SHORT COMMUNICATION

VIRUSES AND VIROIDS OF STONE FRUITS IN ALGERIA

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SUMMARY

Field surveys were carried out in the major stone fruit growing areas of eastern Algeria to assess the sanitary status of stone fruits. A total of 454 samples from peach, apricot, almond, sweet and sour cherry, plum and myrobalan were tested by ELISA or RT-PCR for the presence of *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV) *Apricot latent virus* (ApLV), *Cherry virus A* (CVA), *Cherry green ring mottle virus* (CGRMV), and Plum bark necrosis stem pitting-associated virus (PBNSPaV). The overall average infection level was 10.4%. The most frequent virus was PNRSV (56.8%), followed by PDV (27.2%) and ApMV (22.7%). The most infected species was cherry (21.9%) and the less almond (4.4%). ACLSV, ApLV, CVA, CGRMV and PBNSPaV were not detected. Of 531 samples tested for the presence of viroids by tissue-print hybridization, 28 (5.2%) were infected. *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd) were detected in 15 and 13 samples, respectively. This is the first large-scale study on viruses and viroids of stone fruits in Algeria and reports for the first time the presence of HSVd in the country.

Key words: *Prunus*, ELISA, RT-PCR, tissue-print hybridization, incidence, sanitary status, viroids.

In Algeria, little is known on virus and viroid diseases of stone fruit trees. Records are limited to the occasional finding of *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple chlorotic leaf spot virus* (ACLSV) (Aouane, 2003) and of *Peach latent mosaic viroid* (PLMVd) (Torres *et al*., 2004).

To gather a better insight of the sanitary status of stone fruits in eastern Algeria, a survey was carried out for the presence of PNRSV, PDV, ApMV, ACLSV, *Apricot latent virus* (ApLV), *Cherry virus A* (CVA), *Cherry green ring mottle virus* (CGRMV), Plum bark necrosis stem pitting-associated virus (PBNSPaV), PLMVd and *Hop stunt viroid* (HSVd).

Field inspections were conducted and sample collected in spring 2005 and 2006 in the districts of Setif, Bordj bou Arreridj, M'sila, Mila, Batna and Constantine where a surface of *ca*. 27,000 ha is given over to stone fruit trees. Leaf samples were collected from nearly 5 % of the trees in 23 commercial orchards and 13 mother blocks and from 20% of the trees in a varietal collection (at least two trees per each variety).

Sampled species were peach (109 samples), plum (98), apricot (91), almond, (68), cherry (64) and myrobalan (24) (Table 1). All samples were tested by DAS-ELISA (Clark and Adams, 1977) using commercial kits for PNRSV, PDV and ApMV (Agdia, USA), and an antiserum to ACLSV kindly supplied by Dr. D. Boscia. Thirtyfive additional samples were tested by RT-PCR using protocols and primers designed for ApLV (Nemchinov and Hadidi, 1998), CVA (M. Al Rwahnih, personal communication) and CGRMV (Rott and Jelkmann, 2001), and by nested RT-PCR for PBNSPaV (Abou Ghanem-Sabanadzovic *et al*., 2001; Amenduni *et al*., 2005).

Tissue-print hybridization (TPH) was used for the detection of PLMVd and HSVd (Pallás *et al*., 2003) in the leaves collected from 170 apricots, 128 peaches, 87 cherries, 86 plums, 35 almonds, and 25 myrobalans. Fresh cut ends of leaf petioles were pressed onto nylon membranes from each sample in triplicate, directly in the field in autumn 2004. Membranes were stored at 4ºC, and developed later in the Mediterranean Agronomic Institute of Bari, Italy. Leaves were also stored at 4°C and kept as backup for RT-PCR assays on those samples that gave doubtful responses (Astruc *et al*., 1996; Ambrós *et al*., 1998).

Some of the surveyed trees showed foliar symptoms ranging from yellowing, chlorotic/necrotic ringspots and oak leaf patterns, which were associated with the presence of ilarviruses. As shown in Table 1, a total of 15 cherries, 10 peaches, 10 apricots, 5 plums, 4 myrobalans and 3 almonds tested positive for at least one virus. Whereas the average infection level determined by ELISA was 10.4%, infection rates of individual host species were: cherry, 23.4% (15/64); myrobalan, 16.7%

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Table 1. Relative incidence of stone fruit viruses detected by ELISA.

