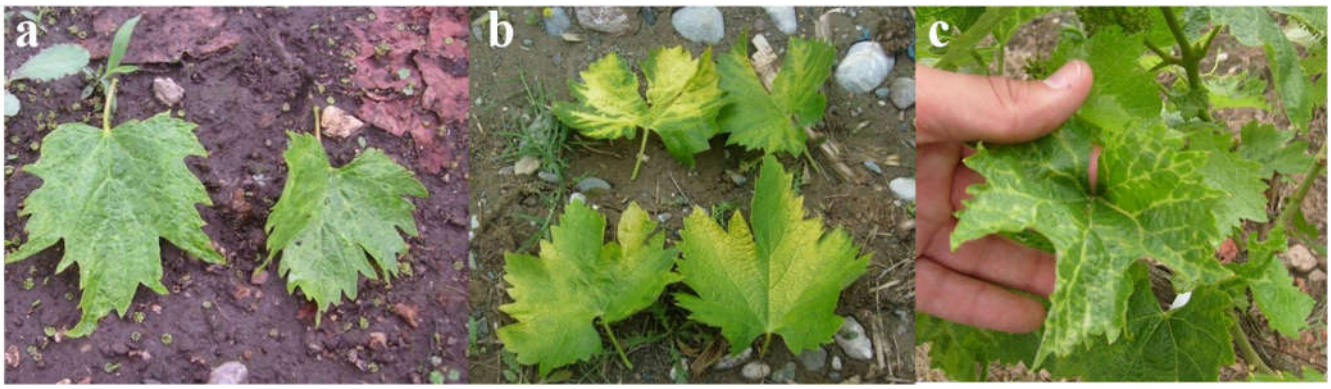

Fig. 1. Infectious Degeneration phenotype on *cv.* Kallmet found in the vineyards during field survey in the district of Skadar. Malformations showing typical fan leaf symptoms (a), yellow mosaic showing bright yellow and discrete areas of yellowing (b) and vein banding symptom (c) resembling those caused by GFLV distorting strains, chromogenic strains and co-infection by GFLV and *Grapevine yellow speckle viroid* (GYSVd), respectively

Table 1. Number of symptomatic plants with Infectious Degeneration disease-like symptom types, positive tests for GFLV and the percentage of positive tests in each symptomatic type found in *cv.* Kallmet vineyards screened visually for the occurrence of malformations, yellow mosaics and vein banding types of symptoms and tested by DAS-ELISA for GFLV in lignified overwintering vines

Symptom type	No. symptomatic plants	No. GFLV positive samples for DAS-ELISA	% detection
Malformation	60	11	18
Yellow mosaic	24	6	25
Vein banding	4	1	25
Total	88	18	

Extraction from phloem scrapings were done in all four canes. The protocol followed a DAS-ELISA, with overnight incubation of the sample at 4C° according to manufacturer instructions (Agritest Srl, Valencano, Italy). A biological positive and negative was employed in addition to those provided with the kit. The samples were tested in duplicate. Absorbance at 405 nm was red in a microplate reader and the threshold for nominating a sample as positive was considered an absorbance reading equal to or more than three times the mean of the biological negative.

RESULTS

Field survey showed that out of the 14 vineyards of *cv.* Kallmet investigated only three of them appeared to be free of the infectious degeneration disease-like symptoms. These vineyards comprised new plantations of 3-4 years old at the time of field survey. The three types of symptoms were found randomly in the investigated vineyards (Fig. 1). Vines with malformed leaves and shoots were found and suspected to

Yellow mosaic and vein banding phenotypes showed similar percentage of positive detection (Table 1).

DISCUSSION

Due to frequently observed syndrome of Infectious Degeneration in Albanian vineyards planted with native cultivars and the recent results showing that GFLV had a low incidence in random samplings of defoliated vines (Sadikaj et al., 2016) the current study was undertaken to understand if the observed field phenotype is associated to GFLV infection. The low association between GFLV and Infectious Degeneration disease-like symptoms in the field indicates that other nepoviruses and/or *Strawberry latent ring spot virus* (SLRSV, *Secoviridae*) may be associated with the phenotype in addition to other possible etiologies. Viruses belonging to the family *Secoviridae*, genus *Nepovirus* incorporate almost all viruses related to Infectious Degeneration disease complex. They are divided into European nepoviruses and North American grapevine nepoviruses (Martelli et al., 2015, Uyemoto, 2015).

European nepoviruses are divided into three subgroups. Subgroup A incorporates GFLV, *Arabis mosaic virus* (ArMV), *Rasberry ringspot virus* (RpRSV) and *Grapevine deformation virus* (GDefV). In the subgroup B three viruses are included; *Tomato black ring virus* (TBRV), *Grapevine chrome mosaic virus* (GCMV) and *Grapevine Anatolian ring sport virus* (GARSV). In subgroup C only one virus, *Cherry leafroll virus* (CLRV), is included. In addition to European nepoviruses listed above *Strawberry latent ringspot virus* (SLRSV), is an unassigned member of the family *Secoviridae* any induces symptoms of vine decline and leaf mottling. The list of North American Nepoviruses includes, in addition to European Nepoviruses, the *Blueberry leaf mottle virus* (BLMV), *Peach rosette mosaic virus* (PRMV), *Tobacco ringspot virus* (TRSV) and *Tomato ringspot virus* (ToRSV). The relatively low incidence of GFLV in Albanian native cultivars complies with earlier findings (Merkuri et al., 1994). Based on the finding that GFLV incidence relative to Infectious Degeneration phenotype in the field shows little association it is suggested that other viruses involved in the Infectious Degeneration disease complex etiology should be tested. This could lead to a re-consideration of viruses to be tested in a national certification scheme for virus-free propagation material in Grapevine.

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