

SHORT COMMUNICATION

VIRUSES AND VIROIDS OF STONE FRUITS IN EGYPT

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SUMMARY

Field surveys were carried out to assess the sanitary status of stone fruits in Egypt. A total of 716 samples was tested by ELISA for *Prunus necrotic ring spot virus* (PNRSV), *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Apple chlorotic leaf spot virus* (ACLSV) and *Apple mosaic virus* (ApMV). The viruses most frequently detected were PNRSV (57%) and PPV (41%); PDV and ACLSV were found in 3% of samples and ApMV was not detected. Plum bark necrosis stem pitting-associated virus (PBNSPaV) and *Apricot latent virus* (ApLV) were detected for the first time in Egypt by using RT-PCR. Of 693 samples tested by tissue-imprint hybridization (TIH), 101 (15%) were found to be infected by either *Peach latent mosaic viroid* (PLMVd) or *Hop stunt viroid* (HSVd).

Key words: *Prunus*, ELISA, RT-PCR, tissue-imprint hybridization, sanitary status.

Stone fruit trees are a major fruit industry in the Mediterranean area. The stone fruit cultivated area in Egypt is 49.209 ha with an approximate yearly production of 476849 tons (6 % of total fruit production). Apricot, peach and Japanese plum are the most important stone fruit crops. The total stone fruit area is made up of the old lands (16 % with production of 86.933 tons/year) and the new reclaimed lands (84 % with production of 389.916 tons/year) (Anonymous, 2005). The production areas are concentrated in north and north-eastern parts of the country.

Prior to this study, several viruses had been detected in stone fruit trees in Egypt: *Plum pox virus* (PPV) in apricot, peach, and plum (Dunez, 1988; Wetzel *et al.*, 1991; Mazyad *et al.*, 1992; Aboul-Ela *et al.*, 1999), *Apple chlorotic leaf spot virus* (ACLSV) in peach (Farrag *et al.*, 2004), *Peach rosette mosaic virus* (PRMV) in peach

(Kheder *et al.*, 2004) and *Prunus necrotic ring spot virus* (PNRSV) (Dunez, 1988). There is one report of *Hop stunt viroid* (HSVd) in peach, apricot, and plum (Torres *et al.*, 2004) and of *Peach latent mosaic viroid* (PLMVd) in peach (Hassan *et al.*, 2006). No information is available on their incidence and distribution. Therefore, a more systematic survey was made to assess the incidence and distribution of PPV, PNRSV, ACLSV, *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *Apricot latent virus* (ApLV), *American plum line pattern virus* (APLPV), Plum bark necrosis stem pitting-associated virus (PBNSPaV), PLMVd and HSVd.

Field surveys and sample collections were done in 72 locations in reclaimed and old lands of the governorates of Fayoum, Qalyoubia, Cairo-Alexandria Desert road, Monoufia, Behaira and Sinai. The collections included apricot (*Prunus armeniaca*), peach (*Prunus persica*) and Japanese plum (*Prunus salicina*). Dormant budwood was collected during the winter of 2005; leaf sample collection and symptom observations were made during spring 2006. Randomly collected samples totaled 716, of which 410 were peaches, 200 were apricots and 106 were plums. All samples were tested by DAS-ELISA (Clark and Adams, 1977) for the presence of PPV, PNRSV, PDV and ApMV and by DAS-simultaneous ELISA (Flegg and Clark, 1979) for ACLSV. Serological reagents were from commercially purchased kits (Loewe, Sauerlach, Germany).

In addition, a total of 39 orchard representative and randomly selected samples were tested by RT-PCR for *Apricot latent virus* (ApLV) (Nemchinov and Hadidi, 1998) and *American plum line pattern virus* (APLPV) (Scott and Zimmerman, 2001). Nested RT-PCR was used for the detection of Plum bark necrosis stem pitting-associated virus (PBNSPaV) (Abou Ghanem-Sabanadzovic *et al.*, 2001; Amenduni *et al.*, 2005).

Tissue-imprint hybridization (TIH) assays were used for the detection of PLMVd and HSVd (Pallás *et al.*, 2003). Assays were made on 304 peaches, 291 apricots and 98 plums. Fresh cut ends of leaf petioles were imprinted in the field in duplicate for each sample. Tissue imprinting and symptom observation were done during autumn 2005. Imprinted membranes were stored at 4°C, and developed later at the Mediterranean Agro-

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

Species	No. of samples		PNRSV		PPV		PDV		ACLSV	
	Tested	Infected	No.	%	No.	%	No.	%	No.	%
Peach	410	30	29	97	0	0	2	7	2	7
Apricot	200	50	6	12	43	86	1	2	1	2
Plum	106	28	27	96	1	4	0	0	0	0
Total	716	108	62	57	44	41	3	3	3	3

nomie Institute of Bari, Italy. Leaf samples were also stored at 4°C and used as backup for RT-PCR assays on those samples that gave doubtful reactions (Astruc *et al.*, 1996; Ambrós *et al.*, 1998).

