Viruses, Viroids and Phytoplasmas Reported to Infect Fruit Trees in Syria: A Review

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Abstract

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In Syria, like most Mediterranean countries, fruit trees industry is considered one of the most important sectors among all Syrian agriculture sectors. Many fruit tree species are grown in Syria since a long time and they are adapted to the local environmental conditions in the country which is a Mediterranean climate. Syria is rich in growing many local varieties of fruit tree species which represent considerable and important genetic resources of fruit tree species all over the world. As most plant species, fruit trees are affected by many biotic and abiotic disease stress factors. Among the biotic factors, viruses, viroids and phytoplasmas are among the most important factors which can cause serious diseases on several fruit tree species. Since the early 1980s, many virus, viroid and phytoplasma diseases were investigated and reported on many fruit tree species grown in Syria such as olive, stone fruits, grapevine, pome fruits, citrus, fig, walnut and pecan. Many surveys were previously conducted to evaluate the sanitary status of these fruit tree species focusing on the occurrence of these pathogens in Syria. Assessment of the sanitary status of these fruit trees, and detection of those pathogens was carried out using the appropriate and available detection methods such as biological, serological and molecular techniques.

Keywords: Diseases, fruit trees, viruses, viroids, phytoplasmas, Syria

Introduction

Fruit trees industry is a very important sector of the Syrian agriculture. In Syria, many species of fruit trees are grown, especially in the western part of the country from Al-Qunaitara, Daraa, Al-Sweida and Damascus countryside governorates in the south, across Homs and Hama in the middle part of the country and to Idleb and Aleppo in the north. Furthermore, they are also grown in the coastal region in Lattakia and Tartous governorates. As for the eastern part of the country, some species of fruit trees are also grown, but in a limited cultivation area. Olive (*Olea europea* L., Genus: Olea, Family: Oleaceae) is a very ancient and important fruit tree species grown widely in Syria. It has the largest area of cultivation and it ranks first in terms of annual production. According to the most recent agricultural statistics issued by the Planning and Statistics Department at the Ministry of Agriculture and Agrarian Reform, the cultivated area with olive trees was estimated to be 693,227 hectares with a total production of 844,316 tones (Anonymous, 2019). The most prevailing olive varieties are of local origin, e.g. Zaiti, Sorani, Khderi, Doaibli, Qaysi, Jlot, Dan and Mosaabi. Stone fruit species are also grown widely in Syria, especially the following species in the genus Prunus, family Rosaceae: apricot (*Prunus armeniaca* L.), almond (*Prunus dulcis*, subg *amygdalus* (Mill.) D. A. Webb), sweet cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L. 1753), plum (*Prunus domestica* L.) and peach and nectarine (*Prunus persica* (L.) Batsch,). The total cultivation area of all stone fruit species is 126,652 hectares with a total production of 270,663 tones (Anonymous, 2019).

In Syria, there are many important local varieties of apricot (Ajami, Baladi, Shakarbara, Hamwi, Sendiani, Tadmouri, Wazari and Dahabi), almond (Auja and Dafadii), plum (Abou Riha, Ajami, Ballowri and Sukkari), sweet cherry (Kaws kozah, Kalb Atter and Feraoni). As for peach and nectarine there are mainly imported varieties. With a cultivation area of 45,180 hectares and a total production of 252,006 tones (Anonymous, 2019), grapevine (*Vitis vinifera* L., Genus: Vitis, Family: Vitaceae) ranks third after olive and stone fruits. Vineyards are spread all over the Syrian territory, with a higher intensity in the southern region and in the central part of the country, particularly in Al-Sweida, Al-Qunaitara, Daraa, Damascus countryside, Homs, Hama, Aleppo governorates and in the coastal region in Tartous and Lattakia governorates. Table grape varieties are the most widely grown, some of them are local, e.g. Helwani, Baladi, Betamouni, Zaini and Shami. Among the local grapevine varieties used for industry, Ashori and Salti are the most important. Many of citrus species are grown in Syria and the majority of citrus orchards are located in the coastal region in the north western part of the country, especially in Lattakia and Tartous. The total cultivation area of all citrus species is around 42,654 hectares with a total annual production of 1,094,808 tons (Anonymous, 2019).

The most common species belong to family Rutaceae, genus Citrus: (i) sweet orange (*Citrus sinensis* L.), including Navel, Jaffa and Valencia varieties; (ii) mandarin (*Citrus reticulate* Blanco), (iii) lemon (*Citrus lemon* L.); and (iv) grapefruit (*Citrus paradise* Macfad.). Pome fruits are grown in the hilly areas with altitudes more than 800 meters above the sea level in southern, central and western coastal regions of Syria in an area around 56,150 hectares with total annual production of 310,216 tons (Anonymous, 2019); ranking fifth among fruit tree species, after olive, stone fruits, grapevine and citrus. The most cultivated species are: apple

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(*Malus domestica* Borkh., Genus: Malus, Family: Rosaceae), pear (*Pyrus communis* L., Genus: Pyrus, Family: Rosaceae) and quince (*Cydonia oblonga* Mill., Genus: Cydonia, Family: Rosaceae). Apple is considered as the main crop, whereas pear and quince are cultivated in limited area. Even the most prevailing apple varieties are imported, e.g. Golden delicious, Star king, Granny smith and Star crimson, there are also some local varieties, e.g. Sukkari, Skarji and Abou Ghabra. Most of pear varieties are imported, e.g. Bartlett, Williams and Coscia.

