

On the possible relationship between Kober stem grooving and grapevine virus A

by

R. GARAU¹), VANDA A. PROTA¹), ROBERTA PIREDDA¹), D. BOSCIA²) and U. PROTA¹)

¹) Istituto di Patologia Vegetale, Università degli Studi, Sassari, Italia

²) Dipartimento di Protezione delle Piante, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari, Italia

S u m m a r y: Investigations were carried out to establish possible correlations of two diseases of the rugose wood complex, i. e. Rupestris stem pitting (RSP) and Kober stem grooving (KSG) with grapevine virus A (GVA) and grapevine leafroll associated viruses I (GLRaV I) and III (GLRaV III). To this purpose 84 clonal accessions of different wine grape cultivars were analyzed by ELISA and by indexing onto the indicators *Vitis rupestris*, Kober 5BB and LN 33. The results obtained clearly indicated that none of the viruses taken into consideration is apparently involved in the etiology of RSP. Conversely, a remarkably close association of GVA with KSG was discovered.

K e y w o r d s: virus diseases, Kober stem grooving, closterolike viruses, indexing.

Introduction

Rugose wood (RW), a grapevine disease first reported in Italy (GRANITI and CICCARONE 1961), is now considered to have a worldwide distribution (BOVEY *et al.* 1980).

In Sardinia, RW is widespread, especially in the southern viticultural districts. Its presence was ascertained by visual observations (GARAU and PROTA 1973) and indexing to *Vitis* indicators carried out in the course of a certification programme (GARAU *et al.* 1989). RW is a complex disease, in which four different components were identified, i.e. Rupestris stem pitting (RSP), Kober stem grooving (KSG), Corky bark (CB) and LN stem grooving (LNSG), based on the differential reactions shown by the indicators *Vitis rupestris*, Kober 5BB and LN 33, following graft-inoculations (GARAU *et al.* 1989; SAVINO *et al.* 1989).

The nature of RW has not yet been ultimately defined although there is increasing evidence that it may have a viral etiology in which two clostero-like viruses, namely grapevine virus A (GVA) and grapevine virus B (GVB), now two tentative species in the *Trichovirus* genus (MARTELLI *et al.* 1993), may be implicated. However, neither of these viruses has been associated for certain with any of the four RW diseases. Considering that GVA was first isolated from a vine with RW symptoms (CONTI *et al.* 1980) it seemed logical to verify whether there was any correlation between RSP and KSG, which are by far the most frequently encountered components of RW, and GVA alone or in association with grapevine leafroll associated virus I (GLRaV I) and III (GLRaV III).

This paper presents the results of this study, a preliminar account of which has already been given (GARAU *et al.* 1993).

Material and methods

Object of this investigation were 84 vines of different cultivars subjected to sanitary selection. Donor vines were candidate clones belonging to 11 *Vitis vinifera* cultivars as

follows: 20 clones of cv. Vermentino, 16 of Malvasia di Bosa; 11 each of Pascale di Cagliari and Vernaccia; 10 of Cannonau; 4 each of Italia, Monica and Nieddera; 2 of Torbato; one each of Aleatico and Trebbiano.

All donors were indexed by omega grafting on *V. rupestris*, Kober 5BB and LN 33 and the symptoms (stem pitting and/or stem grooving) read three years afterwards on indicators that had been uprooted, autoclaved and peeled clean of cortex.

Individual donors were checked serologically (ELISA) for the presence of GVA, GLRaV I and GLRaV III using either commercial kits (Bioreba, Basel), or antisera (polyclonal and monoclonal) raised at Bari (BOSCIA *et al.* 1992).

The tests were repeated several times using petioles from mature leaves and/or cortical scrapings from dormant canes as antigen source. ELISA protocols for detection of GLRaV I and GLRaV III were applied according to manufacturer's instructions. The manufacturer's protocol for GVA was slightly modified by adjusting the dilution of antibodies and conjugate (1:750), the incubation time of the antigen (42 h at 4 °C) and the substrate (12 h before reading). The antisera raised at Bari were used according to protocol n° 5 of BOSCIA *et al.* (1992) in which ELISA plates were precoated with Protein A and a polyclonal antiserum was utilized for trapping and monoclonal antibodies for revealing the antigen.

Results and discussion

The results relative to indexing and virological analysis of the donors are reported in Tab. 1 and 2, whereas Tab. 3 shows the data, expressed as percent values, of the relative distribution of diseases and viruses.

