SHORT COMMUNICATION

VIRUSES OF POME FRUIT TREES IN JORDAN

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SUMMARY

Surveys were conducted in the traditional areas of apple, pear, and quince cultivation in Jordan to assess the phytosanitary status of these species. The presence of virus diseases and their identification was ascertained through symptom observation in the field, sap transmission to herbaceous hosts, and double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). A total of 1,565 samples was collected from 38 commercial orchards, a mother block, 12 nurseries, and a varietal collection. A total of 1,393 apple, 149 pear, and 23 quince were tested individually by DAS-ELISA for *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV), and *Tomato ringspot virus* (ToRSV). All four viruses were identified in a large number of these samples; ToRSV was the most widespread and ASGV was the second most prevalent.

Key words: surveys, DAS-ELISA, ACLSV, ApMV, ASGV, ToRSV.

The pome fruit industry is important in Jordan, especially in the southern part of the country. The total area cultivated with these crops is about 4,240 ha with an annual production of nearly 41,200 tons (Anonymous, 2002). Recently, pome fruit species have come to the attention of growers. In particular, apple cultivation has, within a few years, expanded to a large area, especially in Ash shawbak. This has favored the introduction of a number of foreign varieties as alternatives to traditional ones. However, the recent introduction of apple and pear cultivars from abroad with unknown sanitary status has increased the incidence and severity of disease problems.

Thirty virus and virus-like diseases known under different names have been described in pome fruit trees. Among the virus diseases affecting pome fruits, those caused by *Apple chlorotic leaf spot virus* (ACLSV, genus *Trichovirus*), *Apple mosaic virus* (ApMV, genus *Ilarvirus*), *Apple stem grooving virus* (ASGV, genus *Capillovirus*), and *Tomato ringspot virus* (ToRSV, genus *Nepovirus*) are the most important economically (Nemeth, 1986; Sutic *et al*., 1999).

Information is not yet available on the phytosanitary status of pome fruits with respect to virus diseases and their distribution in Jordan. The areas cultivated with pome fruits are expanding and the current need of fruit growers to establish new orchards with propagating material of known sanitary status has made the gathering of information on the virus status of nursery stocks indispensable.

This study was conducted to identify the most important viruses of pome fruit trees in Jordan and to estimate the general prevalence and incidence of pome fruit tree viruses in commercial orchards, mother block, nurseries, and varietal collection in Jordan.

A total of 1,565 samples was collected during field surveys in 2002-2003 in the most important pome fruits growing areas (Irbid, Ajlun, Jarash, Mafraq, Zarqa, Amman, Balqa, Jordan Valley, Madaba, Tafila and Ash shawbak) of Jordan. Samples were collected from 38 commercial orchards, 12 pome fruit nurseries, a mother block for budwood production established at Al-Hassan Station (Tafila) owned by The Ministry of Agriculture, and a varietal collection at the National Center for Agricultural Research and Transfer Technology (NCARTT) in Ash shawbak.

Observations of symptom expression were made during spring by repeated surveys in an attempt to associate the results of laboratory tests with field syndrome(s).

Samples for laboratory testing were randomly collected from symptomatic as well as symptomless shoots. To obtain reliable data, shoots from selected trees were taken in February, sealed in plastic bags and kept at 4°C prior to forcing in the glasshouse. Each sample was made up of 4-6 one-year-old, 30 cm long sticks, taken from the four compass points and the internal part of the tree. Samples were tested by biological and serological techniques. Occurrence, distribution and relative incidence of ACLSV, ApMV, ASGV and ToRSV were recorded. Incidence was determined as the percentage of trees found to be infected in each location.

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Association of defined field symptoms with the presence of a particular virus was generally difficult due to the poor growth condition of many plantings, mainly because of water shortage. Symptoms on the trees were not especially obvious. However, when present they varied considerably with species and cultivar, and with the locality and growing conditions of the trees. Nevertheless, the results of field observations and their association of symptoms with viruses as detected by DAS-ELISA, and for some samples by mechanical transmission to herbaceous hosts, can be summarized as follows.

In apple, ToRSV was found in plants showing small, yellowish-green leaves and short internodes. The trunks of some older trees were swollen above the graft union and at the union transverse splitting was observed. Severely infected trees had thinner canopies and pale green leaves. ApMV was found both in symptomless trees, and trees showing symptoms of chlorotic discoloration of various forms (mottling, small irregular creamy or yellow spots that became necrotic) on the spring leaves as they expanded. Leaves that had developed during the summer at high temperatures were usually symptomless. Often the symptoms appeared only in single branches or limbs.

