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SHORT NOTES

Phytosanitary evaluation of olive germplasm in Albania

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Summary. A survey on viruses was carried out in 2008 in the main olive-growing areas of Albania (Kruja, Sauk and Vlora). Fifty samples from 14 local and 2 exotic olive cultivars were collected from 10 commercial orchards and one collection field and inspected for *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Strawberry latent ringspot virus* (SLRSV), *Olive latent virus 1* (OLV-1), *Olive leaf yellowing-associated virus* (OLYaV), *Cucumber mosaic virus* (CMV), *Olive latent virus-2* (OLV-2) and *Tobacco necrosis virus* strain D (TNV-D) by a one-step RT-PCR assay using virus-specific primers. None of these viruses were found in the source plants except SLRSV and OLYaV, which were detected in a 'K.M. Berat' olive tree grown in the collection field. These findings are important because the incidence of olive virus diseases is low in Albania but high in other Mediterranean countries. Thus, all efforts should be to directed to maintaining the Albanian olive germplasm pathogen-free and in the best agronomical and phytosanitary condition possible.

Key words: olive cultivars, viruses, phytoplasmas, sanitary selection.

Introduction

Albania has a climate suitable for olive (*Olea europaea* L.). Olive is an ancient crop dating back to the first century AD in Albania. After World War II, olive production in Albania has become affected by the political and economic situations that have prevailed over the years (Fig. 1) (Agolli, 2000; Statistical Yearbook, 2002, 2005, 2006). During the Communist regime, olive growing was a state monopoly, and acreage reached its highest level in 1990. In the nineties, however, privatization reduced the acreage by about 50%. This was harmful since Albania possesses valuable olive germplasm of more than 20 local cultivars, most of which are apparently native (Belaj *et al*., 2003). More than 90% of these cultivars produce fruits with a high oil content and 70% have a good pulp-to-stone ratio. The high quality of the olive oil and a recent increase in olive production has resulted in a resurgence of the Albanian olive industry. This has led to the certification of extra-virgin olive oil and the production of organic olives because neither pesticides nor fertilizers have been used since the nineties.

To improve the quality of Albanian olive oil, primary-source olive plants with reliable genetic and sanitary status must be identified and preserved. Pre-

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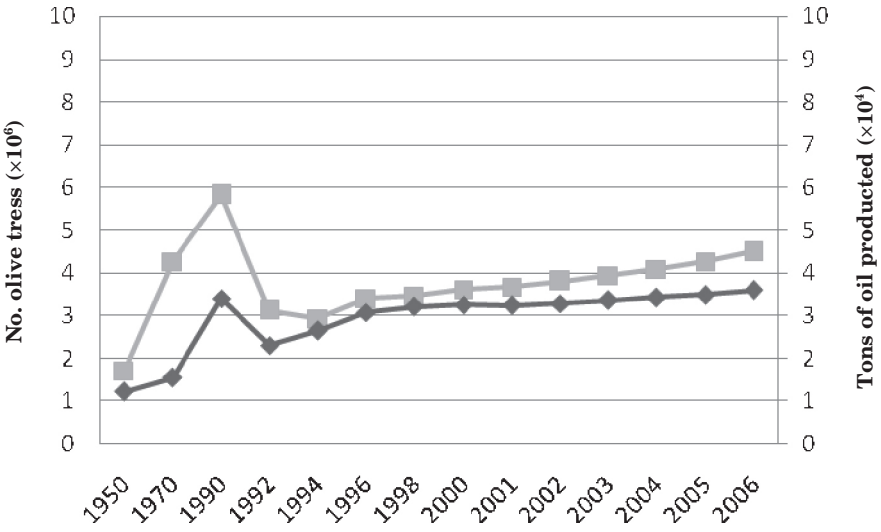

Fig. 1. Olive growing trends in Albania from 1950 to 2006. ■= No. of olive trees, ◆ = tons of oil produced. (Statistical Yearbook 2002, 2005 and. 2006. Albanian Ministry of Agriculture, Food, Directory of Statistic)

viously, some actions were undertaken concerning the agronomical and genetic characterization of Albanian olive cultivars (Kafaki, 1965 and 1980; Thomaj and Panajoti, 2004). However, little is known about the phytosanitary status of primary source plants. The Albanian certification program is similar to most of the EU regulations, in stating that propagation material must be free from any graft-transmissible pathogens (Albanian law No. 9362 March 23, 2005: 'Mbi sherbimin e mbrojtjes se bimeve').