None of the 84 donor vines indexed positive for corky bark or LN stem grooving, whereas 43 (ca. 51 %) proved to be affected by RSP and 16 (20 %) by KSG. Of these, however, 11 had also RSP infections. Apparently disease-

Table 1

Diseases of the rugose wood complex, i.e. Rupestris stem pitting (RSP) and Kober stem grooving (KSG), detected by indexing and their incidence

Varieties	Total	RSP	RSP+KSG	KSG	N° of donors assayed	
					positive reactions	no reaction
Aleatico	1	-	-	-	-	1
Cannonau	10	5	-	-	5	5
Italia	4	2	1	-	3	1
Malvasia di Bosa	16	9	1	-	10	6
Monica	4	3	-	-	3	1
Nieddera	4	1	-	-	1	3
Pascale di Cagliari	11	1	3	2	6	5
Torbato	2	-	-	2	2	-
Trebbiano	1	1	-	-	1	-
Vermentino	20	6	3	1	10	10
Vernaccia	11	4	3	-	7	4
Total	84	32	11	5	48	36
%	100	67	23	10	57	43

free vines (no reactions on any of the indicators) were 36 (ca. 43 %). RSP occurred with a relatively high percentage in almost all cultivars, whereas KSG was detected in cvs Pascale di Cagliari, Torbato, Vermentino and Vernaccia.

A similar proportion of the donor vines (> 72 %) that proved to be apparently free from diseases or were affected by RSP alone, were also apparently deprived of viruses. GVA alone or in mixed infections with GLRaV I was found in 9 % of the donors with RSP alone, and in 11 % of the apparently disease-free donors (3 % in single infection and 8 % in association with GLRaV III).

Higher percentages of GVA were detected in donors indexing positive for RSP and KSG, totalling 18 % in single infection and 55 % in mixed infections with GLRaV I and

III. An even higher incidence of GVA was ascertained in donors indexing positive for KSG alone. In this case, single infections were 20 % and mixed infections with GLRaV I and III were 80 %. Interestingly, none of GVA-free vines positive for KSG contained GLRaV I and III alone or in mixture.

In summary, whereas a high percentage (72 %) of donors inducing RSP symptoms only were apparently deprived of viruses (i.e. ELISA-negative), this proportion dropped considerably (9 %) when the donor vines were affected by both RSP and KSG and became zero when the affecting disease was KSG alone. Furthermore, GVA either by itself or together with leafroll-associated closteroviruses was present in 9 % of the donors apparently affected by

Table 2

Viruses detected by ELISA singly or in mixed infection

Varieties	Total	ELISA negative	ELISA positive	GLRaV			GVA			
				I	III	I + III	alone	+ GLRaV		
								I	III	I+III
Aleatico	1	1	-	-	-	-	-	-	-	-
Cannonau	10	6	4	-	3	-	-	-	1	-
Italia	4	3	1	-	-	-	-	-	1	-
Malvasia di Bosa	16	10	6	-	4	-	-	-	1	1
Monica	4	4	-	-	-	-	-	-	-	-
Nieddera	4	4	-	-	-	-	-	-	-	-
Pascale	11	4	7	-	1	-	2	1	2	1
Torbato	2	-	2	-	-	-	1	1	-	-
Trebbiano	1	1	-	-	-	-	-	-	-	-
Vermentino	20	14	6	-	3	-	1	-	2	-
Vernaccia	11	4	7	1	-	1	2	3	-	-
Total	84	51	33	1	11	1	6	5	7	2
%	100	61	39	3	34	3	18	15	21	6

RSP alone, 73 % of the donors reacting positive for RSP and KSG, and in the totality (100 %) of the donors inducing symptoms of KSG alone (Tab. 3).

These data, together with the lack of detection of leafroll-associated closteroviruses in ELISA-negative but KSG-affected candidate clones, are strongly indicative of the remarkably close association of GVA with Kober stem grooving. On the other hand, it seems plausible to conclude that none of the viruses considered in the present study

show any consistent association with RSP. It is, however, unclear whether KSG symptoms are influenced, and to what extent, by the presence of GLRaV I and III.

The present data are in very good agreement with, and lend support to, a recent study that reported the very close association (about 90 %) of GVA with KSG and the persistence of GVA in heat treated vines that had been freed from leafroll and related closterovirus, but were still affected by KSG (CHEVALIER *et al.* 1993).

Table 3

Viruses detected by ELISA in grapevine clones variously affected by Rupestris stem pitting (RSP) and Kober stem grooving (KSG). Figures are expressed in percent values

Diseases	ELISA negative	GLRaV			GVA			
		I	III	I + III	alone	+ GLRaV		
						I	III	I + III
RSP	72	-	16	3	6	3	-	-
RSP + KSG	9	-	18	-	18	18	28	9
KSG	-	-	-	-	20	40	20	20
No symptoms	75	3	11	-	3	-	8	-

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