During the last three decades, the cultivation of pistachio (*Pistacia vera* L., Genus: Pistacia, Family: Anacardiaceae) trees was increased due to its economic importance as a source of income for many farmers in Syria, especially in the pistachio main growing areas in Aleppo, Idleb and Hama. The cultivation area of pistachio in Syria was estimated around 59,989 hectares with a total production of 31,813 tones (Anonymous, 2019). Most of the grown varieties are of local origin, e.g. Ashouri, Nab aljamal, Ajami, Sen alfeel and Lesan altair. Fig (*Ficus carica* L., Genus: Ficus, Family: Moraceae) is grown in Syria too, mainly in the hilly and cold areas as few trees in some private groves and orchards, but rarely in special big plantations. The total fig cultivation area in Syria is around 9,435 hectares, with a total production of 43,015 tonnes (Anonymous, 2019). The most common fig varieties are of local origin, e.g. Sukkari, Bshari, Ghozlani, Khodairi, Baiadi, Ahmar, Aswad jalladi, Zerkani, Shebli, Shami and Zoaibli. Walnut (*Juglans regia* L., Genus: Juglans, Family: Juglandaceae) is an adapted fruit tree species to the Syrian environmental conditions and it is grown since a long time in most Syrian governorates. The walnut cultivation area in Syria is 3,057 hectares and the total production is 10,569 tones (Anonymous, 2019). Most of walnut varieties are local, e.g. Balahseen, Kalesh, Bukaei, Baladi and Kastal gandar. In addition, some pecan (*Carya illinoensis* (Wangenh) K. Koch, Genus: Carya, Family: Juglandaceae) trees are grown in Syria, most of them represented by few imported varieties, e.g. Riverside, GraTex, GraZona and Choctaw. In addition, there are many other species of fruit trees grown in Syria, but with less importance and limited cultivation areas, e.g. pomegranate (*Punica granatum* L., Genus: Punica, Family: Lythraceae), date palm (*Phoenix dactylifera* L., Genus: Phoenix, Family: Arecaceae), mulberry (*Morus alba* L., Genus: Morus, Family: Moraceae) and loquat (*Eriobotrya japonica* (Thunb.) Lindl., Genus: Eriobotrya, Family: Rosaceae).

In this review article focus is made on the most important viruses, viroids and phytoplasmas previously reported as disease causal agents of different fruit tree species grown in Syria over the past forty years.

Olive trees viruses

Even though olive has a major economic importance and occupies the largest cultivation area among all fruit tree species grown in Syria, until now, only one survey to investigate the presence and spread of viral diseases on it in its main growing regions in the country was conducted by Abdulkader Al-Abdullah and his colleagues in 2003. This survey was conducted by the olive office directorate in Syria in collaboration with the Mediterranean Agronomic Institute of Bari, Italy. In autumn 2003, this survey covered more than 80 commercial orchards belonging to private farmers in six main Syrian olive-growing governorates: Daraa, Hama, Idleb, Aleppo, Tartous and Lattakia. A total of 300 olive tree samples with no clear cut symptoms of viral infections, but some trees were stunted, or had bushy vegetation, or showed malformation of twigs, flattening of branches, deformation, chlorosis or yellowing of the leaves, were collected and tested by reverse transcription-polymerase chain reaction (RT-PCR) technique for the presence of the following eight viruses: Arabis mosaic virus (ArMV, Genus: *Nepovirus*, Family: *Secoviridae*), Cherry leaf roll virus (CLRV, Genus: *Nepovirus*, Family: *Secoviridae*), Cucumber mosaic virus (CMV, Genus: *Cucumovirus*, Family: *Bromoviridae*), Olive latent ringspot virus (OLRSV, Genus: *Nepovirus*, Family: *Secoviridae*), Olive latent virus 1 (OLV-1, Genus: *Alphanecrovirus*, Family: *Tombusviridae*), Olive latent virus 2 (OLV-2, Genus: *Oleavirus*, Family: *Bromoviridae*), Olive leaf yellowing-associated virus (OLYaV, Genus: unassigned, Family: *Closteroviridae*) and Strawberry latent ringspot virus (SLRSV, Genus: unassigned, Family: *Secoviridae*). The results of primary dsRNA analysis showed that 54 out of 125 tested samples were positive, with 43% infection rate. All eight viruses were detected in 51.7% of total samples tested either singly or in mixed infections. CMV was the most prevailing virus with 22.7% of infection rate, followed by CLRV, OLYaV and OLRSV with 15%, 14.3% and 11.5% of infection rates, respectively. The other four tested viruses were recorded with less infection rates: SLRSV (5.7%), OLV-1 (6.0%), OLV-2 (2.0%) and ArMV (0.7%). According to the geographical distribution, the infection rates ranged between 44% in Daraa and 67% in Lattakia and Hama. That was the first report of these eight viruses on olive trees in Syria (Al Abdullah *et al*., 2005, 2007).

Stone fruit trees viruses and viroids

The first report of viruses infecting stone fruit species in Syria was in 1986, within the framework of a limited consultancy survey made by Jean Dunez and supported by the Food and Agricultural Organization (FAO) of the United Nation (Dunez, 1986). Plum pox virus (PPV, Genus: *Potyvirus*, Family: *Potyviridae*) was one of the first viruses reported on stone fruit trees in Syria, especially in apricot trees by observing clear cut symptoms of the virus on the leaves, fruits and stones of many apricot varieties. These visual inspections were confirmed by serological tests (Dunez, 1986, 1989). A decade later and during the period 1995-1997, the sanitary status of selected varieties of stone fruit species was assessed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). In this assessment, a total of 146 local and imported varieties, represented by 1090 trees from all stone fruit species grown in the country were tested for the presence of the main five viruses naturally infecting stone fruits: PPV, Prune dwarf virus (PDV, Genus: *Ilarvirus*, Family: *Bromoviridae*), Prunus necrotic ringspot virus (PNRSV, Genus: *Ilarvirus*,

