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Virus diseases infecting almond germplasm in Lebanon

Zahi H. Kanaan-Atallah, Yusuf Abou-Jawdah and Adeeb Saad

Faculty of Agricultural and Food Sciences, American University of Beirut, PO Box 11-0236, Beirut, Lebanon

Summary. Cultivated and wild almond species were surveyed for virus diseases. Four viruses infected cultivated almonds (*Prunus dulcis*): *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple chlorotic leaf spot virus* (ACLSV) and *Apple mosaic virus* (ApMV). Only ACLSV and ApMV were detected on wild almonds, (*Prunus orientalis* and *P. korschinskii*). The occurence of PNRSV or PDV on seeds used for the production of rootstocks, on seedlings in nurseries, and on mother plants revealed the need for a certification program. There was no evidence for the occurrence of *Plum pox virus* (PPV) or *Tomato ringspot virus* (ToRS).

Key words: *Prunus dulcis, P. orientalis, P. korschinskii,* viruses.

Introduction

Almond is an indigenous tree in the Eastern-Mediterranean region (Grassely and Grossa-Raynaud, 1980). One cultivated (*Prunus dulcis* Mill.) and two wild species [*Prunus orientalis* (Mill.) Koehne and *P. korschinskii* Hand.-Mazz.] currently occur in Lebanon (Post, 1932; Mouterde, 1970). In the ’80s and ’90s the area devoted to almond production in Lebanon increased significantly, with some growers in the Bekaa valley shifting from grape, cherry and apricot to almond. Almond is considered a more interesting and profitable crop, in Lebanon and neighbouring countries, because it may be harvested either in spring to be sold as green fruit for fresh consumption (the whole fruit is consumed), or in summer as mature nuts.

Virus diseases occur worldwide and cause serious reductions in the vigour and production of stone fruit trees (Nemeth, 1986). Several virus diseases have been reported to infect stone fruits: *Plum pox virus* (PPV, genus *Potyvirus*) (Sharka); *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple mosaic virus* (ApMV) (genus *Ilarvirus); Apple chlorotic leaf spot virus* (ACLSV, genus *Trichovirus);* and *Tomato ringspot virus* (ToRSV, genus *Nepovirus),* which are among the most important viruses of stone fruits. A 1995 survey of cultivated almonds in Lebanon detected the occurrence of PNRSV and PDV but not any of the nine other viruses tested for (Jawhar *et al.*, 1996). The present study covered a larger geographical area and included both cultivated and wild almond species in all the almond-growing regions of Lebanon. It reports on two other viruses, ACLSV and ApMV, infecting wild and cultivated almond species in Lebanon besides PNRSV and PDV (found only on cultivated almond species), and confirms previous reports about the non-occurrence of PPV, the most damaging virus of stone fruits.

Corresponding author: Y. Abou-Jawdah
Fax: + 961 1 744460
E-mail: abujawyf@aub.edu.lb

Materials and methods

Major fruit tree nurseries and selected commercial orchards in the almond growing areas in Lebanon were surveyed for virus symptoms, and representative leaf samples were collected for testing. Locations reported by Lubani (1997) to harbour wild almonds *(P. orientalis* and *P. korschinskii)* and wild seedling populations of *P. dulcis* were also surveyed (Fig. 1). Leaf samples were collected at random from symptomless as well as symptomatic shoots. Because researchers have found that the concentration of some viruses (e.g. PNRSV) is season-related, samples for virus detection were collected at different times during the growing season over two consecutive years.