Species	Nr. of samples		Infection rate (%)	Virus-infected trees (No.)		
	Tested	Infected		PNRSV	PDV	ApMV
Peach	109	10	9.2	9	0	1
Plum	98	5	5.1	2	1	2
Apricot	91	9	9.9	7	1	2
Almond	68	3	4.4	2	1	0
Cherry	64	14	21.9	4	9	2
Myrobalan	24	3	12.5	1	0	3
Total	454	44		25	12	10
Mean infection rate (%)			10.4			

Table 2. Viroids detected by tissue-print hybridization.

Species	Number of trees		Infection rate (%)	Viroid-infected trees (No.)	
	Tested	Infected		PLMVd	HSVd
Apricot	170	10	5.8	2	8
Peach	128	18	14.0	13	5
Cherry	87	0	0	0	0
Plum	86	0	0	0	0
Almond	35	0	0	0	0
Myrobalan	25	0	0	0	0
Total	531	28		15	13
Mean infection rate (%)			5.2		

(4/24); apricot, 11% (10/91); peach, 9.2% (10/109); plum, 5% (5/98); and almond, 4.4% (3/68). Viruses detected were PNRSV, PDV and ApMV, in 53.2%, 25.6% and 21.3% of the samples, respectively (Table 1). No infection by ACLSV was found. Double infection by PNRSV+ApMV was detected in one myrabolan tree and by PDV+ApMV in an apricot and a cherry tree.

PNRSV prevailed in infected trees of peach (90%), apricot (78%) and almond (67%). Cherries were mainly infected by PDV (64%), and myrobalan by ApMV (100%); plums by PNRSV (40%) and ApMV (40%). Virus infections were found only in imported cultivars, i.e. cherry (cvs Guillaume, Hedelfingen, Napoléon, and Burlat), Apricot (cvs Bulida and Rouge Roussillon), Prune (cvs Stanley and Golden Japan), Peach (cvs Mycrest, Dixired, GH Hall, and Nectarosa), Almond (cv. Marcona). Setif and Mila were the locations that hosted the most infected orchards. The highest infection rate was observed in commercial orchards followed by mother blocks and varietal collections.

No ApLV, CVA, CGRMV and PBNSPaV were detected by RT-PCR and nested RT-PCR. However, since only 35 samples were analysed, additional testing appears desirable.

TPH assays disclosed that 28 of 531 samples (5.2%) were positive for PLMVd and HSVd, both of which were present in peach (14% infection) and apricot (5.8% infection), but not in cherry, almond, plum and myrobalan (Table 2). These findings were confirmed by RT-PCR assays carried out on doubtful positive samples (not shown). Here again, viroids were detected only in imported peach (cvs Redhaven, Dixired and My Crest) and apricot (cvs Bulida and Bafi). This represents the first record of HSVd in Algeria.

The present survey has shown that the level of viral infection of stone fruit trees from eastern Algeria is lower than that reported from other Mediterranean countries (Myrta *et al.*, 2003). Ilarviruses were the only pathogens detected, PNRSV being the most common, followed by PDV and ApMV.

Although the overall average infection rate by viroids was also relatively low, PLMVd had an incidence in peach comparable with that recorded from other Mediterranean countries like Spain (Badenes and LIácer, 1998), Italy (Barba and Faggioli, 1999), Syria (Ismaeil *et al.*, 2001), Albania (Torres *et al.*, 2004) and Bosnia and Herzegovina (Matic *et al.*, 2005). The same applies to HSVd infections to apricot, which compare well with records from south-east Spain (Cañizares *et al.*, 1998), Syria (Ismaeil *et al.*, 2001), western Turkey and Egypt (Torres *et al.*, 2004).

The fair sanitary status of eastern Algerian fruit tree industry, as it appears from the present work, suggests that the implementation of a national certification pro-

gramme could prove highly beneficial for a rapid improvement of the local nursery and fruit industry and would successfully limit the dissemination of graft-transmissible diseases in the country.