Family: *Bromoviridae*), Apple mosaic virus (ApMV, Genus: *Ilarvirus*, Family: *Bromoviridae*) and Apple chlorotic leafspot virus (ACLSV, Genus: *Trichovirus*, Family: *Betaflexiviridae*). The results showed that a total of 118 stone fruit varieties representing 59.2% of tested trees, were found to be virus-free. With the exception for sour cherry, plum and almond, most of stone fruit species including apricot, peach, nectarine and sweet cherry were infected with the five tested viruses, PDV was the most prevailing virus with 18.8% of infection rate in the tested trees, followed by PNRSV (14.95%), ApMV (12.5%), PPV (9.3%), and finally ACLSV (4.6%) (Al-Chaabi *et al.*, 1997, 2000). Furthermore, during the period 2000-2001, more expanded surveys were carried out in the main stone fruit-growing areas of Syria to evaluate the sanitary status of mother blocks, varietal collections and private commercial orchards. The presence of virus and viroid diseases (the main 5 viruses and 2 viroids) was checked by enzyme-linked immunosorbent assay (ELISA), sap transmission to herbaceous hosts, biological indexing on the woody indicator plants: *Prunus persica* cv. GF305 and *P. serrulata* cv. Kwanzan and dot plot hybridization. A total of 1337 samples representing 444 apricots, 283 peach and nectarine, 246 cherries, 222 almonds and 142 plum trees were tested by ELISA. The overall mean infection rate was 13%, whereas for each single species, the infection rate was as follows: peach and nectarine 24%, sweet and sour cherry 16%, almond 13.5%, apricot 6% and plum 5%. The five tested viruses were detected either singly or in mixed infections. The prevalent viruses in the infected trees were: PNRSV (86.6% in almond and 73.5% in peach and nectarine), PDV (87.5% in sweet and sour cherry and 45.8% in plum) and ACLSV (53.8% in apricot). PPV and ApMV were each found in a single tree. The PPV positive apricot sample was grafted on GF305 and symptoms similar to those induced by PPV were observed two weeks after grafting. The serotyping test of this isolate by indirect DASI-ELISA and using four strain-specific monoclonal antibodies (MAbs), showed that it belongs to strain M (Marcus) of PPV (Ismaeil, 2001, 2006; Ismaeil *et al.*, 2002, 2003a, Al Rwahnih & Ismaeil, 2003). During the same above-mentioned survey, 53 peach and nectarine samples were collected from 15 private commercial orchards in southern and central regions of the country as well as 24 apricot samples from a varietal collection at Douma research station in Damascus countryside. All samples were tested for the presence of Peach latent mosaic viroid (PLMVd, Genus: *Pelamoviroid*, Family: *Avsunviroidae*) and Hop stunt viroid (HSVd, Genus: *Hostuviroid*, Family: *Pospiviroidae*) by dot plot hybridization. PLMVd was recorded only on peach and nectarine (40% infection rate), whereas HSVd was found only on apricot with 62.5% infection rate. Infected trees belonged to both local and imported apricot, peach and nectarine varieties (Ismaeil, 2001; Ismaeil *et al.*, 2001, 2002, 2003a). In order to get preliminary evidence concerning virus strain diversity among the Syrian isolates of PNRSV, 15 isolates from three different *Prunus* hosts (almond, peach and plum) found positive by ELISA test, were chosen for studying their biological, serological and molecular characteristics. Virus transmission from infected scions to GF305 rootstocks was confirmed by indirect ELISA two weeks after grafting. PNRSV isolates tested by DASI-ELISA using ten PNRSV-specific MAbs showed that there were some serological differences between the Syrian PNRSV isolates, which were identified as four different serogroups. However, single-strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) analysis confirmed substantially a high homology among all tested PNRSV Syrian isolates (Ismaeil, 2001; Ismaeil *et al*., 2003b). Furthermore, during 2006, a total of 761 stone fruit samples (sweet cherry, sour cherry, apricot, plum, peach and almond) were collected from mother blocks, varietal collections, nurseries and commercial orchards from six Syrian governorates: Damascus countryside, Al-Sweida, Al-Qunaitera, Homs, Hama and Lattakia and were tested for the presence of ACLSV by DAS-ELISA. The total infection rate was around 7%. ACLSV was recorded on peach with infection rate of 20% and on peach seedlings 2.9%, almond 6.9% and sweet cherry 7.7%. No infections were detected on apricot, plum, sour cherry samples (Al-Jabor *et al.*, 2007, 2008). Two years later, in 2008, a study was conducted to investigate the seed transmission of three ilarviruses: PDV, PNRSV and ApMV reported previously on stone fruit species in Syria. For that purpose, a total of 421 group samples were randomly collected from seven stone fruits nurseries in six Syrian governorates. The samples represented three different phenological stages of stone fruit rootstocks production: 82 seed samples, 67 samples of seedlings with four true leaves and 272 samples of six months old seedlings. Results of DAS-ELISA showed that the total average of viral infection was 1.84% in tested samples of six months old seedlings, whereas it was 0.63% in seed samples. PDV was the most prevailing virus, which was found in 1.45% of tested seedlings, PNRSV in 0.22%, while only three samples were infected with ApMV (0.11%). The highest incidence of viral infection was recorded on the six months old peach seedlings (3.22%), followed by sour cherry (2.64%), almond (1.28%) and apricot seedlings (0.64%). No infections were recorded in tested plum seedlings samples. According to the geographical distribution, the highest incidence of viral infection was recorded in seedling samples collected from Aleppo with 3.88%, followed by Al-Sweida with 2.48%, while, the lowest incidence of viral infections was recorded in seedlings samples collected from Homs (1.08%) and Damascus countryside (1.05%) (Al-Chaabi & Darwesh, 2008; Darwesh & Al-Chaabi, 2007).

Grapevine viruses

The first study on viral diseases of grapevine in Syria was in 1991 by Ramez Al-Daoud and his colleagues. They have studded the incompatibility phenomenon in grafted vines between scion and rootstock and they have indicated by serological tests that Grapevine leafroll associated virus 3 (GLRaV 3, Genus: *Ampelovirus*, Family: *Closteroviridae*) and Grapevine fleck virus (GFkV, Genus: *Maculavirus*, Family: *Tymoviridae*) are both involved in the incompatibility syndrome at the scion and rootstock union in some vineyards in Homs and Hama governorates in Syria (Al-Daoud *et al*., 1991). During the period 1995-1997, the sanitary status of more than 200 selected grapevine varieties