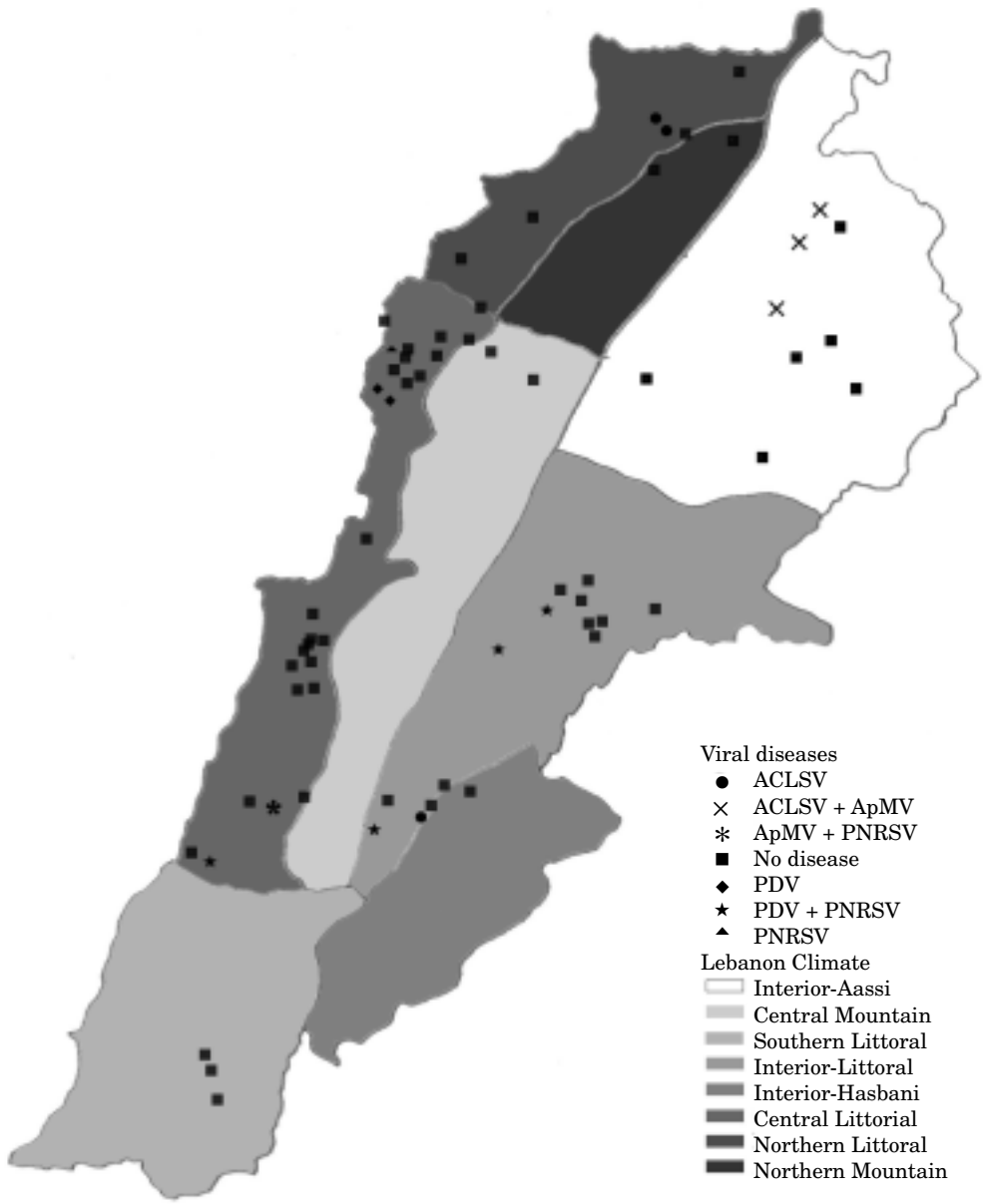

Fig. 1. Occurrence and distribution of viral diseases of almond in Lebanon. The different climatic regions are indicated.

Leaf tissue (1:10 w:v) was extracted in 0.1 M Phosphate Buffer Saline-Tween-Polyvinylpyrolidone (PBST-PVP), pH 7.4, containing 2% PVP, 0.05% Tween-20, 0.15 M NaCl, 4 mM KCl and 0.02% NaN_3. The extracts were kept at –20 °C until analyzed by ELISA. IgGs and alkaline phosphatase conjugated IgGs for six viruses were purchased from commercial companies: ApMV, PDV and ToRSV from Agdia (El Khart, IL, USA); ACLSV and PPV from Sanofi (Paris, France); and PNRSV from Bioreba (Reinach, Switzerland).

Samples were analysed by the standard double-antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977). After addition of the substrate p-nitrophenyl phosphate (Sigma N-2765, Louisiana, USA), the reaction was detected colorimetrically at A405 nm using an ELISA reader (Organon Teknika, Microwell System, Boseind, The Netherlands). Two wells were used per sample. The test was considered positive when the mean absorbance value was greater than twice that of the healthy controls (Singh and Singh, 1995).

Results

Field observations in most of the orchards surveyed did not reveal the characteristic symptoms suggesting viral infections. In a few locations, bud failure was observed leading to sparse foliage on shoots that looked bare with only a few leaves at their tip. Shot holes, which may be caused by viral, bacterial or fungal infections, were commonly observed. Other symptoms such as mottling, oakleaf pattern and mosaics were rare.

Of the 599 plant samples collected for virus testing, 10.3% reacted positive to one or more virus antisera in the ELISA tests (Table 1). The virus pathogens detected were: *Prunus necrotic ringspot virus*, *Apple chlorotic leaf spot virus*, *Prune dwarf virus* and *Apple mosaic virus*. Their average incidence was 3.3%, 2.7%, 2.5% and 1.8% respectively.

