Short Communication

INCIDENCE OF VIRUSES ON AUTOCHTHONOUS AND INTRODUCED OLIVE VARIETIES IN CROATIAN ISTRIA DETECTED BY THREE DIAGNOSTIC TECHNIQUES

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SUMMARY

A survey on viruses infecting olive trees was performed in Croatian Istria. Major olive viruses were detected using molecular (RT-PCR), biological (bioassay on indicator plants) and serological (DAS-ELISA) diagnostic techniques. Specifically, fifteen olive varieties from a total of 62 olive trees have been tested for the presence of eight viruses. *Cherry leaf roll virus* (CLRV), *Strawberry latent ring spot virus* (SLRSV) and *Olive latent virus*-1 (OLV-1) were detected by one-step RT-PCR in ten autochthonous and five introduced olive varieties. The most frequent found was CLRV, detected in 11.3% of tested trees both in autochthonous and in introduced varieties, while OLV-1 and SLRSV were detected only in autochthonous varieties and in lower incidence. In order to confirm the results obtained by one-step RT-PCR, inocula prepared from the flowers were used for inoculation of herbaceous indicator plants. Symptoms on herbaceous hosts were monitored and CLRV and SLRSV were detected by DAS-ELISA, confirming the results of molecular test. The most successful mechanical transmission was on *Chenopodium quinoa*, while the most evident symptoms were observed on *Cucumis sativus* L. cv. Sunčani potok plants.

Keywords: bioassay, CLRV, ELISA, olive, RT-PCR, SLRSV.

Istria is the largest Croatian peninsula and the northernmost growing sub-region of olive cultivation. In Istria olive growing is spreading increasingly. Like other crops, olive is a host of a great number of pathogens including viruses. The first reports of viruses infecting olive trees date from 1979 when *Strawberry latent ring spot virus* (SLRSV) and *Arabis mosaic virus* (ArMV) have been found in Italy (Savino *et al*., 1979). Successively, the largest number of findings has come from Italy, Portugal and Spain and to date, 15 virus species belonging to nine genera have been found in olive. Among them eight most important and most frequent in olive trees are: the nepoviruses ArMV and *Cherry leaf roll virus* (CLRV), the necroviruses *Tobacco necrosis virus* (TNV) and *Olive latent virus*-1 (OLV-1), the sadwavirus SLRSV, the closterovirus *Olive leaf yellowing associated virus* (OLYaV), the Cucumovirus *Cucumber mosaic virus* (CMV) and the oleavirus *Olive latent virus*-2 (OLV-2) (Fernandes Félix and Clara, 2006). There are just three reports of olive viruses in Croatia that highlighted the presence of SLRSV, OLV-2, OLYaV, CLRV and CMV (Bjeliš *et al*., 2007; Luigi *et al*., 2011; Godena *et al*., 2012). In this paper the results of analyses done on 62 olive trees from Croatian Istria are reported.

In 2009 and 2010, samples of olive shoots from the olive collection field of the Institute of Agriculture and Tourism in Poreč (Istria, Croatia) were collected. In total 15 olive varieties have been tested, 10 autochthonous: 'Istarska bjelica', 'Moražola', 'Črnica', 'Črna', 'Bjankera', 'Bova', 'Rosinjola', 'Buža puntoža', 'Buža' and 'Bjelica' and five introduced: 'Moraiolo', 'Itrana', 'Ascolana tenera', 'Frantoio' and 'Leccino'. For isolation of total RNA, RNeasy Plant Mini Kit (Qiagen, Germany) has been used and eight previously mentioned viruses have been tested according to the protocol by Loconsole *et al.* (2010). Moreover, field symptoms on olive trees have been observed and documented from 2009 to 2011.

In order to confirm the results obtained in molecular diagnostic technique, flowers from the RT-PCR positive trees were used for mechanical inoculation of 256 indicator plants grown in green house under controlled conditions using a phosphate buffer (pH 7.2; 0.1 M PO_4 + 1% ascorbate). Specifically, there were seven isolates of three viruses, denominated CLRV-A (A – virus isolate from

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Table 1. Results of molecular diagnostic (RT-PCR) on autochthonous and introduced olive varieties.

Variety	No. of analyzed trees	No. of infected trees	Percentage of infected trees (%)	No. and percentage of trees infected with CLRV (%)	No. and percentage of trees infected with OLV-1 (%)	No. and percentage of trees infected with SLRSV (%)
Autochthonous	51	10	19.6	5 (9.8)	3 (5.9)	2 (3.9)
Introduced	11	2	18.2	2 (18.2)	0	0
Total	62	12	19.4	7 (11.3)	3 (4.8)	2 (3.2)

Table 2. Symptoms observed on different varieties of olive trees in which viruses have been detected.

