

Prevalence and Distribution of Viruses Associated with Fig Mosaic Disease in Iraq

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ABSTRACT

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In the frame to study the prevalence and the distribution of viruses associated with fig mosaic disease in Iraq, surveys were carried out in the main fig producing regions Al-Hashemiya, Al-Diwaniyah, Al-Samawah and Al-Suwayrah. A total number of one hundred leaf samples were randomly collected from major cultivars Aswed Dyala, Waziri and Sultani. All collected samples were analyzed using molecular tests (RT-PCR) for detection of Fig mosaic virus (FMV), Fig leaf mottle associated virus 1 (FLMaV-1), Fig leaf mottle associated virus 2 (FLMaV-2), Fig mild mottle associated virus (FMMaV), Fig cryptic virus (FCV), Fig fleck associated virus (FFkaV) and Fig latent virus 1 (FLV-1). A wide range of foliar symptoms including mosaic, chlorotic mottling, vein banding, chlorotic ringspots and deformations, were observed on fig trees. Molecular analysis detected the presence of at least one virus in 81% of fig trees tested. FCV was the prevailing virus with an incidence of 45% followed by FLMaV-1 (39%), FMV (37%), FMMaV (28%), FFkaV (16%) and FLMaV-2 (10%). Regarding cultivars, the highest infection rate was recorded for cv. Waziri (100%), followed by cv. Sultani (82.2%) and finally cv. Aswed Diyala (74%). This study represents the first report of the presence of FLMaV-1, FLMaV-2, FMMaV and FFkaV in Iraq.

Keywords: Fig mosaic disease, Iraq, prevalence, RT-PCR, viruses

The common fig (*Ficus carica*) belonging to Moraceae family, is one of the oldest and most important cultivated fruit crops in many countries of the world

with temperate climates. Fig fruits are an excellent source of minerals, vitamins and dietary fiber, consumed fresh or dried and are appreciated for their medical traits (Veberic et al. 2008). Originating in Asia Minor, fig has spread throughout the Near- and Middle-East, the Mediterranean and around the world, mainly in subtropical areas (Falistocco 2020), and the worldwide cultivation of fig has achieved great economic importance.

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Fig is considered a sustainable crop; nevertheless, it is vulnerable to the attack by several diseases, pests and disorders. Figs are commercially propagated by grafting or self-rooted cuttings; these methods favor the dissemination of various pests and diseases including viruses and viroids (Preising et al. 2021). In general, viral diseases are a major restrictive production factor causing substantial yield losses in crops. Mosaic is considered the main infectious disease of fig, and associated symptoms are extremely variable and can be transmitted via vegetative propagation of infected plant material (Martelli et al. 1993). Although symptoms have been observed in fig trees for almost a century (Condit and Horne, 1933), the etiological agents associated with fig mosaic disease (FMD) have been investigated only within the past years (Elbeaino et al. 2006; 2007). In recent years, the number of identified fig viruses has increased significantly. The causal agent of FMD has been identified as Fig mosaic virus (FMV) (Elbeaino et al. 2009a), which has an extensive dispersal rate and is transmitted by grafting and vectored by an eriophyid mite, *Aceria ficus* (Caglayan et al. 2012). In addition to FMV, numerous viruses have been reported in fig trees including eight closteroviruses (family Closteroviridae): Fig leaf mottle-associated virus 1, 2 and 3 (FLMaV-1, FLMaV-2, and FLMaV-3), Fig mild mottle-associated virus (FMMaV), Arkansas fig closterovirus 1 and 2 (AFCV-1, AFCV-2) and Fig viruses A and B (FiVA, FiVB); Fig badnavirus 1 (FBV-1) (family Caulimoviridae, genus *Badnavirus*), Fig fleck-associated virus (FFkaV) (family Tymoviridae, genus *Maculavirus*); Fig cryptic virus (FCV, family Partitiviridae, genus

Alphacryptovirus); Fig latent virus 1 (FLV-1; family Betaflexiviridae, genus *Trichovirus*); and Strawberry latent ringspot virus (SLRSV; family Comoviridae, genus *Nepovirus*) (Elbeaino et al. 2006, 2007, 2009b, 2011a, 2012b, 2015; Laney et al. 2012; Park et al. 2021; Tzanetakis et al. 2010) and four viroids : Hop stunt viroid (HSVd), Citrus exocortis viroid (CEVd), Apple dimple fruit viroid (ADFVd) and Fig hammerhead viroid-like RNA (FHVd-LR) (Chiumenti et al. 2014; Olmedo-Velarde et al. 2020; Yakoubi et al. 2007). FMD is the most widespread viral disease of fig, which represents a threat and a continuous constraint for healthy fig production and germplasm exchange worldwide (Shahmirzaie et al. 2012) and is present wherever fig is grown; however, its presence was mostly investigated in European and Mediterranean regions such as Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Hungary, Italy, Egypt, Iran, Lebanon, Palestine, Syria, Tunisia, and Turkey. It was also reported in the USA, Mexico, South Africa, Australia, Japan and China (Ale-Agha and Rakhshandehroo, 2014; Alhudaib 2012; Caglar et al. 2011; Castellano et al. 2007; De Stradis et al. 2007, 2008; Elairet al. 2015; Elbeaino et al. 2009c; Elbeshehy and Elbeaino, 2011; Fernandez et al. 2024; Ishikawa et al. 2012; Jamous et al. 2020; Martelli et al. 1993; Mijit et al. 2017; Perovic' et al. 2016; Shahmirzaie et al. 2012; Tzanetakis et al. 2010).