was evaluated using DAS-ELISA test. A total of 854 vines/grafted seedlings/rootstocks were tested for the presence of three viruses naturally infecting grapevine: Grapevine leafroll associated virus 1 (GLRaV 1, Genus: *Ampelovirus*, Family: *Closteroviridae*), GLRaV 3 and Grapevine fanleaf virus (GFLV, Genus: *Nepovirus*, Family: *Secoviridae*). The results obtained showed that a total of 92 grapevine varieties (69.3% of tested vines) were found to be virus-free. GLRaV 3 was found in 16% of tested vines, GLRaV 1 in 15.1% and GFLV in 4.8% of total tested vines samples (Al Chaabi *et al*., 2000). Later on, and during the period October 2004-May 2005 more expanded surveys for viral diseases on grapevine were carried out in private commercial vineyards and some governmental nurseries in seven different Syrian governorates: Aleppo, Daraa, Al-Sweida, Al Qunaitara, Homs, Hama and Tartous. Samples were collected randomly from 835 individual vines (735 *Vitis vinifera* and 100 rootstock accessions) for laboratory tests. GFLV, ArMV, and Grapevine virus A (GVA, Genus: *Vitivirus*, Family: *Betaflixiviridae*) were the only viruses recovered by mechanical transmission to herbaceous hosts. Vein necrosis developed in around 53% of graft-inoculated 110R indicator plants and vein mosaic in *Vitis riparia*. A total of 71% of the ELISA-tested *V. vinifera* plants (522 out of 735) were infected by one virus (14.8%) or by more than one virus (55.8%) as mixed infection. GVA was the most prevailing virus (54.7%), followed by GLRaV-1, (47.3%), GFkV, (29.7%), and GLRaV-3, (23.9%). The other tested viruses occurred at lower infection rates, i.e. Grapevine leafroll associated virus 2 (GLRaV 2, Genus: *Closterovirus*, Family: *Closteroviridae*) (9%), GFLV (0.8%) and ArMV (0.1%). The most important Syrian grapevine varieties, e.g.: Helwani, Salti, Baladi, and Zaini, had infection rates ranged between 44% and 91%. In terms of geographical distribution, the highest incidence of infections was observed in Damascus countryside (90%), whereas it ranged between 68% and 79% in the other governorates, except for Hama (36%). Rootstocks were in a better sanitary status (25% infection). GFkV was detected in 22% of the tested samples, whereas the presence of GLRaV-3 (3%), GLRaV-1, and GFLV (1%) was very low. Grapevine rupestris stem pitting associated virus (GRSPaV, Genus: *Foveavirus*, Family: *Betaflexiviridae*) was detected in 72.3% of the samples by RT-PCR and a high percentage of GRSPaV-positive vines (80%) induced vein necrosis reactions on 110R indicator plants (Mslmanieh *et al*., 2006, 2007). At the same time, a survey was conducted in southern Syria during 2005 and 2006 to investigate the viruses associated with leaf roll symptoms in grapevine. Around 800 samples were collected and tested for the presence of the following viruses: GLRaV-1, GLRaV-2, GLRaV-3, Grapevine leafroll associated virus 6 (GLRaV-6, Genus: *Ampelovirus*, Family: *Closterovirideae*) and Grapevine leafroll associated virus 7 (GLRaV-7, Genus: *Velarivirus*, Family: *Closteroviridae*) by DAS-ELISA. The grapevine samples were collected from private commercial vineyards and varietal collections distributed in 3 Syrian governorates: Al-Sweida, Daraa and Al-Qunaitara (southern Syria) during autumn and winter of 2005, 2006 and early 2007. Results obtained showed that the highest incidence of leaf roll symptoms observed on vines in autumn was in Al-Sweida (3.4%), mainly on red and black berried varieties. About 40.12% of the samples were found to be infected (35.3% were infected with one virus, and 4.9% were mixed infected with more than one). The highest incidence was recorded in the varietal collections in Al-Qunaitara (78.6%), followed by Daraa (39.3%) and last in Al-Sweida (23.2%). Virus incidence in private commercial vineyards of the three governorates ranged between 25 and 30%. The incidence of GLRaV-1 was the highest (23.5%), followed by GLRaV-3, GLRaV-2 and GLRaV-6 with incidences of 14.38, 7 and 0.25%, respectively. That was the first report of GLRaV-6 on grapevine in Syria (Gharz Eddin *et al*., 2008). Moreover, a survey was carried out for the detection of GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA, Grapevine B virus (GVB, Genus: *Vitivirus*, Family: *Betaflixivirideae*), Tobacco ringspot virus (TRSV, Genus: *Nepovirus*, Family: *Secovirideae*) and two strains of Tomato ringspot virus (ToRSV-ch and ToRSV-PYBM, Genus: *Nepovirus*, Family: *Secovirideae*) associated with graft incompatibility phenomenon. A total of 708 grapevine samples represented private commercial vineyards and varietal collections distributed in 4 governorates: Daraa, Al-Sweida, Damascus countryside and Homs were tested during autumn and winter of 2003, 2004 and 2005, by DAS-ELISA. With the exception to GLRaV-7, TRSV as well as 2 strains of ToRSV which were not found in any tested sample, 28.11% of collected samples were found to be infected with the rest of tested viruses. The highest incidence of viral infection was recorded in the private vineyards (61.5%), whereas it was 20.5% in varietal collections. GLRaV-1 was the most common virus (16.5%), followed by GLRaV-3, GVA, GLRaV-2 and GVB, with incidence of 13.4, 3.8, 1.4 and 0.14%, respectively. 6.64% of tested samples were infected with more than one virus (mixed infection). The viral incidence of tested grapevine samples collected from graft union of private commercial vineyards showing graft-incompatibility was 72.1%, whereas 47.1% of tested samples had mixed infections. The incidence of some viruses varied among different parts of the vine: GLRaV-2 incidence was the lowest (11.8%) in grapevine shoot samples, whereas it was the highest in the samples collected from graft union level (61.8%), followed by GLRaV-3, GLRaV-1 and GVA, with 45.6, 30.9 and 5.9% incidence, respectively. GLRaV-7 and GVB were not detected in the graft union of the tested samples (Al-Chaabi *et al*., 2000). More recently, a survey was conducted during 2016-2018 in five Syrian governorates: Lattakia, Tartous, Homs, Hama and Al-Sweida to assess the distribution of GFLV on different grapevine varieties.