With this end in view, a survey was conducted in the main olive-growing areas of Albania (Kruja, Sauk and Vlora) to identify pathogen-free olive cultivars for the certification program. This survey included 50 samples from 14 local and 2 exotic olive cultivars.

The Albanian olive certification program does not clearly stipulate which pathogens must be absent from the propagation material. Therefore, samples were collected only from trees that did not show symptoms of phytoplasma, olive knot (*Pseudomonas savastanoi* pv. *savastanoi*) or verticillium wilt (*Verticillium dahliae*). The samples were analyzed for eight viruses according to the regulations of the Italian Ministry of Agriculture (DM 20/11/06). These regulations provide for two certified categories: virus-tested (VT) and virus-free (VF). The VT category includes five viruses: *Olive leaf yellowing associated virus* (OLYaV) (Savino *et al*., 1996), *Cherry leaf roll virus* (CLRV) (Savino and Gallitelli, 1981), *Strawberry latent ring spot virus* (SLRSV) (Savino *et al*., 1979), *Arabis mosaic virus* (ArMV) (Savino *et al*., 1979) and *Olive latent virus-1* (OLV-1) (Gallitelli and Savino, 1985). The VF category includes these five viruses plus three more: *Cucumber mosaic virus* (CMV) (Savino and Gallitelli, 1983), *Olive latent virus-2* (OLV-2) (Savino *et al*., 1984) and *Tobacco necrosis virus* strain D (TNV-D) (Cardoso *et al*., 2004). The regulation stipulates that the olive samples must be tested using one-step RT-PCR. All olive samples must also be tested for phytoplasmas. The VF and phytoplasma-free source olive trees identified in the present survey can now be used to produce, for the first time in Albania, primary sources from which certified pathogen-free propagation material can be produced.

Materials and methods

Field survey and collected material

The olive germplasm sampled came from four districts in the central part of Albania, two near Kruja, one near Sauk (10 km from Tirana) and one from the collection field of Shamogjin near Vlore. The most important Albanian cultivars were selected from each area and samples were collected from 3–4 trees of each cultivar. The survey comprised 50 source trees belonging to 14 native cultivars and 2 imported cultivars (Table 1).

RNA and DNA extraction

Phloem tissue was collected from at least six shoots per tree all around the tree canopy in early spring and in autumn. Samples were pulverized with a mortar and pestle in liquid nitrogen; 0.1 g per sample was used for total RNA extraction using an RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. RNA was eluted with 50 μL of RNase-free water. For phytoplasma detection, DNA was extracted using 1.5 g of powdered phloem tissue following the method of Marzachì *et al*. (1999). After washing with 70% ethanol, the pellet was eluted in 100 μL of dietil pirocarbonate treated water.

Pathogen detection

Viruses were detected using one-step RT-PCR (Faggioli *et al*., 2005). Two μL of RNA was added to 23 μL of reaction mixture containing: GoTaq buffer 1× (Promega, Madison, WI, USA); 125 μM each dNTPS; 5 mM DTT; 0.2 μM specific sense and antisense primers; 2.5 U avian myeloblastosis virus (AMV)-RT (Promega); 20 U of RNase Out (Invitrogen Corporation, Paisley, Scotland, UK); 1.25 U GoTaq Polymerase (Promega). The cDNA was synthesised at 46°C for 45 min, followed by denaturation at 95°C for 30 s. Amplification was carried out for 35 cycles under the following conditions: denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 45 s, followed by a final extension for 7 min at 72°C. For each virus a specific primer pair was used according to Loconsole *et al*. (2007). Phytoplasma was detected using a PCR protocol with P1/P7 universal primers (Schneider *et al*., 1995; Deng and Hiruki, 1991), followed by nested PCR with R16(I) F1/R1 (Lee *et al*., 1994) R16(V) F1/R1 (Lee *et al*., 1994) and R2F2C/H (Lee *et al*., 1995). Two μL of total DNA (or 2 μL of the amplification products diluted 1:40 for the nested PCR) was added to 23 μL of the reaction mixture containing: 50 mM KCl, 10 mM Tris-HCl pH 9, 1.5 mM $MgCl_2$, 0.1% Triton®X-100; 0.8 mM dNTPs, 0.25 μM (1 μM for the nested) of each primer, 0.625 U of Taq DNA polymerase (Promega). The mixture con-

Table 1. List of olive samples collected and analyzed. The sample that was positive to SLRSV and OLYaV is shown in bold.