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REFERENCES

Abou Ghanem-Sabanadzovic N., Mahboubi M., Di Terlizzi B., Sabanadzovic S., Savino V., Uyemoto J.K., Martelli G.P., 2001. Molecular detection of a closterovirus associated with Apricot stem pitting in southern Italy. *Journal of Plant Pathology* **83**: 125-132.

Ambrós S., Hernández C., Desvignes J.C., Flores R., 1998. Genomic structure of three phenotypically different isolates of peach latent mosaic viroid: implications of the existence of constraints limiting the heterogeneity of viroid quasi-species. *Journal of Virology* **72**: 7397-7406.

Amenduni T., Hobeika C., Minafra A., Boscia D., Castellano M.A., Savino V., 2005. Plum bark necrosis stem pitting-associated virus in different stone fruit species in Italy. *Journal of Plant Pathology* **87**: 131-134.

Aouane B., 2003. Preliminary studies on stone fruit tree viruses in Algeria. In: Myrta A., Di Terlizzi B., Savino V. (eds.). Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. *Options Méditerranéennes*: *Série B. Etudes et Recherches* **45**: 29-32.

Astruc N., Marcos J.F., Macquaire G., Candresse T., Pallás V., 1996. Studies on the diagnosis of hop stunt viroid in fruit trees: identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. *European Journal of Plant Pathology* **102**: 837-846.

Badenes M.L., LIácer G., 1998. Occurrence of peach latent mosaic viroid in American peach and nectarine cultivars in Valencia, Spain. *Acta Horticulturae* **472**: 565-570.

Barba M., Faggioli F., 1999. I viroidi agenti causali di malattie degenerative dei fruttiferi. *Rivista di Frutticoltura* **2**: 69-73.

Cañizares M.C., Marcos J.F., Pallás V., 1998. Studies on the incidence of hop stunt viroid in apricot trees (*Prunus armeniaca)* by using an easy and short extraction method to analyze a large number of samples. *Acta Horticulturae* **472**: 581-585.

Clark M.F., Adams A.N., 1977. Characteristics of microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**: 475-483.

Ismaeil F., Abou Ghanem-Sabanadzovic N., Myrta A., Di Terlizzi B., Savino V., 2001. First record of Peach latent mosaic viroid and Hop stunt viroid in Syria. *Journal of Plant Pathology* **82**: 227.

Matic S., Al Rwahnih M., Myrta A., 2005. Occurrence of stone fruit viroids in Bosnia and Herzegovina. *Phytopathologia Mediterranea* **44**: 285-290.

Myrta A., Di Terlizzi B., Savino V., Martelli G.P., 2003. Virus diseases affecting the Mediterranean stone fruit industry: a decade of surveys. In: Myrta A., Di Terlizzi B., Savino V. (eds.). Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. *Options Méditerranéennes: Série B. Etudes et Recherches* **45**: 15-23.

Nemchinov L., Hadidi A., 1998. Apricot latent virus: A novel stone fruit pathogen and its relationship to apple stem pitting virus. *Acta Horticulturae* **472**: 159-164.

Pallás V., Amari K., Gómez G., Abou Ghanem-Sabanadzovic N., Di Serio F., 2003. Viroids of stone fruits: Incidence and diseases in the Mediterranean. In: Myrta A., Di Terlizzi B., Savino V. (eds.). Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. *Options Méditerranéennes: Série B. Etudes et Recherches* **45**: 129-133.

Rott M.E., Jelkmann W., 2001. Characterization and detection of several filamentous viruses of cherry: Adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction protocol. *European Journal of Plant Pathology* **107**: 411-420.

Torres H., Gómez G., Pallás V., Stamo B., Shalaby A., Aouane B., Gavriel I., Kominek P., Caglayan K., Sipahioglu M., Michelutti R., Myrta A., Fiore N., 2004. Detection by tissue printing of stone fruit viroids, from Europe, the Mediterranean and North and South America. *Acta Horticulturae* **657**: 379-383.

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