Symptoms / Variety	'Rosinjola'	'Frantoio'	'Buža' seedling	'Ascolana tenera'	'Buža puntoža'	'Buža'
Yellowing or yellow spots	+	+	+	–	+	+
Jaggies and sickle leaves	–	+	–	–	–	+
Defoliation	–	–	+	–	–	–
Irregularities of shoots branching	–	+	–	–	+	–
Ripening delay	+	–	–	–	–	–
Bifurcations	–	+	–	+	–	+
Deformations or twisted leaves	+	–	–	+	+	+
Virus isolate	CLRV-R	CLRV-F	CLRV-Bs	CLRV-A	SLRSV-Bp	SLRSV-B

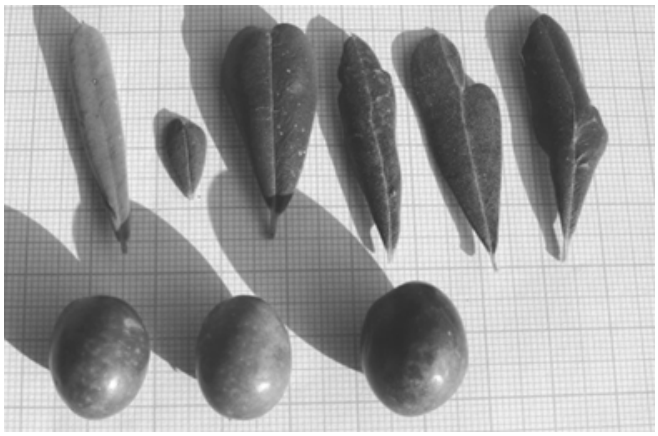

Fig. 1. Fruits and leafs of olive variety 'Rosinjola' infected with *Cherry leaf roll virus* (CLRV-R) showing deformation and yellowing of leaves and univen fruit ripening.

variety 'Ascolana tenera'), CLRV-F (F – isolate from 'Frantoio'), and CLRV–Bs (Bs – 'Buža' seedlings); OLV-1-R (R – 'Rosinjola') and OLV-1-Bp (Bp – 'Buža puntoža'); SLRSV-B and SLRSV-Bp (B – 'Buža'; Bp – 'Buža puntoža'). The intensity of symptoms, the type of symptoms and the number of infected leaves were checked every two to seven days.

Leaves of symptomatic inoculated plants and flowers of infected olive trees were tested by ELISA (Clark and Adams, 1977) using commercial antisera (Loewe Biochemica, Sauerlach, Germany). All tests were performed according to manufacturer's instructions.

In total, 12 out of 62 olive trees tested positive to CLRV, OLV-1 and SLRSV by RT-PCR, whereas ArMV, TNV, OLYaV, CMV and OLV-2 were not found. The most frequent virus was CLRV, which was detected in autochtonous ('Buža' and 'Rosinjola') and introduced ('Ascolana tenera' and 'Frantoio') varieties. Precisely, CLRV was detected in four samples from seedlings of 'Buža' variety and in one sample from the varieties 'Frantoio', 'Ascolana tenera' and 'Rosinjola'. Detailed description of virus infection is given in Table 1. In other countries the percentage of this virus varied from 0.5% in Spain (Bertolini *et al*., 2001) to 33.3% in Italy (Saponari *et al*., 2002).

OLV-1 and SLRSV were detected with lower incidence and only on autochthonous varieties. SLRSV was detected on two samples of varieties 'Buža puntoža' and 'Buža', and OLV-1 in 'Rosinjola', 'Buža' and 'Buža puntoža'. The percentage of infection of OLV-1 in other countries varied from 5.7% in Egypt (Youssef *et al*., 2010) to 34.3% in Tunisia (El Air *et al*., 2010), while incidence of SLRSV varied from 0.3% in Lebanon (Fadel *et al*., 2005) to 29.2% in Italy (Saponari *et al*., 2002). This virus was previously detected in Croatia with an incidence of 6.7% (Bjeliš *et al*., 2007).

Different types of symptoms have been observed associated to specific virus infection (Table 2 and Fig. 1). No symptoms were observed on 'Buža puntoža' and 'Rosinjola' found infected by OLV-1 infection. In bioassay, the viruses, from flowers of RT-PCR positive trees were transmitted on seven different herbaceous indicator species (Table 3). A great variety of types and severity of symptoms were observed (Fig. 2 and Fig. 3). The success of the transmission was confirmed by DAS-ELISA for CLRV and SLRSV. The most successful transmission of CLRV and SLRSV was on *Chenopodium quinoa* in which in the case of isolate OLV-1-Bp infection, unusual shape of leaves reminding the shape of an olive leaf was observed (Fig. 3). Inoculated plants of *Cucumis sativus* L. cv. Sunčani potok reacted with the strongest symptoms for all viral isolates. *C. sativus* L.