Viruses associated with FMD are transmitted by vegetative propagation of infected plant material, some of them are transmitted by vectors. In fact, the Emaravirus FMV is vectored by the eriophyid mite *Aceria ficus* and the

transmission rate reaches 70% (Caglayan et al. 2012) and the closterovirus FLMaV-1 is transmitted by the wax scale insect *Ceroplastes rusci* (Yorganci and Açikgöz, 2019).

The lack of a complete association between FMV and FMD, the detection of many new viruses in fig mosaic trees, and the extreme variability of symptoms indicate that the disease is more complicated than previously thought and symptoms can be caused not only by FMV, but also by mixed virus infections (Laney et al. 2012). However, symptoms similar to mosaic disease have been observed in FMV-free fig plants infected with a mixture of fig viruses (Elbeaino et al. 2006, 2007, 2010, 2011a, 2011b).

Symptoms of presumable viral nature and mosaic disease had been observed in Iraqi fig orchards, few data exist on the presence of fig-infecting viruses in the country. In fact, only Fig mosaic virus (FMV) and Fig badnavirus1 have shown to be present in Iraq (Mohammed et al. 2019; Zagier et al. 2021). Accordingly, the main objective of the present study was to investigate the

prevalence and the geographical distribution of seven known viruses infecting figs in Iraqi fig orchards.

MATERIALS AND METHODS

Field surveys and sample collection.

Field surveys were conducted in commercial fig orchards in different fig-growing regions Al-Hashemiya (Babil province), Al-Diwaniyah (Al-Qadisiyah province), Al-Samawah (Al-Muthanna province), and Al-Suwayrah (Wasit province) (Fig. 1). A wide range of foliar symptoms including deformations, mosaic, chlorotic mottling, vein banding and clearing, and chlorotic ringspots, were observed on fig trees. One hundred leaf samples were randomly collected from the most important fig cultivars Aswed Dyala (50 samples), Waziri (15 samples) and Sultani (35 samples). At least four leaves of different ages were collected from the quadrant of the tree canopy and combined as one sample.

The collected samples were subjected to laboratory tests (RT-PCR) for the detection of FMV, FLMaV-1, FLMaV-2, FMMAV, FCV, FFkaV and FLV-1.

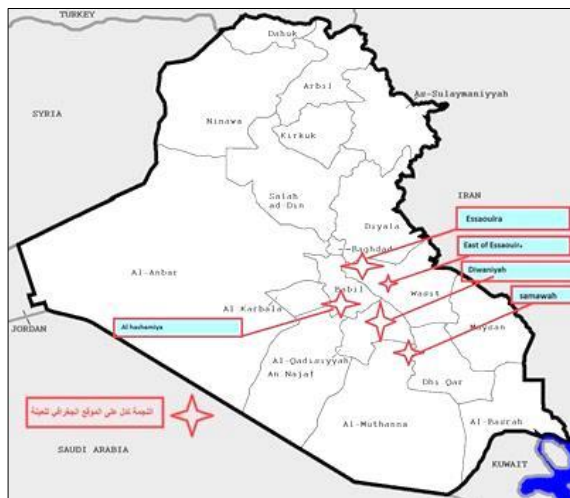


Fig. 1. Map of different surveyed regions.

Total nucleic acids extraction.

Total nucleic acids (TNA) were extracted according to Foissac et al. (2001). About 0.3 g of leaf vein from each sample were powdered in liquid nitrogen and ground in 1 ml of extraction buffer (4 M guanidine thiocyanate, 0.2 M sodium acetate ($C_2H_3NaO_2$) pH 5.2, 25 mM EDTA, 1.0 M potassium acetate ($C_2H_3KO_2$) pH 5.0 and 2.5% w/v PVP-40), and then mixed with 2% sodium metabisulfite as antioxidant. The mixture was transferred into an Eppendorf tube containing 100 μ l N-Lauroylsarcosine sodium salt (10%) and incubated at 70°C for 10 min, then placed on ice for 5 min. After centrifugation at 13,000 rpm for 10 min, 300 μ l of supernatant was transferred to an Eppendorf tube to which were added 150 μ l absolute ethanol, 300 μ l of 6 M sodium iodide (NaI) and 50 μ l of SiO_2 solution (12%, pH 2.0). The mixture was stirred for 30 min at room temperature and then centrifuged at 6,000 rpm for 1 min. The pellet was recovered and washed with 500 μ l of washing buffer [50% STE 1 \times (10 mM Tris-HCl, pH 8.0, 1 mM EDTA and 100 mM NaCl), 50% absolute ethanol]. It was then re-suspended in 120 μ l of sterile distilled water, incubated for 3 min at 70°C and then centrifuged at 13,000 rpm for 3 min. The supernatant was transferred to a new Eppendorf tube and stored at -20°C.

