Detection of Olive-Infecting Viruses in the Mediterranean Basin

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Abstract

Olive (*Olea europaea)* is host of 13 different viruses but, it is possible that other viruses, which are either non mechanically transmissible or occur in low concentration in plant tissues, are present in nature. This likelihood is supported by the widespread occurrence of double-stranded RNAs (dsRNAs) in plants negative to biological tests. As very little information is available on the geographical distribution of olive-infecting, investigations were conducted for gathering information on the prevalence and distribution of olive viruses in the Mediterranean basin based on the presence of double stranded RNA (dsRNA). A total of 527 samples were collected throughout the surveys covering 10 countries and 83 locations. Out of 286 tested samples collected from 6 Italian regions, 210 (73.4%) were dsRNA positive, whereas the average of viral infections in the Mediterranean basin was 64.5%. Molecular hybridisation tests, on 25 % of dsRNA-positive samples collected in Apulia, revealed the presence of the three nepoviruses (ArMV, CLRV and SLRSV), OLYaV and OLV-1.

INTRODUCTION

Olive (*Olea europaea)* is the host of 13 different viruses belonging in seven different genera, the majority of which cause latent infections, whose effect on the host is yet to be determined. However, the widespread occurrence of double-stranded RNAs (dsRNAs) also in symptomless plants negative to bioassays indicates that a number of other viruses, difficult to isolate are present in nature. Very little information is available on the geographical distribution of virus infecting olive in nature. Although most of the current records come from Italy and Portugal, the recovery of a number of viruses from Jordan, Turkey and Spain provided evidence that viruses occur in olive crops also elsewhere. A real problem resides in the lack of information on the prevalence and distribution of olive viruses and their mode of spreading in nature, which would be essential for a successful control. Because of this, we have analysed a large sample of olive plants coming from 10 Mediterranean countries, using as basic infection marker the presence of double stranded RNA (dsRNA). Molecular diagnosis (i.e. dot blot molecular hybridisation and RT-PCR) has also been used to study the distribution of the most important olive-infecting viruses in Apulia (Southern Italy). Data regarding the current sanitary status of olive crops in different Mediterranean countries are reported and commented upon.

MATERIALS AND METHODS

The number of samples to be collected was established according to information on crop distribution in the region, surface area covered, economic importance of the different varieties, types and origin of propagating materials, presence of nurseries and mother plant blocks, and age of the plants. Each sample consisted of 5-6 cuttings about 30 cm in length collected from one- to two-year old twigs from the quadrant of the tree. Samples were labelled and stored in plastic bags in the cold (4°C).DsRNAs extraction

procedure was carried according to Grieco et al. (2000). Eight primer pairs, specific to CMV, SLRSV, ArMV, CLRV, OLRSV, OLV-2, OLV-1 and OLYaV, were designed for RT-PCR (Grieco et al., 2000). RT-PCR was as described by Martelli et al. (1996). dsDNA fragments, specific to the eight above viruses and cloned in the *EcoRI/HindIII* sites of a pSPT64 vector, were transcribed in vitro to produce specific digoxigenin-labelled riboprobes (DIG-RNA Labelling Kit, Boehringer). dsRNA were extracted from 500 mg of olive cortical scrapings, denatured by treating with 50 mM NaOH and spotted onto a nylon membrane (Hybond N+, Amersham). Dot-blot hybridisation with Dig-riboprobes was carried out as described (Saldarelli et al., 1996).

RESULTS

A total of 527 samples was collected throughout the surveys covering 10 countries and 83 locations (Fig.1) and tested for the presence of dsRNA analysis (Fig.2). As shown in Table 1, out of 286 tested samples collected from 6 Italian regions, 210 (73.4%) were dsRNA positive, a very high percentage was considered very high by comparison with what was previously estimated on the base of bioassays. Surprisingly, a high level of infection was found in Apulia (80.1%) where *ca.* 32 % of Italian olives is grown (Godini and Murolo, 1997). Nearly the same percentage was found in Lazio (78.6%). The average of viral infection in the Mediterranean basin, was also relatively high (64.5%). The highest incidence of infection was observed in Tunisia, Italy, Lebanon and Palestine, while the lowest was in Albania, Malta and Cyprus. All the attempts to isolate any of olive viruses from symptomatic samples by mechanical inoculation failed. The sanitary status of some widespread varieties grown in different countries was seriously degraded. In Greece, the widely known varieties Kalamata and Pilion had the highest level of infection (75% and 70%, respectively). Molecular hybridisation tests were made on 25 % of dsRNA-positive samples collected in Apulia. Three nepoviruses ArMV(50%), CLRV (33.3%) and SLRSV (29.2%), as well as OLYaV (41.7%) and OLV-1 (8.3%) were detected, but not CMV, OLRSV and OLV-2.

