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Research Note

Occurrence of the Redglobe strain of Grapevine leafroll-associated virus 2 in table and wine grape varieties in Italy

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Summary: The Redglobe strain of Grapevine leafroll-associated virus 2 (GLRaV-2-RG) is a recently discovered closterovirus so far reported to infect solely cv. Redglobe. A survey for this virus 2 in about 380 accessions of table and wine grape varieties conducted during 2001-2003 proved that GLRaV-2-RG was present also in other table grape varieties, though to a smaller extent, while it was never detected in wine varieties.

Key words: GLRaV-2, graft-incompatibility, grapevine, GLRaV-2-RG, Redglobe.

Introduction: So far at least 8 ampeloviruses and one closterovirus serologically unrelated have been found in grapevine where they are usually associated with grapevine leafroll (LR) disease (Martelli 1997; Monis 2000; Alkowni *et al*. 2002). Grapevine leafroll-associated virus 2 (GLRaV-2) causes LR symptoms in grapevine, but it seems to be also involved in graft-incompatibility (Greif *et al.* 1995). Recently, a new closterovirus, with a 74 % sequence homology to GLRaV-2, has been identified in cv. Redglobe (Rowhani *et al.* 2000). This virus did not induce symptoms in LR indicators, but proved to be closely associated with a disorder of young grafted vines in California, where cv. Redglobe scions grafted on 4 rootstocks (Kober 5BB, 5C, 3309C and 1103P) declined and died within a couple of years from grafting. This virus, which was originally identified as a new viral species denoted Grapevine rootstock stem lesion-associated virus (GRSLaV) (Uyemoto *et al*. 2000; Rowhani *et al.* 2003) is now regarded as a strain of GLRaV-2 to be called GLRaV-2-RG.

This study aims to provide insight into the presence of GLRaV-2-RG in cv. Redglobe and in other table and wine grape varieties collected in Italy.

Material and Methods: In 2001, 2002 and 2003 about 380 samples of table and wine grape varieties were collected in 10-year-old vineyards both in the North and in the South of Italy. They were tested by DAS-ELISA with antibodies for specific detection of GLRaV-2 (Agritest, Italy). About 140 samples were obtained from the table grape varieties Redglobe, Crimson and Italia, about 40 samples from other table grape varieties, such as Autumn Royal, Cardinal, Victoria, Regina, whereas the remaining 200 samples were accessions from different wine varieties.

RNA was extracted from 145 samples, 68 of which were from table grape varieties and 77 from wine varieties, according to MacKenzie *et al.* (1997). RT-PCR assays were then carried out using two specific primer pairs: GLR2CP1/GLR2CP2, which amplify the entire CP cistron (597 bp) of several GLRaV-2 strains (Abou Ghanem *et al.* 2000), and RGHSP 227V/777C, which amplify a fragment of 546 bp in the HSP70 putative gene of GLRaV-2-RG (Rowhani *et al.* 2000).

Results and Discussion: Almost all grapevine accessions of cvs. Redglobe and Crimson scored positive with antiserum to GLRaV-2. Only 6 samples of cv. Redglobe did not prove positive in the ELISA test. Occasionally positive samples were found among the other table and wine grape varieties, with an incidence varying from 5 to 6 % (Tab. 1).

Table 1

Serological results for GLRaV-2 on grapevine samples of table and wine grape varieties. Redglobe accessions came from 4 vineyards in Apulia and one vineyard of the ISV variety collection of Conegliano, Veneto; Crimson accessions came from two vineyards in Apulia; Italia accessions came from a vineyard in Apulia and one in Friuli Venezia-Giulia; Autumn Royal accessions came from two vineyards in Apulia; the other table grape and the wine variety accessions came from various vineyards in Italy. Some wine grape accessions were introduced from California about 10 years ago

Grapevine variety	Tested samples	Positive samples	Positive samples, %
Redglobe	48	42	87
Crimson	55	55	100
Italia	33	3	9
Autumn Royal	10	1	10
Other table grape varieties	25	4	16
Wine varieties	200	10	5
Total	371	115	31