Reverse transcription and amplification.

A two-step protocol was used for reverse transcription (RT) and amplification (PCR) of the target RNA.

Five hundred ng of TNA extracts were mixed with 1 μ l random primers (1 μ g μ l⁻¹) and 1.5 μ l of sterile distilled water, denatured at 95°C for 5 min and quickly chilled in ice. Reverse transcription was done for 1 h at 39°C in 1 μ l Moloney Murine Leukemia Virus M-MLV (200 U μ l⁻¹) (Invitrogen Corporation), 4 μ l

buffer 5 \times M-MLV buffer (50 mM Tris HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 2 μ l DTT (0.1 mM) and 0.5 μ l dNTPs (10 mM), adjusted to a final volume of 25 μ l with sterile distilled water. A final step for enzyme denaturation was conducted at 70°C for 10 min.

The amplification was performed using 2.5 μ l of the reverse transcription reaction, in a total volume of 25 μ l containing 2.5 μ l of 10 \times Taq polymerase buffer, 0.5 μ l of 50 mM $MgCl_2$, 0.5 μ l of 10 mM dNTPs, 0.5 μ l of 10 μ M primer (sense), 0.5 μ l of 10 μ M primer (anti-sense) (Table 1) and 0.25 μ l of Taq polymerase (5 unit/ μ l). Amplification was carried out in a thermocycler (Applied biosystem) after a preliminary denaturation at 94°C for 12 min, followed by 35 cycles at 94°C for 30 sec, annealing at 58°C (55°C for FLV-1) for 35 sec, and extension 72°C for 30 sec. Final elongation was carried out at 72°C for 7 min. The PCR products were analyzed by electrophoresis in 1.2% agarose gel in 1 \times TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3), and visualized under UV light after staining with ethidium bromide

RESULTS

Typical fig mosaic and other leaf alterations resembling to virus-like symptoms were observed in all visited orchards on young and old leaves. Trees showed a wide array of leaf discolorations, including mosaic, chlorotic ringspot, chlorosis, mottling, chlorotic blotching, chlorotic blistering, vein clearing, yellowing, and vein feathering recorded on leaves in compared to healthy ones. Leaf malformations were associated with discolorations and chlorotic mottling with a contrast ranged from yellow to green color. Yellow ringspot and mosaic appeared also on some immature fruits which were prematurely dropped (Fig. 2).

Table1. Primers used for RT-PCR detection of fig infecting viruses

Virus	Primer sequences	Amplified product bp	Target gene region	References
FMV	E5-s CGGTAGCAAATGGAATGAAA E5-a AACACTGTTTTGCGATTGG	302	RdRp	Elbeaino et al. 2009a
FLMaV-1	N17-s CGTGGCTGATGCAAAGTTTA N17-a GTTAACGCATGCTTCCATGA	350	HSP70	Elbeaino et al. 2006
FLMaV-2	F3-s GAACAGTGCCTATCAGTTTGATTTG F3-a TCCCACCTCCTGCGAAGCTAGAGAA	360	HSP70	Elbeaino et al. 2007
FMMaV	LM3-s AAGGGGAATCTACAAGGGTCCG LM3-a TATTACCGCTTGAGGTTGC	311	HSP70	Elbeaino et al. 2010
FCV	R1-s TCGATTGTCTTTGGAGAGG R1-a CGCATCCACAGTATCCCATT	353	RdRp	Elbeaino et al. 2011b
FFKaV	d8-s ATGACGACTGTCAACTCCCT d8-a TTAAGCCAGGGTGGGAGTGTG	270	RdRp	Elbeaino et al. 2011a
FLV-1	CPtr1-s CCATCTTCACCACACAAATGTC CPtr2-a CAATCTTCTTGGCTCCATAAG	389	RdRp	Gattoni et al. 2009