ACLSV and ASGV were mostly detected in symptomless trees. In some cases, ACLSV-infected apple trees exhibited general decline, small leaves, asymmetrically distributed chlorotic spots on the young leaves, asymmetric leaf growth, distortion and crinkle. ACLSV induced a mosaic ring pattern on leaves of infected pear and many cultivars were showing diffused symptoms of indistinct mottle as numerous pale-green or greenish yellow spots.

Viruses were found in all inspected orchards. None of the four viruses were detected in large number of samples collected from symptomatic pome fruit trees. Some apple trees were showing virus-like symptoms, in which leaves were reduced in size, with abnormally coarse serrations, but they tested negative for these viruses. The symptoms on these trees may have been caused by other pathogens or viruses, e.g., *Apple stem pitting virus* (ASPV, genus *Foveavirus*) or *Cherry rasp leaf virus* (CRLV, genus *Cheravirus*), which were not part of this investigation. Additionaly, other factors such as low virus concentration in the samples, abiotic agents causing virus-like symptoms, or uneven distribution of viruses in the trees may also have affected the negative results in DAS-ELISA.

During each survey, representative samples from symptomatic and healthy looking trees were collected and used to inoculate a panel of herbaceous hosts to test for the presence of mechanically transmissible viruses. Indicator hosts included: *Chenopodium amaranticolor*, *Chenopodium quinoa*, *Cucumis sativus*, *Nicotiana glutinosa*, *Phaseolus vulgaris*, and *Vigna unguiculata*. Flowers and/or young leaves from glasshouse-forced budsticks were ground in 0.01 M phosphate buffer pH 7.8, containing 0.001 M sodium diethyldithiocarbamate (Na-DIECA), 2.5% nicotine and 100 mg ml^{-1} activated charcoal.

Based on biological assay, several isolates were recovered from leaves collected from naturally growing pome fruit trees in Jordan. Four isolates were selected to represent other isolates based on the symptoms elicited in *C. amaranticolor*, *C. quinoa*, *C. sativus*, *N. glutinosa*, *P. vulgaris*, and *V. unguiculata.* The representative isolates were identified as ACLSV, ApMV, ASGV, and ToRSV.

ACLSV produced small chlorotic local lesions in the inoculated *C. quinoa* leaves 5-7 days after inoculation. These lesions became necrotic 24-36 h later. Within 10-20 days, a chlorotic ring mottle appeared in the young leaves of the growing point. Concurrently, growth was severely reduced. Chlorotic local lesions, surrounded by necrotic dots were observed in inoculated leaves of *C. amaranticolor*. ApMV induced systemic chlorotic lines and rings in *V. unguiculata*. Systemic infection was readily established in *C. quinoa* leaves.

ASGV caused primary necrotic lesions in the inoculated leaves of *C. quinoa* and systemic infection characterized by chlorosis, leaf distortion and reduction in growth. *N. glutinosa* developed systemic yellow mosaic and line patterns mainly in young leaves. The inoculated leaves of *P. vulgaris* showed brown necrotic spots 5-7 days after the inoculation and in some cases long necrotic areas developed along veins. In systemically infected plants, downward curling of the leaf margins occurred after 10-14 days. ToRSV produced local chlorotic or necrotic ringspots in mechanically inoculated leaves of *C. sativus* within 8-10 days post-inoculation. One week later, systemic chlorotic ringspots, tip necrosis, mottling and oak leaf patterns were produced in subsequent growth. The symptoms of ToRSV in *V. unguiculata* at 5-8 days after inoculation included red or purple lesions in the inoculated leaves followed by systemic tip necrosis.

Inoculated and tip leaves from all herbaceous plants that had been mechanically inoculated with extracts from plants infected with ACLSV, ApMV, ASGV or ToRSV were back-inoculated to more herbaceous hosts. ELISA (see below) showed them all to be infected. All symptomless plants tested negative by DAS-ELISA.

In addition to the biological assay, DAS-ELISA (Clark and Adams, 1977) was used for detecting ACLSV, ApMV, ASGV, and ToRSV using commercial kits from Bioreba (Bioreba AG, Reinach, Switzerland) following the manufacturer's protocol. Standard negative and positive controls (Bioreba AG, Reinach, Switzerland) were included in each plate to verify the assay performance. ELISA readings were considered positive when sample wells were a visually detectable yellow color and had absorbances greater than twice the mean absorbance reading of two healthy control samples. Among a total of 1,393 apple, 149 pear, and 23

quince trees, remarkable differences in incidence were observed between the different viruses; the most prevalent was ToRSV, followed by ASGV, ACLSV and then ApMV (Table 1).

