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Phloem-limited viruses of the grapevine in the Mediterranean and Near East: a synopsis

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SUMMARY - An account is given on the distribution of phloem-limited viruses (closteroviruses, vitiviruses, foveaviruses and fleck-like viruses) in the Mediterranean countries, as determined by analyzing *ca.* 17,000 grapevine samples collected in the course of numerous field surveys. ELISA tests were conducted in several laboratories using different reagents and procedures. Grapevine leafroll associated closteroviruses 1 and 3 (GLRaV-1 and GLRaV-3), grapevine vitivirus A (GVA) and grapevine fleck virus (GFkV) proved widely distributed in all surveyed countries, confirming the high incidence in the Mediterranean basin of leafroll, rugose wood and fleck diseases, which they are associated with. Preliminary surveys carried out in Italy and in Portugal by PCR revealed a high percentage (74% and 44%, respectively) of grapevine rupestris stem pitting associated virus (GRSPaV), the putative agent of one of the syndromes of the rugose wood complex.

Key words: grapevine, Mediterranean, Near East, closteroviruses, vitiviruses, GFkV, GRSPaV

RESUME - *On illustre la distribution des virus localisés dans le phloème de la vigne (closterovirus, vitivirus, foveavirus et virus de type marbrure) dans les pays méditerranéens, estimée en analysant environ 17.000 échantillons de vigne collectés au cours de plusieurs prospections au champ. Les tests ELISA ont été exécutés dans de nombreux laboratoires, en utilisant différents réactifs et procédés. Les closterovirus 1 et 3 associés à l'enroulement foliaire de la vigne (GLRaV-1 et GLRaV-3), le vitivirus A de la vigne (GVA) et le virus de la marbrure de la vigne (GFkV) se sont avérés être largement répandus dans tous les pays considérés, confirmant ainsi l'incidence élevée dans le bassin méditerranéen des maladies de l'enroulement foliaire, du bois strié et de la marbrure, auxquelles ces virus sont associés. Les enquêtes préliminaires réalisées en Italie et au Portugal en utilisant la PCR ont mis en évidence un pourcentage élevé (74% et 44%, respectivement) du virus associé au rupestris stem pitting (GRSPaV), agent probable de l'un des syndromes du complexe du bois strié.*

Mots clés*: vigne, Méditerranée, Proche Orient, closterovirus, vitivirus, GFkV, GRSPaV*

The consensus is that phloem-restricted viruses are involved in the aetiology of leafroll, rugose wood, and fleck, three of the major virus diseases of *Vitis* (Martelli, 1993; Walter and Martelli, 1997). These viruses belong to different genera, all have a positive sense single-stranded RNA genome but particles of two types, isometric and filamentous. Most of these viruses are not transmissible, or are transmitted with difficulty by inoculation of sap, and some have mealybug vectors (reviewed by Boscia *et al.,* 1997b; Martelli *et al.,* 1997). These viruses are:

1. Closteroviruses. Seven (Boscia *et al.,* 1995; Choueiri *et al.,* 1996), possibly eight (Monis and Bestwick, 1997; J. Monis, personal communication) such viruses, denoted grapevine leafroll-associated viruses (GLRaV), have been consistently detected in leafroll-diseased vines and are largely suspected to be the causal agents of the disease. Notwithstanding the fact that there is ample circumstantial evidence and, for some of them (GLRaV-1, -2, -3, -7), experimental evidence of the induction of leafroll-type response in healthy vines when these become singly infected either by grafting (Belli *et al.,* 1995; Choueiri *et al.,* 1996) or through the intermediary of vectors (Engelbrecht and Kasdorf, 1990; Rosciglione and Gugerli, 1989; Belli *et al.,* 1994), all these viruses continue to be cautiously referred to as "leafroll-associated". Whether all closteroviruses are agents of leafroll only is still under scrutiny. For instance, GLRaV-2 is allegedly involved in a graft incompatibility condition expressed by Kober 5BB, reported from Italy and France (Garau *et al.,* 1994b; Greif *et al.,* 1995).