In this study, a total of 360 samples showing different symptoms suggestive of virus infection, e.g. mosaic, vein yellowing, chlorosis, vein banding, mottling, leaf distortion, and shortened internodes were collected and tested by DAS-ELISA. Out of 360 samples, 42 were infected with GFLV (11.66% of the total tested samples). In terms of geographical distribution, the highest infection rate was in Hama (22.72%) followed by Homs (17.1%). When varieties were compared, the highest infection rate was recorded on Zaini variety with infection rate of 22.22% (Akel *et al*., 2020).

Pome fruits viruses and phytoplasma

The first report on apple viruses in Syria was by Kawas (2009), who investigated the spread of viral diseases on apples in southern Syria during 1998-2000. A total of 108 samples were collected from apple orchards and were tested by biological assays and serologically by ELISA against six viruses: ApMV, ACLSV, ToRSV, TRSV, Tomato black ring virus (ToBRV, Genus: *Nepovirus*, Family: *Comoviridae*) and ArMV. Results obtained showed that ACLSV and ApMV were the most prevailing viruses, with relative occurrence of 24% and 26.9%, respectively. ToRSV, TRSV, ToBRV and ArMV were recorded for the first time on apple in Syria at relative occurrence rates of 13%, 14.8%, 12.03% and 2.43%, respectively. More expanded surveys were conducted to evaluate the sanitary status of pome fruit trees in Syria during spring 2003 and 2004 in 6 Syrian governorates: Damascus countryside, Al-Qunaitara, Al-Sweida, Homs, Hama and Lattakia, as the main production areas of pome fruits. Leaf samples from 1077 apples, 54 pears and 14 quinces were collected from 70 commercial orchards and three varietal collections and tested by ELISA for the presence of four viruses: ACLSV, ApMV, Apple stem grooving virus (ASGV, Genus: *Capillovirus*, Family: *Betaflexiviridae*) and Apple stem pitting virus (ASPV, Genus: *Foveavirus*, Family: *Betaflexiviridae*). Results obtained showed that virus infection rates were 34% in apple and 2% in pear, whereas no virus infections were recorded from quince trees. ACLSV was the most prevailing virus on apple with 34%, whereas ASGV and ApMV were found in 2 and 0.2% of tested trees, respectively. Pear trees were infected only with ACLSV (2%). For biological indexing, bud wood samples from 21 apple trees and 15 pear trees were indexed by grafting on the following woody indicators: *Malus pumila* cvs. Virginia Crab and Radiant for apple and *M. pumila* cv. Virginia Crab and *Pyrus communis* cv. Nouveau Poiteau for pear. Virus infection rates obtained by woody plants indexing were higher than ELISA testing, as ASPV and ASGV were found in 86 and 82% of apple tested samples, and 80 and 60% on pear tested samples, respectively. An additional RT-PCR test which was carried out for a limited number of samples, confirmed the high incidence of ACLSV, ASPV, ASGV and ApMV (Al-Jebr *et al*., 2005; Ismaeil *et al.*, 2006, 2007). Moreover, a survey was conducted during 2006 in six Syrian governorates: Damascus countryside, Al-Sweida, Al-Qunaitara, Homs, Hama and Lattakia. A total of 999 samples of pome fruits [apple, pear, quince and hawthorn (*Crataegus rhipidophylla* Gand, Genus: Crataegus, Family: Rosaceae)] were collected from mother blocks, varietal collections, commercial orchards and nurseries and were tested for the presence of ACLSV using DAS-ELISA. Results showed that the total infection rate of all pome fruits tested samples was 21.9%. The highest infection rate was recorded in apple (42.5%). No infections were detected on pear and hawthorn samples. ACLSV was recorded on quince with 5% infection rate for the first time in Syria. (Al-Jabor *et al.*, 2007, 2008). In order to characterize some of ACLSV Syrian isolates serologically, a total of 120 samples of leaves, flowers and fruits were collected from apple, pear and some stone fruit trees species (almond, cherry and peach) showing viral symptoms from a varietal collection at the Agricultural Scientific Research Centre in Al-Sweida and were tested for this virus in 2008. Modified double antibody sandwich (DAS-ELISA) and in triple antibody sandwich ELISA (TAS-ELISA) with monoclonal antibodies were used. The results obtained showed that 57 virus isolates were divided into 23 different serological subgroups, the MAb C1 reacted with 38 isolates, whereas MAb A2 reacted with 22 isolates. The reaction of some monoclonal antibodies (MAb C1, Mab C2, MAb C3, MAb A2 and MAb B2) with apple isolates was strong with the exception of MAb C2, whereas their reaction with isolates from other plant hosts was weak. Virus isolates collected from apple reacted positively with the above mentioned five MAbs and with polyclonal antibodies, but the ELISA readings obtained with MAbs were higher than that with polyclonal antibodies. With the exception of one ACLSV isolate obtained from peach flowers, the reactions of polyclonal antibodies with other isolates were not consistent with monoclonal antibodies reactions. The reaction level of this isolate with three MAbs: MAb C1, MAb C2 and MAb C3 was weak, whereas its reaction with polyclonal antibodies was strong (Al-Jabor *et al*., 2009, 2010). Furthermore, a limited survey conducted in Al-Sewida Governorate to investigate the presence of apple proliferation phytoplasma (*Candidatus phytoplasma mali*, Seemüller and Schneider, Genus: Phytoplasma, Family: Acholeplasmataceae), showed that this phytoplasma was associated with some abnormal symptoms on apple trees, such as witch`s broom, leaf reddening, small fruit size with elongated peduncle and sometimes fruits deformation. A total of 160 samples from apple trees of the two varieties (Golden delicious and Star king) were collected and tested by DAS-ELISA using antiserum against this phytoplasma. The infection rate was 8.1% of all tested samples (Al-Jabor, 2012).