ToRSV was detected in all pome fruit orchards surveyed. The virus incidence ranged from 4.5 to 11% and the mean level was about 6.8%. Results showed that the highest rate of infection was recorded in nurseries (11%), while the rates of infection in the varietal collection, commercial orchards and the mother block were 9.3, 6.0, and 4.5%, respectively. ASGV was not detected in nurseries, while percent infections of samples from the mother block, the varietal collection, and commercial orchards were 12.5, 8.9, and 3.7%, respectively. More than 11.8% of the samples from the varietal collection contained ACLSV, whereas the infection rates of the mother block, nurseries, and commercial orchards were 7.9, 3.7, and 1.5%, respectively. ApMV was detected in very few samples (Table 1).

A distinct difference in infection level was found between the regions examined. The virus incidence ranged very widely among regions from a high at Ash shawbak, Tafila, and Jarash to a low at Balqa, Zarqa, and Amman (Table 2).

This study shows for the first time the identities of some of the viruses infecting commercially grown pome fruits in Jordan. According to field investigations, herbaceous host reactions, and ELISA, the presence of ACLSV (Lister, 1970a), ApMV (Fulton, 1970), ASGV (Lister, 1970b), and ToRSV (Smith, 1970) was confirmed in the area.

Field investigations and laboratory work revealed that ToRSV was widespread in the surveyed area. The high frequency of occurrence of ToRSV is very probably related to the infestations of its nematode vector *Xiphinema americanum* and infection of other plant species, including stone fruits, grapevines and weed dandelion, found in that area. Moreover, most of the pome fruit trees are on Malling Metron 106 (MM 106) rootstock, which is the most frequently naturally infected clone (Fridluund, 1989).

The conducted survey, which is the first in Jordan to

Table 1. Incidence of ACLSV, ApMV, ASGV, and ToRSV in pome fruit trees/seedlings surveyed in Jordan irrespective of location, in commercial orchards, a mother block, nurseries, and a varietal collection during 2002-2003, using DAS-ELISA.

Type of orchards	No. of samples tested	Viruses			
		ACLSV	ApMV	ASGV	ToRSV
Commercial orchards	1,165	18 [a] (1.5) [b]	13 (1.1)	43 (3.7)	71 (6.0)
Mother block	88	7 (7.9)	1 (1.1)	11 (12.5)	4 (4.5)
Nurseries	109	4 (3.7)	1 (0.9)	0 (0.0)	12 (11)
Varietal collection	203	24 (11.8)	1 (0.4)	18 (8.9)	19 (9.3)
Total	1,565	53 (3.4)	16 (1.0)	72 (4.6)	106 (6.8)

[a] Number of infected plants
[b] Percentages

Table 2. Incidence of ACLSV, ApMV, ASGV, and ToRSV in pome fruit trees/seedlings from different locations in Jordan, irrespective of pome fruit species, surveyed during 2002-2003, using DAS-ELISA.

Region	No. of samples tested	Viruses			
		ACLSV	ApMV	ASGV	ToRSV
Irbid	183	4 [a] (2.1) [b]	2 (1.0)	12 (6.6)	11 (6.0)
Ajlun	74	3 (4.0)	1 (1.4)	3 (4.0)	2 (2.7)
Jarash	121	4 (3.3)	0 (0.0)	0 (0.0)	23 (19.0)
Mafraq	131	1 (0.8)	1 (0.7)	1 (0.7)	8 (6.1)
Zarqa	46	0 (0.0)	1 (2.2)	0 (0.0)	1 (2.2)
Amman	88	0 (0.0)	2 (2.2)	0 (0.0)	0 (0.0)
Balqa	256	1 (0.4)	4 (1.6)	6 (2.3)	1 (0.4)
Madaba	249	4 (1.6)	2 (0.8)	9 (3.6)	21 (8.4)
Tafila	88	7 (8.0)	1 (1.1)	11 (12.5)	4 (4.5)
Ash shawbak	329	29 (8.8)	2 (0.6)	30 (9.1)	35 (10.6)
Total	1,565	53 (3.4)	16 (1.0)	72 (4.6)	106 (6.8)

[a] Number of infected plants
[b] Percentages

date, provided a relatively clear picture of the phytosanitary status of pome-fruit species. Based on the survey, the level of virus infection of pome fruits is in an acceptable range, considering the lack of certification programs regulating the production of certified virus-free propagative material in Jordan. The absence of legislation organizing fruit tree nurseries and mother block allows production and marketing of seedlings without formal health quality control or varietal identification.

The major concern coming from the present study is the alarming infection rate observed in apple mother blocks. This is the major source of budwood for propagation. This means that, if prophylatic measures are not urgently adopted, apple orchards established in the years to come can be expected to have poor phytosanitary status. The current plant health situation of these crops calls for the urgent implementation of a sanitation programme based mainly on the use of virus-free propagating material.

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