2. Vitiviruses. Four vitiviruses (Grapevine virus A to D) have been so far recovered by mechanical transmission from grapevines generally affected by rugose wood (reviewed by Boscia *et al.,* 1997). Of these, GVA was proven to be the agent of Kober stem grooving (Chevalier *et al.,* 1993; Garau *et al.,* 1994a; Choueiri *et al.,* 1997) and GVB the agent of corky bark (Boscia *et al.,* 1993; Garau *et al.,* 1993), two of the rugose wood syndromes (Martelli, 1993). The aetiological role of GVC and GVD has not been ascertained.

3. Foveaviruses. Grapevine rupestris stem pitting-associated virus (GRSPaV), a member of the recently established genus Foveavirus (Martelli and Jelkmann, 1998) is currently regarded as the likely agent of rupestris stem pitting (Meng *et al.,* 1998, 1999), another of the rugose wood syndromes (Martelli, 1993). Curiously enough, only the RNA genome of this virus is known, for its particles, supposed to be filamentous, have not yet been observed, unless the unidentified capillovirus-like particles recovered from a Canadian vine affected by rupestris stem pitting and LN33 stem grooving (Monette and Godkin, 1995) belong to GRSPaV.

4. Fleck-like viruses. In *Vitis* there appears to be a family of non mechanically transmissible viruses with isometric particles 30 nm in diameter, exhibiting a rounded contour and a surface structure distinctively organised in pentameric and hexameric capsomeres. The

most known of these is Grapevine fleck virus (GFkV) the agent of fleck disease (Boscia *et al.,* 1991), another is the Grapevine asteroid mosaic-associated virus (GAMaV) (Boscia *et al.,* 1994), and a third such virus, provisionally called Grapevine Red globe virus (GRGV) has recently been identified in Italian and Albanian grapevine accessions (Sabanadzovic *et al.,* 1999). All fleck-like viruses are apparently latent in *Vitis vinifera* and GRGV also in *Vitis rupestris*, which, however, reacts with differential symptoms to the infection by GFkV and GAMaV (Martelli, 1993).

For many years, surveys for the presence of the above viruses and the diseases they elicit have been conducted in Mediterranean and Near East countries, first by the Department of Plant Protection of the University of Bari (DPPM), then jointly with the Mediterranean Agronomic Institute of Bari (IAM-BA). These surveys gained momentum when the Mediterranean Network for the Study of Grapevine Closteroviruses (MNGC) fostered by IAM-BA came to life, whereby a wealth of information on the presence, relative incidence, and distribution of grapevine viruses was gathered. Commercial vineyards, nurseries, and varietal collections, which are often used as budwood sources for propagation, were the object of field investigations and sampling. Samples were collected at random, except in the course of sanitary selection programmes (mostly in Central and Southern Italy), when an effort was made to select as many apparently symptomless vines as possible. Since the early 80s, viruses were identified at the DPPM and IAM-BA from foliar tissues and, more recently, from cortical scrapings of mature canes by immunoenzymatic assays using standard DAS-ELISA protocols (Clark and Adams, 1977) or TAS-ELISA when monoclonal antibodies became available (Al Moudallal *et al.,* 1984). For GVA, ELISA plates were pre-coated with protein A (Boscia *et al.,* 1992), and biotynilated antibodies were sometimes utilised for the detection of some closteroviruses. The serological reagents for these tests were mostly produced by the DPPM. Currently, molecular assays (dot blot hybridization or PCR) accompany ELISA, or substitute for it whenever necessary. For example, due to the persistent unavailability of antisera, GRSPaV can only be detected by molecular assays. Because GRSPaV primers for PCR became available only recently (Minafra *et al.,* 1997) quantitative data on are still scanty.

