Phytopathol. Mediterr. (2011) 50, 150–153

SHORT NOTES

Detection of viruses in olive trees in Croatian Istria

MARTA LUIGI[1,2], SARA GODENA[3], EDYTA ĐERMIĆ[4], MARINA BARBA[1] and FRANCESCO FAGGIOLI[1]

[1]CRA-Centro di Ricerca per la Patologia Vegetale, 00156 Roma, Italy
[2] Dipartimento di Gestione dei Sistemi Agrari e Forestali (GESAF) - Università degli Studi Mediterranea di Reggio Calabria, Loc. Feo di Vito, 89060 Reggio Calabria, Italy
[3] Institute of Agriculture and Tourism Poreč, Karla Huguesa 8, 52 440 Poreč, Croatia
[4] University of Zagreb, Faculty of Agriculture, Department of Plant Pathology, Svetošimunska 25, 10 000 Zagreb, Croatia

Summary. Following identification of four viruses in a general survey of olive trees throughout Croatia, a detailed survey was conducted in 2009 in the field collection of the Institute of Agriculture and Tourism in Poreč (an important reservoir of Istrian native olive germplasm) in order to evaluate the sanitary status of the most important Croatian Istria olive cultivars. Twenty five samples from symptomatic or symptomless trees were collected from five autochthonous and four exotic cultivars. All the samples were tested by RT-PCR for the presence of: *Olive leaf yellowing associated virus* (OLYaV), *Cherry leaf roll virus* (CLRV), *Strawberry latent ring spot virus* (SLRSV), *Arabis mosaic virus* (ArMV), *Olive latent virus-1* (OLV-1), *Cucumber mosaic virus* (CMV), *Olive latent virus-2* (OLV-2) and *Tobacco necrosis virus D* (TNV-D). Six of the 25 plants were found positive to CLRV; all infected plants showed leaf and fruit deformation and leaf yellowing. Four positive samples were from the native cv. Buža whereas the other two were from two exotic cultivars: Ascolana tenera and Frantoio. The presence of CLRV, either in native or imported plants, highlights the importance of strict phytosanitary regulations to prevent incursion of key virus diseases.

Key words: *Cherry leaf roll virus*, native olive germplasm, sanitary selection.

Introduction

Olive trees (*Olea europaea* L.) are among the most ancient and important fruit tree crops in Croatia, and there has been continuous expansion in the number of planted olive trees during recent years. In 2008, the production area had almost reached 15,000 ha (Anonymous, 2009a). There are six sub-regions of olive cultivation in Croatia: Istria, Kvarner and Primorje, North Dalmatia, Central Dalmatia, South Dalmatia and the Dalmatian hinterland.

Corresponding author: F. Faggioli
Fax:+39 06 82070246
E-mail: francesco.faggioli@entecra.it

Istria is the largest Croatian peninsula and is the north-eastern border of the olive cultivation area. The first description of Istrian olive varieties was by Carlo Hugues in 1902 (Hugues, 1999). Since 1994, the olive growing area has increased threefold and, according to the latest official statistical data, more than 600,000 olive trees are now being cultivated in Istria (Anonymous, 2008). The autochthonous Istrian olive varieties, along with the Mediterranean climate and the favourable geographic position, allow for the production of recognizably high-quality olive oil. For this reason, a phytosanitary evaluation of the most widespread local varieties becomes very important. Phytosanitary status of olive plants in Istria is still

unknown. The Istrian olive germplasm consists of about 30 local varieties; the most widespread in old plantations are presented in Figure 1. There are about twenty registered nurseries in Croatia and five of them are situated in Istria (Anonymous, 2009b). From 1999 to 2003, 12,890 olive grafted plants of the autochthonous Istrian olive variety 'Buža' were produced, along with 9,496 plants of the 'Istarska bjelica' variety and 2,850 plants of the 'Rosulja' variety (Bulimbašić, 2004).