Citrus viruses and viroids

The first report on the presence of virus and viroid diseases on citrus species in Syria was made by Joseph Marie Bovè in 1995 based on visual observations (Bovè, 1995). The first extensive survey of viral diseases on citrus species in Syria was conducted by Raied Abou Kubaa and his colleagues in 2006. The main citrus growing areas of Syria in Lattakia and Tartous governorates were surveyed to investigate the presence and spread of the most infectious virus disease on citrus: Citrus tristeza virus (CTV, Genus: *Closterovirus*, Family: *Closteroviridae*) and its main insect vectors. Eight nurseries (130 plants per each), two bud-wood source fields and 19 orchards of the main citrus varieties were inspected, and samples were collected and tested by the direct tissue blot immunoassay (DTBIA). A total of 89 CTV-infected plants out of 2653 samples tested were positive by the DTBIA test in two nurseries, two bud-wood source fields and six orchards with 4% infection rate. Five citrus varieties were found to be infected, and the variety Meyer lemon showed the highest incidence of 16%. Numerous sweet orange varieties were found to be highly infected in the orchards, whereas only the Washington navel sweet orange was found

to be infected in the nurseries. Almost all infected samples induced moderate vein clearing, leaf cupping, stunting, and stem pitting symptoms when grafted on Mexican lime indicator plant. Only one CTV strain from the sweet orange variety Valencia induced severe symptoms on Mexican lime seedlings by graft-transmissions. The viral infection was widely and randomly distributed in the commercial orchards, especially in southern Tartous in some nurseries. Positive samples by DTBIA were also confirmed by biological indexing on Mexican lime and by immunocapture reverse transcription-PCR. Based on the reaction with several monoclonal antibodies (MAbs), CTV Syrian isolates clustered in 4 serogroups. To determine the genetic diversity of CTV strains present in the country, infected samples were tested by single strand conformation polymorphism (SSCP) of the coat protein (CP) gene of the virus, multiple molecular markers analysis (MMMA), and sequence analysis of the CP gene. SSCP analysis of CP gene yielded two distinct simple patterns and CP sequence analysis showed that both SSCP profiles belonged to viral isolates genetically related to the severe CTV-VT strain. This finding was also confirmed by MMMA. In fact, all infected samples reacted only with VT-specific markers (primers VTPOL, VT5, VTK17). None of the other known CTV genotypes were detected in the survey. In addition, molecular hybridization of PCR amplicons from the CP gene of the virus was done with a set of strain-specific probes. The results revealed that the CP gene sequences obtained from the five selected clones of a Syrian CTV isolate deposited in Genbank under accession No. EU626555 showed more than 99 and 98% nucleotide sequence identity to a Jordanian CTV isolate (GenBank accession No. AY550252) and the VT isolate (GenBank accession No. U56902), respectively (Abou Kubaa *et al*., 2008, 2009a, 2009b, 2012). During the same survey, the occurrence of CTV aphid vectors in the main citrus growing areas at the coastal region of Syria (Lattakia and Tartous) was also investigated in 18 different commercial orchards. Four aphid species were identified: the green citrus aphid (*Aphis spiraecola* (Patch), Genus: Aphis, Family: Aphididae) which was the most prevailing species, representing 50% of the total aphid population, followed by the cotton aphid (*A. gossypii* Glover, Genus: Aphis, Family: Aphididae) with 27.3%, the brown citrus aphid (*Toxoptera aurantii*, Boyer de Fonscolombe, Genus: Toxoptera, Family: Aphididae) with 20.3%, whereas the black bean aphid (*A. fabae* (Scopoli), Genus: Aphis, Family: Aphididae) represented only 2.3% of the aphid population collected in this survey (Abou Kubaa *et al*., 2009c). A survey for the presence of HSVd in Syrian citrus species was also conducted in Lattakia and Tartous, where, a total of 120 samples from citrus trees were collected from four commercial orchards. Total RNA was extracted from leaves and used in RT-PCR using two primer pairs: HSVd-R and HSVd-F of the HSVd reference sequence (GenBank, accession No. NC-001351). The results of RT-PCR showed that 39 samples were HSVd-positives. There were 35 infected samples of Jaffa and Valencia sweet orange varieties in one orchard in Tartous. The remaining four infected trees of the variety Valencia were detected in another orchard in Lattakia. That was the first report of HSVd on citrus species in Syria (Abou Kubaa *et al*., 2011). Moreover, in 2010, 336 samples were collected during a survey for investigating the presence of CTV, Satsuma dwarf virus (SDV, Genus: *Nepovirus*, Family: *Comoviridae*), and Citrus tatter leaf virus (CTLV, Genus: *Capillovirus*, Family: *Flexiviridae*) on citrus species in different orchards in Syria. Samples were tested by DTBIA and results showed that CTV and CTLV were the most common viruses in Banyas region with virus incidence of 35.37% and 20.73% respectively. SDV was the most common virus in Lattakia (20.06%), Whereas CTV was the most prevailing virus in the surveyed area with 25.89% infection rate. That was the first record of SDV and CTLV on citrus species in Syria (Hamdan & Ismail, 2011; Hamdan *et al*., 2013). Furthermore, Citrus psorosis virus (CPsV, Genus: *Ophiovirus*, Family: *Aspiviridae*) was reported for the first time in Syria by Raied Abou Kubaa and his colleagues. During 2011, a total of 250 symptomatic and asymptomatic citrus tree samples (100 from a mother block located in Lattakia and 150 from six commercial orchards in Jableh, Tartous and Lattakia) were collected to investigate for the presence of CPsV. All collected samples were tested by DAS-ELISA and RT-PCR. The results showed that CPsV was found in two Navel orange trees in Lattakia. The results were confirmed by RT-PCR using primer pairs: consF/consR which target a part of CP gene of the virus. The RT-PCR product was cloned and sequenced, the sequence of the Syrian CPsV isolate (SYR-C7) was deposited in GenBank under the accession number HG964696. The phylogenetic tree analysis showed 97% nucleotide identity with two CPsV isolates from Italy (AM235964 and AY194917) (Abou Kubaa *et al*., 2014). The Effect of CTV on growth of sweet orange (Baladi variety) and satsuma trees grafted on sour orange rootstock in Hraisoon and Al-Thawraa areas (Syrian coastal region) during 2012 and 2013 was investigated. Results obtained showed that CTV infection caused leaf deformation which appeared as boat or spoon-shaped leaves. The symptoms were more visible on satsuma trees compared with Baladi sweet orange trees. In addition, leaf size and length of both varieties were reduced in infected trees. CTV infection reduced fruits quality generally in both citrus varieties, but had no significant effect on trunk circumference in both varieties (Hamdan *et al*., 2014a, 2014b, 2015). In summer 2013, another survey on citrus species was conducted to investigate the presence of viroids other than HSVd reported previously in Syria. Leaf samples were collected from 65 stunted trees (4 commercial orchards in Lattakia and Tartous). Each sample was tested for the presence of two viroids infecting citrus: Citrus exocortis viroid (CEVd, Genus: *Pospiviroid*, Family: *Pospiviroidae*) and Citrus dwarf viroid (CVd-III, Genus: *Apscaviroid*, Family: *Pospiviroidae*) by RT-PCR. Results obtained revealed that 24 out of 65 samples tested were infected, including 10 and eight that reacted positively for the presence of CEVd and CVd-III, respectively, and six samples showed mixed infection with both viroids. Furthermore, biological indexing showed mild epinasty, leaf curling and typical stunting symptoms, confirming the presence of both viroids. That was the first molecular identification of CEVd and CVd-III on citrus trees in Syria (Abou Kubaa *et al*., 2016). Lately, during 2016 and 2017, a study was conducted to assess the prevalence of different variants of CTV along the Syrian coast by biological indexing. Samples from different citrus