Table 1. Virus infections in different Mediterranean countries

Countries	Samples tested	Infection rate by single viruses (%)										
		GVA	GVB	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-4	GLRaV-5	GLRaV-6	GLRaV-7	GRSPaV
Afghanistan (1)	5	40			0		0					
Albania (2); (3)	1311	42	1*	15	6		39				**12***	
Algeria (1)	10	60		30	10		80					
Armenia (1)	27	4	0	0	11	19	0				19	
Cyprus (4)	928				12		31	10				
France (5)	80				66	18	38					
Greece (6); (7)	557				49		55					
Italy, imported from Greece (8)	842	37	20	83	23		66				13	
Central-Southern Italy (9); (10); (11); (12); (13); (14)	5923	41	6*	52	15	25*	61			9*	0.5*	74*
Northern Italy (15); (16); (17); (18)	397	49*			57		28					
Jordan (19)	938	48	2	18	37		14			0*	0.3	
Lebanon (20)	1536	32	4	20	1		12					
Malta (21)	322	12*		40	42		61					
Morocco (1)	24	83	33	83	0		96				0	
Palestine (22)	566	66	4	16	46	8	22				0.2	
Portugal (23)	133	6	39		10	10	20	7	7		10	44
Spain (24)	2000	0		38	7		25					
Tunisia (25)	1010	55	10	47	30	18	72				0	
Turkey (26); (27); (28)	381	55*	3*	27*	35	12*	36			16*	4*	
Yemen (1)	130	23			1.5		3				0	

- value determined on a limited number of samples

References in table 1 *(notes)*

(1) G.P. Martelli *(unpublished information)*

(2) Merkuri *et al.*, 1994

(3) J. Merkuri and M. Digiaro *(surveys for sanitary selection; unpublished information)*

(4) Ioannou, 1993

(5) Walter and Zimmermann, 1991 *(samples with LR only)*

(6) Avgelis *et al.*, 1997 *(samples with LR only)*

(7) Katis *et al.*, 1991

(8) Boscia and Demarinis, 1998 *(cv. Victoria only)*

(9) Digiaro *et al.*, 1994

(10) Garau *et al.*, 1994a

(11) Faggioli *et al.*, 1997 *(surveys for sanitary selection)*

(12) V. Savino and M. Digiaro *(surveys for sanitary selection; unpublished data)*

(13) M. Digiaro and V. Savino *(survey of nearly introduced table grape cultivars; unpublished information)*

(14) A. Minafra *(GRSPaV detection by PCR - unpublished information)*

(15) Fortusini *et al.*, 1991

(16) Fortusini *et al.*, 1993

(17) Lenzi *et al.*, 1993 *(cv. Moscato bianco only)*

(18) Credi and Giunchedi, 1996

(19) Al-Tamimi *et al.*, 1998

(20) Haidar *et al.*, 1996

(21) Martelli *et al.*, 1992

(22) Alkowni *et al.*, 1998

(23) Mansinho *et al.*, 1999 *(data from PCR only)*

(24) Fresno *et al.*, 1997

(25) Mahfoudi *et al.*, 1998

(26) Ozaslan *et al.*, 1993

(27) Yilmaz *et al.*, 1997

(28) Koklu *et al.*, 1998

Table 1 summarises the outcome of the tests made over the last six years or so at the DPPM and IAM-BA and in other European and Mediterranean laboratories, on about 17,000 vines altogether. Unfortunately, the data available are incomplete, for not all the viruses were searched for in all samples. Moreover, because these data were obtained in different laboratories, with different reagents, or at different times, they may be biased by the variation in the efficiency of techniques and sensitivity of reagents that took place over time. For example, it is known that the serological detection of GLRaV-2, GVA, and GVB still poses problems (Boscia *et al.*, 1997a) and is less efficient than their molecular detection (by PCR in particular). Notwithstanding these limitations, Table 1 provides an enlightening scenario of the distribution and incidence of phloem-restricted viruses in many of the viticultural countries of the Mediterranean and Near East, confirming the alarming deterioration of the sanitary status of their grapevine industry. In particular, the striking infection levels by some closteroviruses (GLRaV-1 and GLRaV-3) and vitiviruses (GVA) (Fig. 1) reaffirms the widespread occurrence of leafroll and rugose wood throughout the Region, as determined by field surveys (Martelli, 1989). Furthermore, the high rate of PCR detection of GRSPaV in Portugal (59 of 133 samples = 44%) (Mansinho *et al.*, 1999) and Italy (91 of 123 samples = 74%) (A. Minafra, personal communication) unravels an alarming incidence of rupestris stem pitting, thus adding to the already remarkable presence of rugose wood in the area. GFkV is, on the whole, as widespread as some of the closteroviruses (GLRaV-1 and GLRaV-3) and vitiviruses (GVA). It is not known, however, if this is consequent only to dissemination of infected propagative material, or to both infected plant material and vector-mediated transmission, as with clostero- and vitiviruses. Finally, nothing is really known on the distribution and incidence of the two fleck-like viruses GAMaV and GRGV, a gap that will be filled shortly, now that virus-specific PCR primers have been designed (Sabanadzovic *et al.*, 1999).