Regulation NN 100/09 of the Croatian Ministry of Agriculture, Fisheries and Rural Development (Ministry of Agriculture, Fisheries and Rural Development, 2009) defines which pathogens must be absent from the propagative material in accordance with the EPPO Standard PM 4/17(2) (OEPP/EPPO, 2006). This Regulation foresees that the olive propagative material and seedlings of the CAC category should at least be checked for disease symptoms. Furthermore, the grafted plants should be free from harmful organisms which could affect their quality. Moreover, the propagative material should not show any disease-like symptoms caused by some harmful organisms such as: insect *(Saissetia oleae)*, mite (*Eusophera pinguis)*, nematodes (*Meloidogyne* spp.), olive knot bacterium (*Pseudomonas savastanoi)*, fungus (*Verticillium dahliae)* and all viruses and virus-like organisms. Furthermore, plants should be grown using soil free from nematodes belonging to the genera *Longidorus* and *Xiphinema*.

Because of the great importance of olive in Croatia, a survey of the phytosanitary status of olive trees was conducted in 2005 and 2006. As far as we know, this was the first analysis that focused on the presence of olive viruses in Croatia. The survey included assays for eight viruses: OLYaV (Savino *et al*., 1996), CLRV (Savino and Gallitelli, 1981), SLRSV (Savino *et al*., 1979), ArMV (Savino *et al*., 1979), OLV-1 (Gallitelli and Savino, 1985), CMV (Savino and Gallitelli, 1983), OLV-2 (Savino *et al*., 1984) and OLRSV (Savino *et al*., 1983). The study was carried out on olive orchards in 25 different locations across Croatia. The level of virus infection was of about 25% (at eight locations). In total, four viruses were detected: OLYaV, SLRSV, CMV and OLV-2. In Istria, two positive samples were found on two varieties: 'Istarska bjelica' (OLYaV and CMV) and 'Bjelica' (OLYaV) (Bjeliš *et al*., 2007).

Based on this knowledge, our study focussed only on the Istrian native cultivars and some other cultivars introduced into the region. The sampled olive germplasm was collected from trees that were 15–20 years old from the field collection of the Institute of Agriculture and Tourism

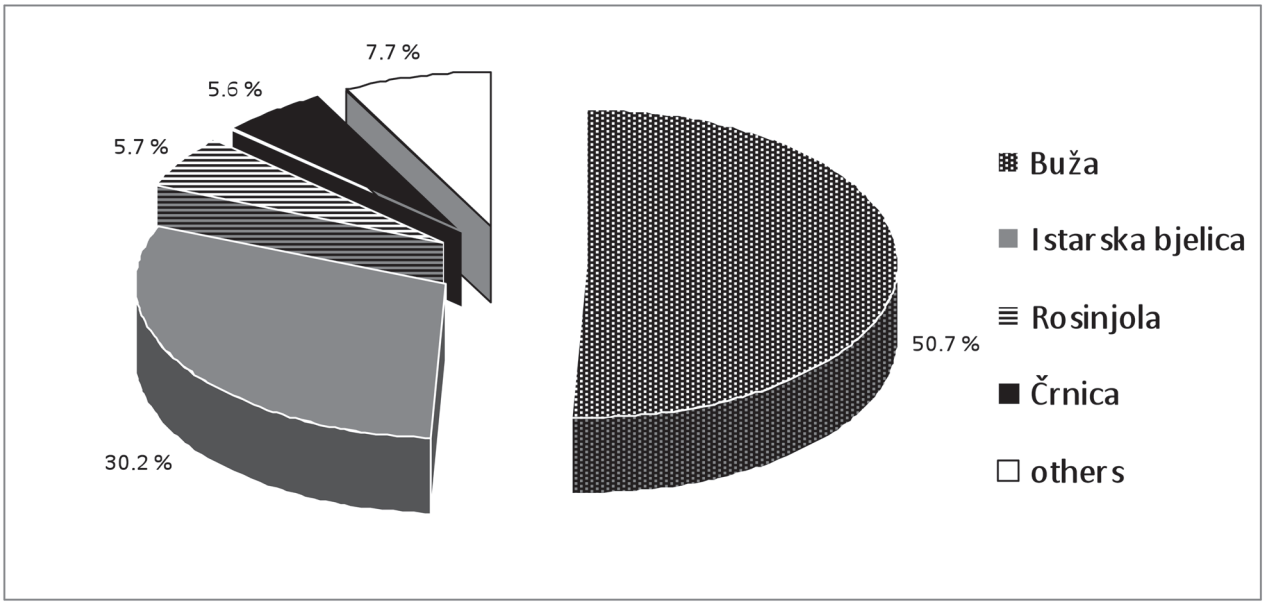

Figure 1. Proportion of area in production of the local olive varieties in Croatian Istria (Pribetić, 2006).