Fig. 2. Symptoms of twisted leaves on *Chenopodium quinoa* Willd. caused by CLRV-Bs isolate.

Fig. 3. Deformed leaves of test plant *Chenopodium quinoa* Willd. induced by OLV-1-Bp isolate.

Table 3. Herbaceous indicator plants (used in biotest and DAS-ELISA) and olive flowers (used in DAS-ELISA). CLRV and SLRSV have been detected by DAS-ELISA (n.t. – not tested).

Indicator plant and flowers of different olive varieties	No. of plant in biotest	Virus isolate tested	N° infected/N° total in DAS-ELISA	Percentage (%)
Chenopodium quinoa Willd.	43	CLRV (-Bs, -A, -F)	4/19	21.0
		OLV-1 (-Bp, -R)	n.t.	–
		SLRSV (-B, -Bp)	7/13	53.8
Cucumis sativus L. cv. Dugi zeleni	39	CLRV (-Bs, -A, -F)	0/17	0
		OLV-1 (-Bp, -R)	n.t.	–
		SLRSV (-B, -Bp)	0/13	0
Cucumis sativus L. cv. Pariški kornišon	41	CLRV (-Bs, -A, -F)	0/17	0
		OLV-1 (-Bp, -R)	n.t.	–
		SLRSV (-B, -Bp)	0/14	0
Cucumis sativus L. cv. Sunčani potok	13	CLRV (-Bs)	0/2	0
		SLRSV (-Bp)	1/7	14.3
Nicotiana benthamiana Domin.	16	OLV-1 (-Bp)	n.t.	–
		SLRSV (-Bp)	0/6	0
		CLRV (-A)	0/5	0
Nicotiana glutinosa L.	40	OLV-1 (-Bp, -R)	n.t.	–
		SLRSV (-B, -Bp)	0/17	0
Nicotiana tabacum L. cv. Burley	64	CLRV (-Bs, -A, -F)	2/27	7.4
		OLV-1 (-Bp, -R)	n.t.	–
		SLRSV (-B, -Bp)	0/18	0
Variety 'Buža'	–	CLRV-Bs	0/1	0
Variety 'Ascolana tenera'	–	CLRV-A	0/1	0
Variety 'Frantoio'	–	CLRV-F	0/1	0
Variety 'Buža'	–	SLRSV-B	0/1	0
Variety 'Buža puntoža'	–	SLRSV-Bp	1/1	100.0
TOTAL	256	CLRV	6/90	6.7
		SLRSV	9/90	10.0

cv. Sunčani potok showed to be the most sensitive to virus infections while *Nicotiana glutinosa* showed the lowest symptoms expression for all viral isolates. The isolate SLRSV-Bp induced average peak intensity of symptoms in all indicator plants (on five plants it induced the maximum intensity of symptoms and on four indicator plants the symptoms were observed on all leaves). This isolate induced the greatest number of infected leaves per total leaves number on *C. sativus* cv. Sunčani potok, while on indicator plant *C. sativus* cv. Dugi zeleni it induced the lowest number of infected leaves. The isolate CLRV-A had the lowest pathogenic potential observed in this survey on *C. sativus* cv. Dugi zeleni, since it induced the maximum of intensity of symptoms only once, but no plants developed symptoms on all leaves. In general, systemic symptoms appeared on *C. quinoa*, *C. sativus* cv. Pariški kornišon,

N. benthamiana and *N. glutinosa*, while local symptoms appeared on *C. sativus* cv. Dugi zeleni, *C. sativus* Sunčani potok and *N. tabacum* cv. Burley.

ELISA was performed on in total 180 samples (Table 3). The results confirmed both the precence of the two viruses and species for which *C. quinoa* has been the most successful indicator plant (11 positive samples). SLRSV-B resulted as the isolates with the highest transmission capability.

Various considerations arise from the analysis of the obtained results. First, the Istrian olive germplasm analyzed during this survey seems to be quite healthy. Only 19.4% of tested trees were infected; this, in comparison to some other olive growing countries, is a quite encouraging result. Indeed, in other countries, the average percentage of infestation was much higher: 34% in Lebanon (Fadel *et al*., 2005); 43% in Siria (Al Abdullah *et al*., 2005); from 32.8% (Faggioli *et al*., 2005) to 73.4% (Grieco *et al*., 2002) in Italy; 86.3% in Tunisia (El Air *et al*., 2010) and 93.8% in California (Al Rwahnih *et al*., 2011). In addition, the results obtained in one-step RT-PCR were also confirmed by biological and serological assays. This is very important considering the difficulty generally found in managing olive tree samples for viral diagnosis.

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