No symptoms of infection by viruses and viroids were observed in visited orchards during surveys, except for those of Sharka disease in old lands on local apricot cultivars, as reported by Mazyad *et al.*, (1992). The hot climate and iron deficiency conditions in several orchards probably masked the symptoms usually induced by the detected viruses.

Of all samples tested by ELISA, 15% were infected (Table 1). A total of 50 apricots, 30 peaches and 28 plums proved to be infected by at least one virus. The infection level of the different species was: plum (26%), apricot (25%) and peach (7%). The detected viruses were PNRSV in 57% of samples, PPV in 41%, PDV in 3% and ACLSV in 3%. No infection by ApMV was detected. Double infections by PNRSV+ACLSV were found in two peaches and by PNRSV+PDV in one peach and one apricot. PNRSV was the virus most frequently found in peach (97%). The cvs Swelling, Desert Red and Florida Prince had infection rate of 12%, 12% and 7%, respectively. Plum showed the highest infection rate by PNRSV (96%). The imported cv Hollywood was the most affected by PNRSV (39%). In addition, one single infection by PPV was found in plum cv Balady growing inside an apricot orchard. No other viruses were detected in plum. Apricot was the species most commonly infected by PPV (86%). The virus was found in the local apricot cvs El Amar (48%) and Bala-

dy (23%). No PPV infection was found in the imported cv Canino. PPV was found only in old lands of Fayoum and Delta area. No infection was detected in new reclaimed lands of Cairo-Alexandria Desert road and Sinai Peninsula, where most of the cultivation were peaches and apricot cv Canino.

By using nested RT-PCR, PBNSPaV was detected in 8 samples (21%) and by using RT-PCR ApLV was detected in 3 samples (8%). No APLPV was found in these tests. ApLV was found in apricot and plum and PBNSPaV in peach and plum. Two mixed infections by PBNSPaV+ApLV were found in plum. All infected cultivars were of imported origin, with the exception of native apricot cv Balady.

In TIH assays, 101 samples (15%) tested positive for viroids (PLMVd or HSVd) (Table 2). PLMVd was found in peaches (8%), whereas HSVd was found in apricots (26%) and plums (2%). No mixed infections were detected. RT-PCR, carried out on doubtfully positive samples, confirmed the hybridization results (data not shown). Imported peach cvs Florida Sun, Swelling, Desert Red and Florida Prince were found to be infected by PLMVd at rates of 80%, 15%, 8% and 6% respectively. HSVd was found in the imported apricot cv Canino (78%) and local cv Amal (13%). The only viroid found in plum was HSVd in 11% of samples of imported cv Santa Rosa. The infection rate by viroids was high in the new lands (22% in the Desert road and 20% in Sinai) compared with the old lands (8% in the Delta). This difference can be explained by the exclusive use of the apricot cv Canino in the new lands that was infected

Table 2. Viroids infection detected by tissue-imprint hybridization (TIH).

Species	No. of trees		Infection rate (%)	Viroid infection	
	Tested	Infected		PLMVd	HSVd
Peach	304	24	8	24	0
Apricot	291	75	26	0	75
Plum	98	2	2	0	2
Total	693	101	15	24	77

when imported. In addition, most of the peach cvs of Florida origin were infected with PLMVd and used extensively for establishing orchards in the new lands.

This study showed a high incidence of virus diseases, specially in the old lands. Apricot was highly infected by PPV but the virus was recorded only in old lands, where it had been detected before with higher incidences (67.5%, 60% and 12.5% in apricot, plum and peach respectively) (Aboul-Ela, 1994). This may be due to the length of the study that started in 1987, when the virus was first reported, and to the replacement of many of those stone fruit orchards in old lands with new crops e.g. citrus and mango since the end of the 90s. The results confirmed the prevalence of PNRSV in peach in the Mediterranean region (Myrta *et al.*, 2003). PDV incidence was low, perhaps because no cherry samples were collected. ACLSV is often found in the Mediterranean region (Myrta *et al.*, 2003), but we found it only infrequently in Egypt. PBNSPaV and ApLV are reported here for the first time in Egypt, although more samples should be tested to establish a more meaningful incidence of these viruses.

The incidence of PLMVd in peach was lower than that found in other southern Mediterranean countries: Western Turkey (Torres *et al.*, 2004), Syria (Ismaeil *et al.*, 2001), Lebanon (Choueiri *et al.*, 2001), and Jordan (Al Rwahnih *et al.*, 2001). This difference may be linked to the limited number of peach cvs cultivated in Egypt. The incidence of HSVd in apricot was similar to that in other southern Mediterranean countries: Syria (Ismaeil *et al.*, 2001), Jordan (Al Rwahnih *et al.*, 2001), and Lebanon (Choueiri *et al.*, 2002). Torres *et al.*, (2004) found a higher incidence of HSVd in apricot in Egypt (58%), but relatively few samples were tested.

The present study showed similar incidences of virus and viroid diseases in Egypt. Thus the sanitary status of the stone fruit industry in both old and new cultivated areas is poor. Therefore, the implementation of a national certification program is highly desirable to limit further dissemination of graft-transmissible diseases in the country.

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