in Poreč (an important reservoir of Istrian native olive germplasm). Within the field collection, the most important Istrian cultivars were selected, and samples were collected (one to three trees of each variety) either from symptomatic or symptomless plants. The survey included 25 source trees belonging to five local varieties and four introduced varieties (Table 1). Alongside these, four young trees of the 'Buža' variety about 4 years of age were also sampled. For virus detection, eight different shoots located in eight zones around the tree canopy of each individual plant were used as sources of phloem tissue. Samples were pulverized with a mortar/pestle in liquid nitrogen. Total RNA extraction was performed starting from 0.1 g of tissue per sample and using RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's protocol. All collected samples were analyzed for the presence of the following viruses: OLYaV, CLRV, SLRSV, ArMV, CMV, OLV-1, OLV-2 and *Tobacco necrosis virus* strain D (TNV-D) (Cardoso *et al.*, 2004) through a one-step RT-PCR protocol (Faggioli *et al*., 2005) using virus specific primers (Loconsole *et al*., 2007).

All of the samples collected from the field collection of the Institute of Agriculture and Tourism in Poreč were found to be free of viruses, except for samples from 'Frantoio', 'Ascolana tenera' and four young 'Buža' trees which were found positive for CLRV (Table 1).

In order to investigate the virus distribution within individual trees, shoots collected from eight zones around the entire tree canopy of each CLRV infected plant were separately analyzed. Results confirmed the uneven distribution of viruses within the infected plants (Loconsole *et al*., 2007), and emphasised the need for accurate sampling procedures to increase the reliability of the detection.

This is the first report of olive trees infected by CLRV in Croatia. Moreover this is the first evidence of a possible association of specific symptoms (yellowing and deformation) to CLRV in olive. CLRV (genus *Nepovirus*, family *Comoviridae)* is included in the list of plant viruses that should be closely monitored during selection of propagation material, mainly for walnut and olive trees, due to the capability of this virus for wide propagation and dispersal transmission by seed and pollen (Rumbou *et al.,* 2009).

It seems that most of the mother plants currently utilized in Croatia have been selected regardless to their phytosanitary condition; plants were only visually inspected. Since sanitary selection based exclusively on visual examination is not effective due to the widespread occurrence of latent infections, laboratory tests are necessary to give full description of the health status of plants. Among different techniques, PCR-based methods have proved to be the most effective for identification and detection of olive viruses.

The low virus incidence found in Istria contra-

Table 1. Plant material assayed for presence of viruses in this study. The numbers in parentheses indicate positive samples.

Variety	No. of samples
Bova	3
Buža	7 (4)
Buža puntoža	3
Istarska bjelica	3
Rosinjola	3
Ascolana tenera	2 (1)
Frantoio	2 (1)
Itrana	1
Moraiolo	1
Total	25

dicts the results of surveys carried out in other Mediterranean countries, where virus infection has reach about 50% of the trees tested (Saponari *et al*., 2002; Al Abdullah *et al*., 2005; Fadel *et al*., 2005). From a phytosanitary perspective, Istrian olive germplasm should be preserved and protected to maintain high health status. This is particularly important because of the high quality extra virgin oil that can be produced from the region due to specific prevailing agronomic conditions. The presence of CLRV, which was detected by this work either in native or imported plants, highlights the importance of implementing strict phytosanitary regulations. This may be achieved by preventing the import of non-certified olive plant material and by developing and qualifying domestic olive propagation material in accordance with international regulations. For this purpose, virus-free stock materials have been identified in this study. These source plants can be used to set up an effective certification system.

Literature cited

Al Abdullah A., T. El Beaino, M. Saponari, H. Hallak and M. Digiaro, 2005. Preliminary evaluation of the status of olive-infecting viruses in Syria, *Bulletin OEPP/EPPO Bulletin* 35, 249–252.

Anonymous, 2008. Central Croatian Bureau of Statistics, *http://www.dzs.hr*.