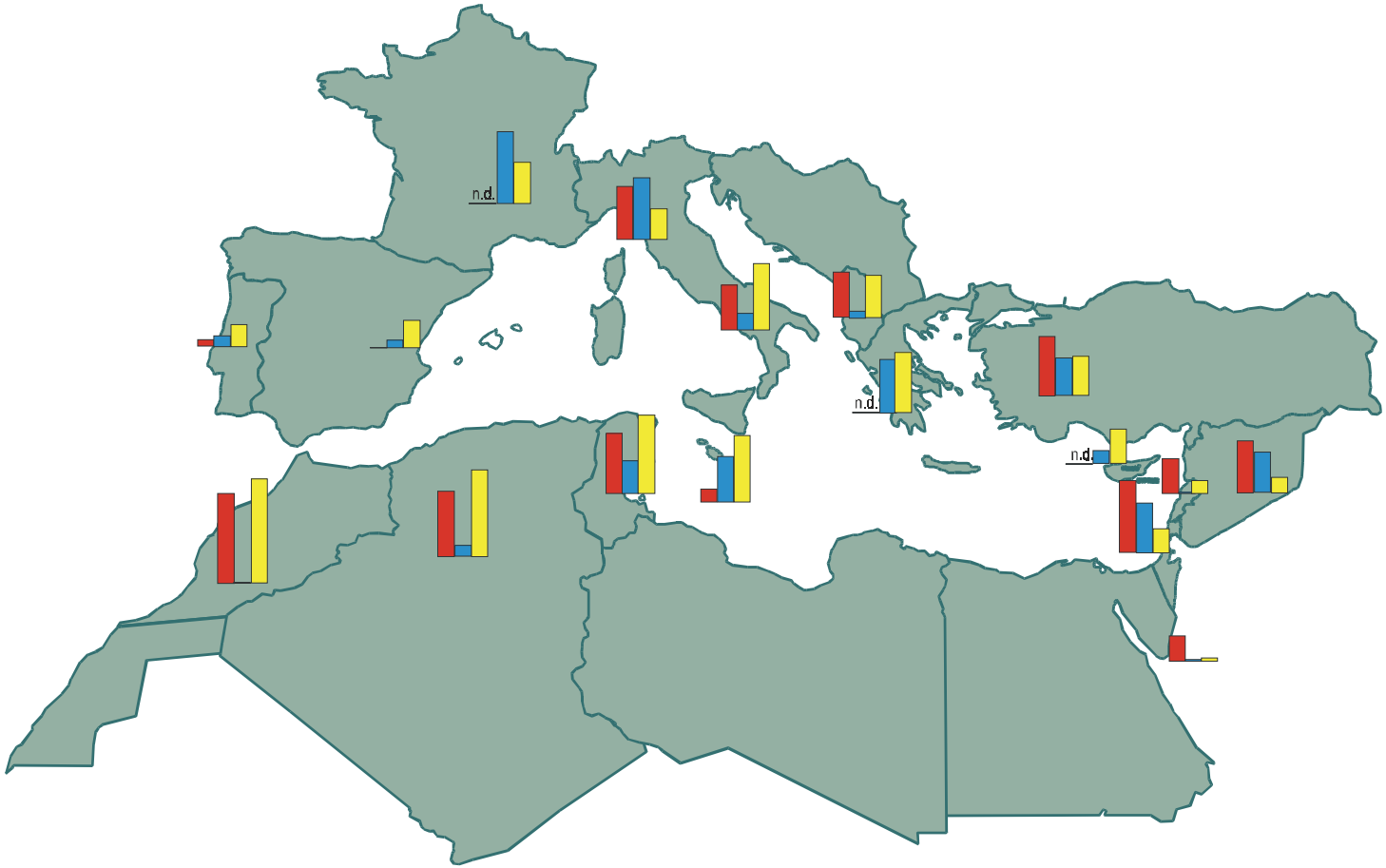

Fig. 1. Distribution of GVA ( ), GLRaV-1 ( ) and GLRaV-3 ( ) in different Mediterranean countries (n.d. = not determined)

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