species were collected and tested for the presence of CTV by DTBIA test. Three CTV isolates were chosen and grafted on different citrus indicator plants, e.g. Mexican lime and sour orange. Different symptoms were produced by the three isolates on the same citrus indicator plants. Symptoms ranged between mosaic, chlorosis, leaf malformation, and leaf curling. Moreover, no symptoms were observed on stems of the indicator plants such as stem pitting and quick decline, up to one year after grafting. Results obtained suggested the possibility of having two CTV strains in Syria: yellowing and mild strains (Akel *et al*., 2019).

Fig tree viruses and viroids

Until recently, no surveys were carried out in Syria to investigate occurrence of fig virus and viroid diseases. A survey was carried out during spring and summer of 2010 in fig orchards and gardens in 10 Syrian governorates: Damascus countryside, Al-Qunaitara, Daraa, Al-Sweida, Homs, Hama, Idleb, Aleppo, Tartous and Lattakia, and in one varietal collection in Idleb. A total of 90 fig tree samples were collected and tested by RT-PCR for the presence of seven viruses: Fig leaf mottle-associated virus 1 (FLMaV-1, Genus: *Closterovirus*, Family: *Closteroviridae*), Fig leaf mottle-associated virus 2 (FLMaV-2, Genus: *Closterovirus*, Family: *Closteroviridae*), Fig mild mottle associated virus (FMMaV, Genus: *Closterovirus*, Family: *Closteroviridae*), Fig mosaic virus (FMV, Genus: *Emaravirus*, Family: *Fimoviridae*), Fig latent virus 1 (FLV-1, Genus: *Trichovirus*, Family: *Betaflexiviridae*), Fig cryptic virus (FCV, Genus: *Deltapartitivirus*, Family: *Partitiviridae*), Fig fleck-associated virus (FFkaV, Genus: *Maculavirus*, Family: *Tymoviridae*), and one viroid: HSVd using sets of specific primers for each virus and viroid. Results obtained showed that around 84% of the trees were infected with at least one virus. FMV was the most prevailing virus with 56.7%, followed by FFkaV (36.7%), FLMaV-2 (31.1%), FMMaV (12.2%), FLV-1 (11.1%) and FLMaV-2 (4.4%), whereas FCV was not detected. HSVd was detected in 13.3% of the tested samples. The phylogenetic tree of HSVd isolates was constructed and showed that the nucleotide sequences of most Syrian HSVd-Fig isolates grouped with those reported in Lebanon from the same host and from mulberry, forming a distinct clade (M-group). That was the first report of FMMaV, FMV, FLV-1 and HSVd on fig trees in Syria (Elbeaino *et al.*, 2012).

Walnut and pecan viruses

In Syria, no surveys have been implemented to identify virus diseases that affect walnut and pecan trees, except for one survey carried out during May and October of 2008 by Salah Al Chaabi and Faiz Ismaeil and covered seven Syrian governorates: Damascus-countryside, Al Qunaitara, Homs, Hama, Idleb, Aleppo and Lattakia. Different symptoms were observed on many local walnut varieties, and ranged between dieback of some shoots and branches to chlorotic and pale yellow discolorations of leaves. In Hama, some of the trees grafted on *Juglans nigra* L. rootstocks showed a black line at the graft union region. No clear cut symptoms were observed on pecan trees except for some shedding of leaves. A total of 339 samples consisted of leaves, shoots and flower clusters from five local walnuts (297 samples) varieties: Balahseen, Kalesh, Bukaei, Baladi and Kastal Gandar, and from four imported pecan (24 samples) varieties: Riverside, GraTex, GraZona and Choctaw and from *J. nigra* rootstocks (18 samples), were collected and tested for the presence of CLRV by DAS-ELISA. Results obtained showed that CLRV was detected in 22 walnut samples with 7.4% infection rate in the two varieties Balahseen and Baladi, and in four samples of the four pecan varieties with 16.7% infection rate. CLRV positive infections were found mainly in the north western and coastal region (13 infected samples in Idleb and three in Lattakia) and in the central region (six infected samples in Hama and four in Homs). No infected trees were found in Damascus countryside, Al-Qunaitara in the southern region, and Aleppo in northern region. That was the first record of CLRV on walnut and pecan trees in Syria (Al-Chaabi & Ismaeil, 2009, 2011).