PNRSV was detected in samples from Tamboureet (Saïda region; incidence 47%) Mazraet al-Dahr (Shouf, 10%); Rashana (Batroun, 14.3%); Ferzol (Zahle, 12.2%); Aitanit (western Bekaa, 10%) and a nursery in Taalabaya (Zahle, 7.4%) (Table 1). Some infected trees showed growth failure of vegetative buds, chlorotic and necrotic patches on the leaves and/or shotholes that did not produce any fungal or bacterial growth when plated on culture media. Incidences of PNRSV in all virus-infected samples, in samples from locations with virus infected-plants, and in the total leaf samples collected from Lebanon were 32.2%, 5.6% and 3.3% respectively. PNRSV was detected in samples collected from one-year-old seedlings in a nursery in Taalabaya (central Bekaa). The mother plants of these scion seedlings were also found to be infected with this virus. PNRSV infections were not detected in seedling populations of *P. dulcis* or in wild almond populations, even though some of their populations were located close to infected almond orchards.

PDV was detected in Zighrine (Hermel, 2%); Aitanit (western Bekaa, 15%); Ferzol (Zahle, 2.4%) and in Jedayel and Kfarmashoun (Jbeil, 13.3 and 25% respectively) (Table 1). PDV symptoms were observed on a few infected trees in the field. Symptoms included general stunting, shorter internodal distances and, more rarely, large chlorotic patches on the leaves. Incidences of PDV in all virus-infected samples, in samples collected from locations with virus-infected plants, and in the total leaf samples collected were 24.2%, 4.2% and 2.5% respectively.

PDV was also detected in samples from a nursery in Jibshit (Nabatieh) on one-year-old grafted seedlings. A sample of seeds used for production of rootstocks in a nursery in Taalabaya (Zahle) was tested for virus infection, and some of the seeds were found to be infected with PDV. No PDV infection was detected in any of the sampled wild almonds or wild seedling populations, even though these populations grew close to orchards with PDV.

ACLSV was detected in almond samples from Zighrine (10%), Wadi Faara (10.5%) and Wadi al-Turkman (Hermel, 6.7%); Baaloul (western Bekaa, 9.5%); Ilat and Idbel (Akkar, 11.8 and 2.5% respectively) (Table 1). Some ACLSV-infected trees in Akkar showed small, light-coloured rings on their leaves; however, no symptoms were observed elsewhere on infected trees. Incidences of ACLSV in all virus-infected samples, in samples from locations with virus-infected trees, and in the total leaf samples collected were 25.8%, 4.5% and 2.7% respectively. The positive samples from Baaloul came from seedling trees of *P. dulcis*; those from Zighrine and Wadi al-Turkman were from symptomless *P. orientalis*, while some positive samples from

Table 1. Locations and areas where viral diseases on almonds were detected.

Area	Location	No. of samples tested	Virus			
			ACLSV[a]	ApMV[b]	PDV[c]	PNRSV[d]
Akkar	Idbel	40	1			
	Ilat	34	4			
Batroun	Rashana	15				2
Hermel	Wadi Faara[e]	19	2	1		
	Wadi al-Turkman[e]	30	2	5		
	Zighrine[e]	50	5	3	2	
Jbeil	Jedayel	15			2	
	Kfarmashoun	16			4	
Nabatieh	Jibshit[f]	3			1	
Saïda	Tamboureet	17			2	9
Shouf	Mazraet al-Dahr	10		4		2
Western-Bekaa	Aitanit	20			3	2
	Baaloul	21	2			
Zahle	Ferzol	41			1	5
	Taalabaya[f]	7			1	2

[a] ACLSV= *Apple chlorotic leaf spot virus*.
[b] ApMV= *Apple mosaic virus*.
[c] PDV= *Prune dwarf virus*.
[d] PNRSV= *Prunus necrotic ringspot virus*.
[e] Includes samples from wild almond species.
[f] Samples from grafted seedlings in nurseries.

Wadi Faara came from *P. korschinskii* trees. ACLSV was also detected on the cultivated *P. dulcis* varieties in the last locations.

ApMV was detected in samples collected from Zighrine (4%), Wadi Faara (10.5%) and Wadi al-Turkman (Hermel, 6.7%); and from Mazraet al-Dahr (Shouf, 30%) (Table 1). Incidences of ApMV in all virus infected samples, in samples from locations with virus-infected trees, and in the total leaf samples collected were 17.8%, 3.1% and 1.8% respectively. No ApMV symptoms were observed in the field and the almond trees seemed healthy. Some positive samples from Zighrine and Wadi al-Turkman came from *P. orientalis* populations and those collected in Wadi Faara came from *P. korschinskii*. ApMV-positive samples were also detected in *P. dulcis* orchards at the same locations.