RT-PCR assays confirmed that GLR2CP1/GLR2CP2 primer pair did not produce amplimers for samples infected only with GLRaV-2-RG, while RGHSP 227V/777C primer pair yielded a PCR product only for samples infected with GLRaV-2-RG but not for samples infected with other GLRaV-2 strains. Thirty-nine grapevine samples, positive in the serological test, gave positive signals with GLR2CP1/GLR2CP2 primer pair or GLRaV-2-RG specific primer pair or both. Furthermore 22 samples, negative in the serological assay, scored positive with one or both primer pairs. In particular, among the latter samples, 6 (4 Redglobe accessions, one Autumn Royal accession and the Leopoldo III accession) were found infected only with GLRaV-2-RG, 14 samples (one Italia accession, one Autumn Royal accession, 9 accessions of other table grape varieties and three accessions of wine varieties) were infected with GLRaV-2 strains different from GLRaV-2-RG and two samples (one Italia and one Redglobe) were infected with both closteroviruses. The results con-

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firmed a higher sensitivity of the RT-PCR assay compared to the ELISA test.

Cross-reaction of GLRaV-2-RG with GLRaV-2 antibodies was observed in some samples infected only with GLRaV-2-RG and positive in the ELISA test. Weak cross-reaction was reported also by ROWHANI *et al*. (2000).

Infection rates in table and wine grape varieties, calculated by molecular data, confirmed to be very different: among wine varieties 64 samples out of 77 (83 %) were not infected with any strain of GLRaV-2 and only 20 out of 68 (29 %) among table grape varieties.

The presence of GLRaV-2-RG was detected in table grape cvs Redglobe, Italia, Crimson, Autumn Royal and in the unique accession of Leopoldo III. Infection rates of GLRaV-2-RG (in single or in mixed infection with other strains of GLRaV-2) were quite different in the varieties: 89 % (16 out of 18) for Redglobe, 30 % (3 out of 10) for Crimson and 23 % (3 out of 13) for Italia (Tab. 2).

On the whole, 16 % (11 out of 68) samples of table grape varieties were infected only with GLRaV-2-RG, 31 % (24 out of 77) were infected only with other strains of GLRaV-2 and 19 % (13 out of 68) were infected with both closteroviruses. It is worth noting that no trace of GLRaV-2-RG was detected in samples of wine varieties since infected accessions of wine varieties contained only GLRaV-2 variants different from the RG-strain.

In conclusion, the serological test did not reveal the presence of GLRaV-2 and GLRaV-2-RG in all the infected samples, while the PCR assay did. Moreover, the biomolecular test was necessary to distinguish other strains of GLRaV-2 from GLRaV-2-RG, which our results confirmed to be clearly different.

This is the first report of GLRaV-2-RG to be present in varieties other than Redglobe. Samples infected with GLRaV-2-RG were accessions of cvs Italia, Crimson, Autumn Royal, collected in Apulia in the area of Redglobe vineyards; only the GLRaV-2-RG-infected sample of cv. Leopoldo III came from Northern Italy, from the ISV grapevine variety collection of Conegliano.

At the moment no data on the presence of a graft-incompatibility are available, because most scions were grafted on previously grafted grapevines. Further research and specific trials are required in order to understand the effect of GLRaV-2-RG on table grape varieties in Italy.

Table 2

Comparison of molecular results obtained from RT-PCR assays on 68 accessions of table grape varieties and 77 accessions of wine grape varieties with primer pairs specific for GLRaV-2-RG and GLRaV-2 variants different from the RG-strain

Variety	Number of samples infected with				
	GLRaV-2-RG and other strains of GLRaV-2	GLRaV-2-RG	Other strains of GLRaV-2	No GLRaV-2 strain	Total
Redglobe	8	8	1	1	18
Crimson	3	0	7	0	10
Italia	2	1	3	7	13
Other table grape varieties	0	2	13	12	27
Total table grape varieties	13	11	24	20	68
Wine varieties	0	0	13	64	77

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