DISCUSSION

To dismiss viral problems of olive as negligible is no longer possible. In fact, it conflicts with European Union specifications on quality of nursery productions (CAC), the implementation of the Italian voluntary certification scheme, and the increasing international demand for propagation material with high sanitary standard. The first step in meeting these requirements has been the assessment of olive sanitary status, in order to increase the knowledge on the prevalence and distribution of viruses in the countries where olive crop is dominant. The main field surveys were carried out in Italy where olive is prevailing. Samples were collected from different regions i.e. Apulia, Umbria, Sardinia, Tuscany, Latium and Abruzzo. The work was extended so as to cover most of Mediterranean basin where nearly 98% of the world's olive trees are grown (FAO, 1992). DsRNAs are considered as markers for viral infections and their analysis is invaluable for fishing out unknown viruses which cannot be sorted out and identified by traditional means (Martelli, 2000) and also proved to be useful for quick screening of quarantine material for determining the sanitary status (Grieco et al., 2000). By using dsRNA analysis, it was confirmed that the sanitary status of olive in the main Italian orchards is highly degraded (73.4%). This condition does not differ much from that of other Mediterranean countries, that had a mean viral infection of 64,5%. In conclusion, viral infections of olive were found to be more frequent than it was estimated on the basis of bioassays. The above findings give an insight of the distribution and prevalence of olive viruses and give useful hints for implementations of preventive measures, such as sanitary selection and sanitation, which are likely to be the only effective strategy to control the dissemination of these pathogens.

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Tables

Table 1. Presence of dsRNA in olive samples from the Mediterranean basin.

Country	**Samples**			**DsRNA positive**	
	Nr.	**Location**	**Varieties**	**Nr.**	**%**
ITALY	286	27	36	210	73,4
Apulia	*151*			*121*	*80,1*
Umbria	*58*			*31*	*53,4*
Sardinia	*35*			*25*	*71,4*
Tuscany	*20*			*14*	*70,0*
Latium	*14*			*11*	*78,6*
Abruzzo	*8*			*8*	*100*
GREECE	78	13	9	49	62,8
ALBANIA	37	7	12	9	24,3
EGYPT	32	9	14	16	50,0
LEBANON	30	5	5	21	70,0
SPAIN	24	3	6	10	41,7
PALESTINE	12	3	3	8	66,7
MALTA	12	5	1	5	41,7
TUNISIA	10	9	3	10	100
CYPRUS	6	2	3	2	33,3
TOTAL	527	83	(74)	340	64,5

Figures

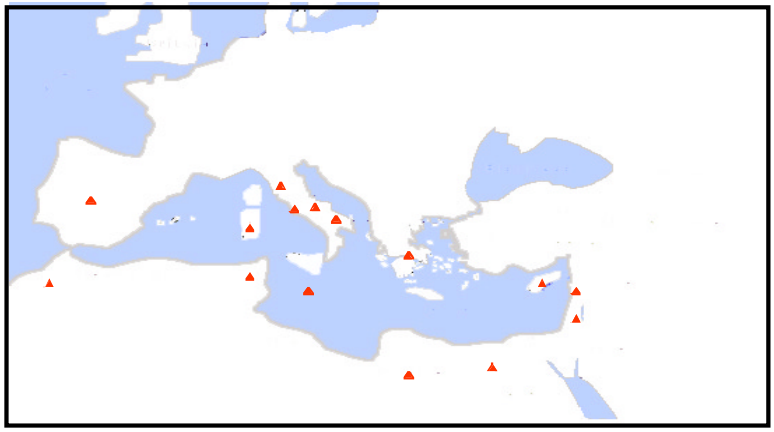

Fig. 1. Field survey and sample collections in the Mediterranean Basin.

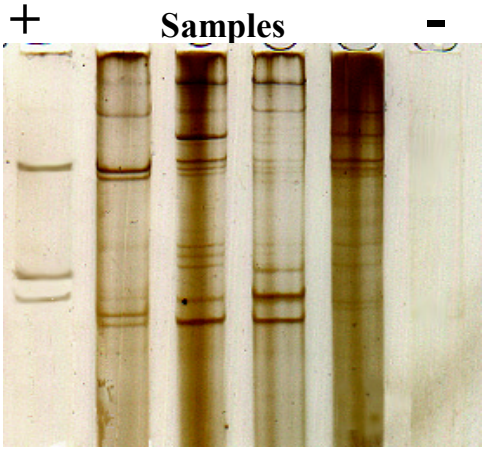

Fig. 2. Typical electropherogram of dsRNA extracted from cortical scapings of sampled olive trees. + = ds RNA extracted from OLV-1-infected *Nicotiana benthamiana*; - = dsRNA extracted from healthy olive.