Mixed infections occurred in only three samples.

PPV and ToRSV were not detected in any of the samples tested. Symptoms of PPV were not observed even on other species of stone fruits in the areas covered by the survey.

Discussion

Almond production in Lebanon is becoming increasingly important, especially on marginal lands. This survey assessed the phytosanitary situation of almond germplasm in both cultivated and wild almond populations.

ELISA tests showed that PNRSV and PDV infections of cultivated *P. dulcis* are widespread

throughout Lebanon, and the geographical area covered is larger than that reported by Jawhar *et al*. (1996). Compared with the incidence of virus diseases on almonds reported by Jawhar *et al*. (1996), the percentage of positive samples detected in this study was lower (10.3% vs. 21%). This difference may be due to the time of sampling. Samples for this survey were collected from March until the end of July; those of Jawhar *et al*. (1996) during March and May. Varveri *et al*. (1997) found a drastic reduction in the efficiency of detection of the *Ilarviruses* PNRSV and PDV after May and concluded that the increase in temperature during summer inactivates the virus, rendering its detection by ELISA more difficult. Even though populations of wild almond species and wild seedling populations of *P. dulcis* sometimes grew in close proximity to orchards of cultivated almonds that had infections of PNRSV and PDV, no evidence was found that these viruses also infected the wild populations. The relatively low incidence of PNRSV and PDV in cultivated populations and the absence of infection on wild seedling populations growing nearby suggest that transmission by pollen is not an efficient way of spreading *Ilarvirus*es under field conditions.

In Lebanon, ACLSV has been detected previously on cherry and peach, but not on almond, while ApMV has not been reported to occur on stone fruits (Jawhar *et al*., 1996). This survey reveals the occurrence of ACLSV on almond and reports the occurrence of ApMV in Lebanon for the first time.

The occurence of ACLSV and ApMV on wild almonds from the Hermel area, of ACLSV on seedling trees in Baaloul (western Bekaa), and of both these viruses in almonds grown from seedlings obtained from local sources in the Akkar and Shouf areas indicate that ACLSV and ApMV infections in Lebanon have a long history. The existence of wild pear populations (*Pyrus syriaca* Boiss.) growing mixed with wild almonds in these areas suggests that these viruses may also occur on apples and pears.

The present survey confirmed earlier reports indicating the absence of PPV and ToRSV in Lebanon (Jawhar *et al*., 1996). PPV is not important on almond; for even though natural infections may occur, the trees remain symptomless (Diekmann and Putter, 1996; Dallot *et al*. 1997). ToRSV has not been reported in the Mediterranean region except for one unconfirmed report from Turkey (Azery and Çyçek 1997; Martelli and Savino, 1997).

There will be a high risk for dissemination of the above-mentioned viruses if there are no restrictions on importing non-certified or poorly certified propagation material, such as seeds for the production of rootstocks, scions or seedlings. The use of healthy germplasm is absolutely fundamental when establishing fruit tree orchards. The occurrence of PNRSV and PDV in seeds used for the production of rootstocks, in nursery seedlings and in mother plants used by nurseries poses a serious threat for the economic production of almonds and other stone fruits. Farmers should be very selective about the source of their seedlings. At present there is no certification system in Lebanon and facilities needed for the detection and control of quarantine diseases are not yet available. The current phytosanitary situation of almonds in particular and stone fruits in general, (Jawhar *et. al*., 1996) is satisfactory in Lebanon, when compared with that of other countries in the Mediterranean region. The percentage of infected almond trees is 10.3-21% in Lebanon, compared with 94% in Greece and 86% in Italy (Savino *et al*., 1995; Varveri and Ben, 1995). Furthermore, even though PPV is present in most Mediterranean countries, including neighbouring Syria and Turkey (Dunez, 1989) it has not yet been detected in Lebanon, which makes it more urgent to establish a fruit-tree certification system and enforce inspections and quarantine regulations. Only certified planting material or seedlings from approved organizations or institutions must be allowed entrance into the country.

Wild almonds should be preserved to maintain the national biodiversity, and their resistance/tolerance to biotic or abiotic factors should be assessed. They may become sources of desirable horticultural or disease resistance characteristics, such as tolerance to the above-mentioned viruses and other pests; and tolerance to drought and soil alkalinity.

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