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SHORT NOTES

Grapevine leafroll-associated closterovirus 7 in Greece

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Summary. An extensive survey was carried out to assess, by ELISA, the occurrence of grapevine closteroviruses in several grape-growing areas of Greece, with particular regard to GLRaV-7. Samples were collected in commercial vineyards and varietal collections from apparently symptomless and symptomatic leafroll vines. GLRaV-7 was widely distributed and frequently associated with GLRaV-1 and GLRaV-3. ELISA was suitable for GLRaV-7 detection but the reagents utilized in this study must be improved.

Key words: vineyard, GLRaV-7, leafroll, survey, diagnosis.

Introduction

Grapevine leafroll disease is one of the most important graft-transmissible diseases of grapes, causing important losses in the viticultural industry. Grapevine leafroll-associated virus 7 (GLRaV-7) is one of eight serologically distinct closteroviruses reported to be associated with leafroll disease of grapevine (Boscia *et al*., 1995; Choueiri *et al*., 1996; Monis and Bestwick, 1997). The virus has been recorded in several Mediterranean countries (Italy, Turkey, Palestine) and in Armenia (Alkowni *et al*., 1997; Martelli, 1997; Yilmaz *et al*., 1997). GLRaV-7 was first reported in Greece by the Mediterranean Network on Grapevine Closteroviruses 1992–1997 (Martelli, 1999).

An extensive survey carried out in several grape-growing areas of the Hellenic islands and mainland confirmed the presence of the virus in Greece.

Materials and methods

Two hundred and thirty commercial vineyards and varietal collections were surveyed and sampled in 1997–1999. Samples were collected from vines exhibiting leafroll symptoms and apparently symptomless vines, belonging to the most important Greek varieties [Roditis (165), Sultana (25), Savvatiano (55), Black of Nemea (60), Corinthiaki (35), Razaki (15), Black of Mesenicola (16), Lemnio (26), Muscat d'Ambourge (28), Muscat of Sa-

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mos (22), Mandilaria (20), Liatiko (17), Kotsifali (15), Monembasia (18), Vaftra (12), etc.] and selected in the course of a sanitary selection programme in the islands of Crete, Samos, Lemnos and Paros, and in Epirus and Macedonia.

Mature canes were collected from all branches of all vines each winter and were taken to Heraklion to be checked for viruses. Tests were done by ELISA using cortical scraping extracts from at least four canes per vine. A polyclonal antiserum to GLRaV-7 supplied by the Dipartimento di Protezione delle Piante e Microbiologia applicata, University of Bari, was cross-absorbed with virus-free *Vitis vinifera* cv. Razaki extracts prior to immunoglobulin purification by protein A chromatography and conjugation with alkaline phosphatase (Clark *et al*., 1988). Commercial kits (Agritest, Valenzano, Italy; Bioreba AG, Basel, Switzerland; Sanofi Pasteur, Marnes la Coquette, France) were used for detecting GLRV-1, -2, -3, -5 and -6). Each grapevine was tested at least twice. Extracts were incubated for 2–3 h at room temperature but some experiments required overnight incubation at 4°C. Readings of each plate were scored as the ratio of the average A_{405} value of each sample (2 wells/plate) to the average A_{405} readings of the healthy control (4 wells/plate). Scores of -, +, 2+ and 3+ were assigned to treatments with the following ratios: lower than 1.99, 2.00–2.49, 2.50–2.99, and above 3 respectively.

To study the reproducibility of ELISA in the same vine for a number of years, the test was repeated every year for three years on vines from a collection maintained in pots under shade houses.

Results and discussion

ELISA reagents for GLRaV-7 detection required long incubation times for the complete development of the reaction (overnight at 4°C to reach values of 1.482±0.229 O.D.) and had a fairly low specificity since they developed a significant background even with the healthy controls (0.427±0.129 O.D.). This may have reduced the sensitivity of the test, especially in the case of low virus concentration, as it probably affected the infected/healthy absorbance ratio.

The results of DAS-ELISA on GLRaV-7 in 665 grapevines with leafroll are summarized in Table 1**.** More than 12% of the grapevines were certainly infected with GLRaV-7 (score 3+), while a further 6% was probably infected (scores + and 2+). Only in a few cases (about 1.8%) was GLRaV-7 found alone; in these cases grapevines frequently showed very mild or uncertain leafroll symptoms. By contrast, in strongly symptomatic vines GLRaV-7 was associated with other closteroviruses commonly occurring in Greek vineyards (Avgelis *et al*., 1997). In the present study, in 285 samples from widely grown Hellenic cultivars, GLRaV-7 was associated mainly with GLRaV-3 (28%) and to a much less extent with GLRV-1 (slightly more than 1%) (Table 2). It is worth

Table 1. ELISA for GLRaV-7 in 665 grapevines showing leafroll symptoms.