No.	Cultivar	District	No. of samples
1	Kripsi i Krujes	Kruja	4
2	Frengu	Kruja	4
3	Ulli i Bardhë i Krujës	Kruja	4
4	Boç	Sauk	3
5	Ulli i zi	Sauk	2
6	Leccino	Sauk	1
7	Olivastër e kuqe	Sauk, Vlora	3+1
8	**K. M. Berat**	Sauk, **Vlora**	3+4 **(1)**
9	Frantoio	Sauk, Vlora	1+1
10	Kalinjot	Sauk, Vlora	4+3
11	Ulli i Bardhë i Tiranë	Sauk, Vlora	3+2
12	Kotruvs	Vlora	2
13	Managel	Vlora	1
14	Unafka	Vlora	1
15	Mixan	Vlora	2
16	Kushan	Vlora	1
Total			50

taining the DNA was denatured for 3 min at 95°C, then amplification was carried out for 35 cycles under the following conditions: denaturation at 94°C for 45 s, annealing at 55°C (50°C for the nested) for 60 s, extension at 72°C for 2 min, followed by a final extension for 7 min at 72°C. All amplified products were analyzed by electrophoresis in 1.2% agarose gel and stained with ethidium bromide.

Primary-source production

One-year-old branches of about 4 nodes were harvested from pathogen-free olive source-plants. The basal cut was made 2–3 mm from the nodes instead of in the apical region and a couple of leaves were left at the last node. To promote root production, each scion was previously treated with indolebutyric acid (IBA). The scions were placed in a rooting box with a basal temperature of 24°C and a relative humidity of 100%.

Results and discussion

No symptoms of viruses, phytoplasma, bacteria or fungi were found in the Kruja area, whereas in Sauk, two 'Frantoio' plants showed clear yellowing symptoms and in the experimental field at Vlore, 'Frantoio', ' Ulli i Bardhë i Tiranës', 'Kalinjot' and 'K. M. Berat' trees showed Verticillium wilt and olive knot. All trees sampled were asymptomatic for viruses, phytoplasma, bacteria and fungi, with the exception of the two 'Frantoio' plants collected in Sauk. The one-step RT-PCR with specific primer pairs for eight viruses showed that all samples were free of the tested viruses except for one 'K. M. Berat' olive tree, which tested positive for both *Strawberry leaf ring spot virus* (SLRSV) and *Olive leaf yellowing associated virus* (OLYaV). All eight samples collected in the Sauk region, including those with symptoms of yellowing, tested negative for phytoplasmas.

Most Albanian olive cultivars are native and previous studies have focused only on their genomic and agronomic aspects (Kafaki, 1965 and 1980; Thomaj and Panajoti, 2004). With regard to their phytosanitary status, one study, using double-strand RNA analysis, reported a virus incidence of 22% in the cultivars it tested (Cakalli *et al*., 2001). Thus, it was important to carry out more tests on the pathogen status of Albanian olive trees. The molecular assays performed in the present study showed a near-total absence of virus and phytoplasma infections in the main olive growing regions. The yellowing symptoms in the 'Frantoio' cultivar in Sauk probably arose because this was a foreign cultivar less well adapted to local agronomic conditions (soil, climate). The occurrence of a mixed infection of SLRSV and OLYaV on an asymptomatic tree collected in Vlore could be due to the import of foreign cultivars that grow in the collection field. Based on these findings the phytosanitary state of the Albanian olive cultivars with regard to viruses and phytoplasmas is better than reported. This may be due to the prevalence of using native olive cultivars as propagation material.

The findings in Albania contrast with those of other Mediterranean countries where olive infection rates of about 50% have been reported (Saponari *et al*., 2002; Al Abdullah *et al*., 2005; Fadel *et al*., 2005; Bjelis *et al*., 2007). From a phytosanitary perspective, Albanian olive germplasm should therefore be preserved and protected on account of its agronomical as well as its general pathogen-free qualities. This may be achieved by preventing the imported non certified olive material and by conserving the quality and the characteristics of the Albanian olive oil. At the same time, Albania should develop and qualify olive propagation material in accordance with international laws. For this purpose, primary sources free of tested pathogens were identified in this present study. These source plants can be used to produce olive propagation material that meets the standards of an international certification system.

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