So far, no virus or viroid and phytoplasma diseases were reported on the other fruit tree species (pistachio, pomegranate, date palm, mulberry and loquat) grown in Syria. As a conclusion of the above-mentioned reports, 35 different viruses, four viroids and one phytoplasma were recorded on olive, stone fruits, grapevine, pome fruits, citrus, fig, walnut and pecan in Syria (Table 1). This is to confirm that the health status of the most fruit tree species which were investigated in Syria was unsatisfactory and most of these species and maybe other non-investigated species could be infected with non-tested pathogens. The production and distribution of healthy certified propagating materials of fruit tree species are the most important and urgent approach need to be adopted in order to reduce the economic loss caused by these disease agents on fruit tree species grown in Syria. Furthermore, healthy plant materials selection from fruit tree species combined with the genetic selection are considered as the most effective method to obtain true to type and healthy certified varieties of fruit tree species, especially from the local varieties (healthy and true to type). Finally, it is strongly recommended to establish a national program that aims to select and produce healthy propagating materials of fruit tree species free from viruses, viroids and phytoplasmas and other biotic agents through a country-wide formal program to distribute certified healthy propagation materials to growers. At the same time, control of the nurseries and applying very strict agricultural quarantine procedures at the borders (points of entry) must be strictly followed to prevent the random entry of any uncertified plant propagating materials, especially from the neighbouring countries to Syria.

Table 1. Viruses, viroids and phytoplasmas reported to infect fruit tree species in Syria

Fruit trees	Viruses	Viroids and phytoplasmas
Olive	Arabis mosaic virus (ArMV), Cherry leaf roll virus (CLRV), Cucumber mosaic virus (CMV), Olive latent ringspot virus (OLRSV), Olive latent virus 1 (OLV-1), Olive latent virus 2 (OLV-2), Olive leaf yellowing-associated virus (OLYaV) and Strawberry latent ringspot virus (SLRSV)	
Stone fruits	Plum Pox Virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Apple mosaic virus (ApMV), and Apple chlorotic leafspot virus (ACLSV)	Peach latent mosaic viroid (PLMVd) and Hop stunt viroid (HSVd)
Grapevine	Grapevine leafroll associated virus 1 (GLRaV 1), Grapevine leafroll associated virus 2 (GLRaV 2) Grapevine leafroll associated virus 3 (GLRaV 3), Grapevine leafroll associated virus 6 (GLRaV-6), Grapevine leafroll associated virus 7 (GLRaV-7), Grapevine fleck virus (GFkV), Grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV), Grapevine virus A (GVA), Grapevine B virus (GVB), Grapevine rupestris stem pitting associated virus (GRSPaV), Tobacco ringspot virus (TRSV) and two strains of Tomato ringspot virus (ToRSV-ch and ToRSV-PYBM)	
Pome fruits	Arabis mosaic virus (ArMV), Apple chlorotic leafspot virus (ACLSV), Tomato ringspot virus (ToRSV), Tobacco ringspot virus (TRSV), Apple mosaic virus (ApMV), Tomato black ring virus (ToBRV), Apple stem grooving virus (ASGV) and Apple stem pitting virus (ASPV)	Apple proliferation phytoplasma (Candidatus phytoplasma mali)
Citrus	Citrus tristeza virus (CTV), Satsuma dwarf virus (SDV), Citrus tatter leaf virus (CTLV) and Citrus psorosis virus (CPsV)	Hop stunt viroid (HSVd), Citrus exocortis viroid (CEVd) and Citrus dwarf viroid (CVd-III)
Fig	Fig leaf mottle-associated virus 1 (FLMaV-1), Fig leaf mottle-associated virus 2 (FLMaV-2), Fig mild mottle associated virus (FMMaV), Fig mosaic virus (FMV), Fig latent virus 1 (FLV-1) and Fig fleck-associated virus (FFkaV)	Hop stunt viroid (HSVd),
Walnut and pecan	Cherry leaf roll virus (CLRV)	

الملخص

إسماعيل، فايز وجمال مندو. 2022. الفيروسات والفيرويدات والفايتوبلازما التي تصيب الأشجار المثمرة المسجلة في سورية: مراجعة علمية. مجلة وقاية النبات العربية، 40(1): 78−88. https://doi.org/10.22268/AJPP-040.1.078088

تُعدّ زراعة الأشجار المثمرة في سورية، كما في معظم بلدان حوض البحر المتوسط، واحدةً من بين أهم القطاعات الزراعية السورية، حيث يُزرع فيها منذ القدم أنواعٌ عديدة من الأشجار المثمرة ، وقد تلاءمت معظمها مع الظروف البيئية السائدة محلياً والمشابهة لمناخ منطقة البحر المتوسط. إنّ سورية غنية بزراعة العديد من الأصناف المحلية من مختلف أنواع الأشجار المثمرة والتي تُمثل مصادر وراثية مهمة جداً لا يستهان بها على مستوى العالم. تُصاب الأشجار المثمرة، كمعظم الأنواع النباتية، بالعديد من عوامل الإجهاد المرضية الحيوية واللاحيوية؛ وتُعدّ الفيروسات والفيرويدات والفايتوبلازما من بين أهم العوامل المرضية الحيوية، والتي بإمكانها أن تُسبب العديد من الأمراض الخطيرة على مختلف أنواع الأشجار المثمرة. لقد بدأ منذ أوائل ثمانينيات القرن الماضي تقصي انتشار العديد من الأمراض الفيروسية

والفيرويدية والفايتوبلازمية، وتمّ تسجيلها في أنواعٍ عديدة من الأشجار المثمرة المزروعة في سورية، مثل: الزيتون، اللوزيات، العنب، التفاحيات، الحمضيات/الموالح، التين، الجوز والبيكان؛ حيث أُجريت سابقاً عدّة مسوحات حقلية بهدف تقييم الحالة الصحية لأنواع الأشجار المثمرة تلك، مع التركيز على حدوث هذه المسببات المرضية في سورية. وتمّ تقييم الحالة الصحية لتلك الأشجار المثمرة، والكشف عن تلك المسببات المرضية باستخدام طرائق الكشف المناسبة والمتاحة مثل التقانات الحيوية والمصلية/السيرولوجية والجزيئية.

كلمات مفتاحية: أمراض، أشجار مثمرة، فيروس، فيرويد، فيتوبلازما، سورية.

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