S/H[a]	Grapevine samples	
	N°	%
0.50–1.99	544	81.8
2.00–2.49	18	2.7
2.50–2.99	22	3.3
3.00–12.0	81	12.2

[a] Ratio of readings of absorbance values (A_{405}) of tested (S) and healthy (H) samples

Table 2. Closteroviruses in 285 grapevines showing leafroll symptoms, belonging to 4 grapevine cultivars.

Cultivar	No. of samples ELISA negative	No. of samples ELISA positive						
		LR1	LR3	LR7	LR1+LR3	LR1+LR7	LR3+LR7	LR1+LR3 +LR7
Roditis	0	2	83	2	0	0	78	0
Soultana	1	10	4	5	0	3	2	0
Black of Nemea	0	53	1	3	0	0	0	3
Corinthiaki	1	14	3	0	14	0	0	3
Total	2	79	91	10	14	3	80	6

noting that GLRaV-2, -5 and -6 were not detected in any of the 665 samples assayed, and that in 12 vines (1.8%) none of the closteroviruses tested for were found.

In 1284 putative clones from the sanitary selections of 1998 and 1999, GLRaV-7 occurred in 0.6% of accessions (score 3+), most of which were table grapes. GLRaV-1 and GLRaV-3 were associated with GLRaV-7 in one and four of these samples respectively, while in four samples GLRaV-7 occurred alone. GLRaV-2, -5 and -6 were found only in 2, 3 and 2 symptomless putative clones respectively.

When the reproducibility of ELISA for the detection of GLRaV-7 was tested in 42 putative clones over three years (Table 3), 20 clones gave a consistent negative response, two a consistent positive response, while the remaining clones gave erratic responses. Of these erratic clones, only 10 of the 16 accessions that were ELISA- positive in 1997 were also positive in 1998, and of the 4 clones that reacted positively in 1998 for the first time, only one was again positive in 1999. One possible explanation of these inconsistent results is the limiting effect on sensitivity caused by the high background of the negative controls. However, other factors may also have played a role. For instance it is not unlikely that the very low rate of positive reactions in 1999 was due to aging of the IgG lot.

Based on the above results

GLRaV-7 is a virus with a wide distribution in Greek vineyards, and is frequently associated with GLRaV-1 and GLRaV-3. In view of the low sensitivity of the serological reagents used, its incidence may be even higher than indicated by the findings.

ELISA is suitable for GLRaV-7 detection in grapevine tissues, but the low sensitivity of the reagents utilized makes an improvement in their quality (sensitivity and specificity) desirable. For this reason new antisera to GLRaV-7 are now under production at the University of Bari.

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Table 3. Reproducibility of ELISA for detection of GLRaV-7 over three years, in 42 clones from different grapevine cultivars.

Clone	1997	1998	1999
A16 (41B)	-	-	-
A17 (110R)	-	-	-
A18 (red cv.)	-	-	-
A19 (white cv.)	-	-	-
A21 (red cv.)	-	-	-
A22 (red cv.)	-	-	-
A28 (red cv.)	-	-	-
A33 (white cv.)	-	-	-
B4 (red cv.)	+	-	-
B8 (red cv.)	+	-	-
B10 (white cv.)	-	+	+
B12 (white cv.)	+	+	+
B15 (red cv.)	+	?	-
B20 (white cv.)	+	+	+
E2 (red cv.)	+	-	?
E34 (white cv.)	-	-	-
E35 (white cv.)	-	-	-
P3 (red cv.)	-	-	-
R1 (white cv.)	-	-	-
R4 (red cv.)	-	-	-
R7 (white cv.)	-	-	-
R8 (red cv.)	-	-	-
R9 (white cv.)	-	-	-
S1 (red cv.)	-	+	-
S11 (red cv.)	-	+	-
S15 (red cv.)	+	-	-
S17 (red cv.)	+	?	-
X1 (red cv.)	+	+	-
X2 (red cv.)	+	+	-
X3 (red cv.)	-	-	-
X7 (red cv.)	-	-	-
X12 (red cv.)	+	?	-
X13 (red cv.)	+	+	-
X14 (red cv.)	+	+	-
X16 (red cv.)	+	+	-
X21 (red cv.)	-	-	-
Y21 (red cv.)	-	+	-
Z4 (red cv.)	?	-	-
Z12 (red cv.)	-	?	-
Z18 (red cv.)	-	-	-
Z22 (red cv.)	?	-	-
Z25 (red cv.)	-	?	-

+, ELISA positive (score of 3+);
? , ELISA positive (scores of + and 2+);
- , ELISA negative.

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