Anonymous, 2009a. Statistical Yearbook of the Republic of Croatia, http://www.dzs.hr/Hrv_Eng/ljetopis/2009/PDF/15-bind.pdf

Anonymous, 2009b. Proizvođači voćnog i loznog sadnog materijala. Zavod za sjemenarstvo i rasadničarstvo, Osijek, *http://www.zsr.hr*.

Bjeliš M., G. Loconsole and M. Saponari, 2007. Presence of viruses in Croatian olive groves. *Pomologia Croatica* 13, 165–172.

Bulimbašić S., 2004. Proizvodnja sadnica, Maslina časopis za maslinarstvo i uljarstvo, br.1, 16–17.

Cardoso J.M.S., M.R. Felix, S. Oliveira and M.I.E. Clara, 2004. A tobacco necrosis virus D isolate from Olea Europea L.: viral characterization and coat protein sequence analysis. *Archives of Virology* 149, 1129–1138.

Fadel C., M. Digiaro, E. Choueiri, T. El Beaino, M. Saponari, V. Savino and G.P. Martelli, 2005. On the presence and distribution of olive viruses in Lebanon, *Bulletin OEPP/EPPO Bulletin* 35, 33–36.

Faggioli F., L. Ferretti, G. Albanese, R. Sciarroni, G. Pasquini, V. Lumia and M. Barba, 2005. Distribution of olive tree viruses in Italy as revealed by one-step RT-PCR. *Journal of Plant Pathology* 87, 49–55.

Gallitelli D. and V. Savino, 1985. Olive latent virus 1. A single spherical virus isolated from olive in Apulia (southern Italy). *Annals of Applied Biology* 106, 295–303.

Hugues C., 1999. Maslinarstvo Istre - Elaiografia Istriana, CERES, Zagreb (manuscript transcribed and translated by M. Manin)

Loconsole G., M. Saponari, F. Faggioli, G. Albanese, H. Bouyahia, T. Elbeaino, M. Nuzzaci, V. Prota, G. Romanazzi and V. Savino, 2007. Standardization of common protocol for olive virus detection. *Journal of Plant Pathology* 89, S44–45.

Ministry of Agriculture, Fisheries and Rural Development, 2009. Pravilnik o stavljanju na tržište reprodukcijskog sadnog materijala i sadnica namijenjenih za proizvodnju voća, Narodne novine (NN 100/09).

OEPP/EPPO, 2006. EPPO Standards PM 4/17(2) Schemes for the production of healthy plants for planting. *OEPP/EPPO Bulletin* 36, 77–83.

Pribetić Đ., 2006. Sorte maslina u Istri, MIH, Poreč.

Rumbou A., S. von Bargen, C. Büttner, 2009. A model system for plant-virus interaction-infectivity and seed transmission of Cherry leaf roll virus (CLRV) in Arabidopsis thaliana. *European Journal of Plant Pathology* 124, 527–532.

Saponari M., R. Alkowni, F. Grieco, N. Driouech, M. Hassan, B. Di Terlizzi, 2002. Detection of Olive-infecting virus in the Mediterranean basin. *Acta Horticolturae* 586, 787–790.

Savino V. and D. Gallitelli, 1981. Cherry leaf roll in olive. *Phytopathologia Mediterranea* 22, 76–77.

Savino V. and D. Gallitelli, 1983. Isolation of Cucumber mosaic virus from Olive in Italy. *Phytopathologia Mediterranea* 22, 76–77.

Savino V., D. Gallitelli and M. Barba, 1983. Olive latent ringspot virus, a newly recognized virus infecting olive in Italy. *Annals of Applied Biology* 103, 243–249.

Savino V., M. Barba, D. Gallitelli and G.P. Martelli, 1979. Two nepoviruses isolated from olive in Italy. *Phytopathologia Mediterranea* 18, 135–142.

Savino V., P. Piazzola, A. Di Franco and G.P. Martelli, 1984. Olive latent virus-2, a newly recognized virus with differently shake particles. In: *Proceeding of the 6th Congress of the Mediterranean Phytopathological Union*, Cairo, Egypt, 1984, 24–26.

Savino V., S. Sabanadzovic, G. Scarito, C. La Viola and G.P. Martelli, 1996. Due giallumi di possibile origine virale in Sicilia. *Informatore Fitopatologico* 46(5), 55–59.

Accepted for publication: January 31, 2011