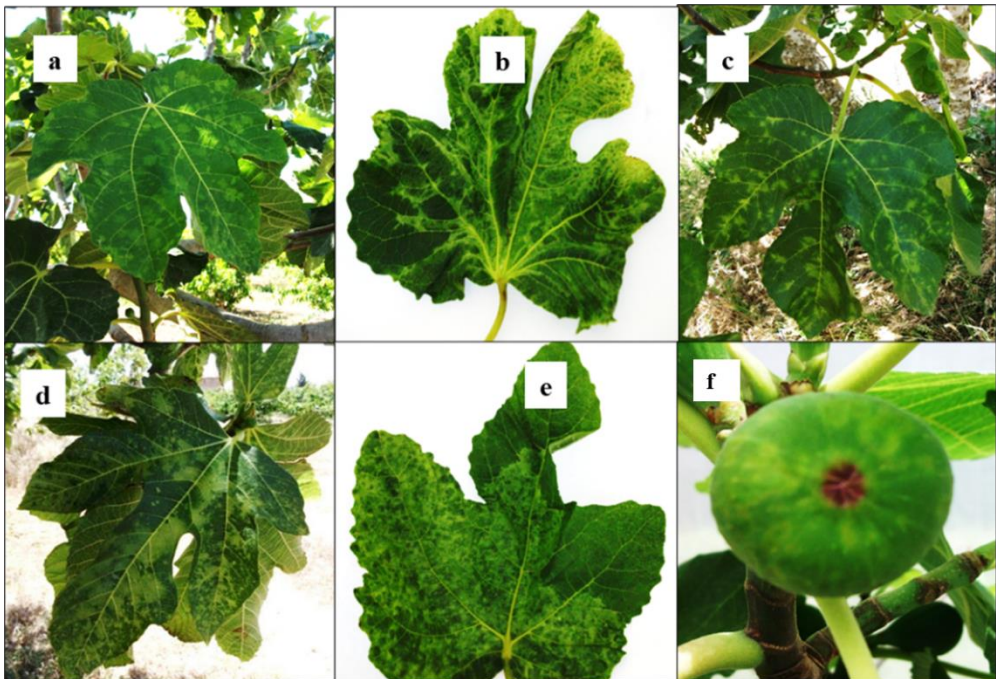


Fig. 2. Leaves from affected fig trees showing fig mosaic disease symptoms. (a) chlorotic yellowish and ringspot, (b) leaf discoloration and deformation, (c) vein clearing and mosaic (d) vein banding and chlorotic spot, (e) mottling and deformation, (f) chlorotic ringspot on fruits.

Molecular analysis demonstrated that at least one virus was present in 81% of fig trees tested. Among the infected samples, 25% were singly infected and the remaining 75% of samples were infected by at least two viruses. FCV was the prevailing virus with an incidence of 45% and was found in cvs. Sultani (34.3%), Aswed Diyala (54%) and Waziri (40%) (Table 1). Samples collected from Al-Diwaniyah region were the most infected by FCV (60%), followed by those collected from Al-Hashemiya (48%), Al-Samawah (40%) and less infected were samples from Al-Suwayrah (34.3%). FLMaV-1 was the second prevalent virus (39%), which was found in cvs. Sultani (45.7%), Aswed Diyala (38%) and Waziri

(26.6%). Samples from Al-Hashemiya were the most infected by this last virus (60%), followed by those from Al-Suwayrah (45.7%), Al-Samawah (26%) and less infected was Al-Diwaniyah region (16%). FMV was the third virus in importance (37%), samples from Al-Hashemite region were the most affected (72%), followed by the those from Al-Samawah area (46.6%), then the samples from Al-Diwaniyah region (24%), and the lowest FMV infection rate was reported in the samples from the city Al-Suwayrah (17.1%). Concerning cultivar infections, cv. Aswed Diyala was the most infected by FMV (48%), followed by cv. Waziri (46.6%), while the lowest infection rate was recorded for cv. Sultani (17.1%) (Table 2).

Table 2. Incidence of fig infecting viruses in fig cultivars and in different fig growing regions in Iraq

Infection traits	Tested samples	Total infection (%)	FMV	FLMaV-1	Infection FLMaV-2	(%) FCV	FFkaV	FMMaV	FLV-1
Cultivars									
Sultani	35	82.8	17.1	45.7	5.7	34.3	20	20	0
Aswed Diyala	50	74	48	38	6	54	8	30	0
Waziri	15	100	46	26	33	40	33	40	0
Total	100	81	37	39	10	45	16	28	0
Regions									
Suwayrah	35	82.8	17.1	45.7	5.7	34.3	20	20	0
Hashemiya	25	80	72	60	4	48	4	40	0
Diwaniyah	25	68	24	16	8	60	14	20	0
Samawah	15	100	46	26	33	40	33	40	0

FMMaV was detected in 28% of tested samples and cv. Waziri was the most infected cultivar (40%). Results demonstrated that the less represented viruses were FFkaV (16%) and FLMaV-2 (10%). Finally, FLV-1 was absent in all tested cultivar and collected samples.

According to cultivars, all tested samples of cv. Waziri were infected and among them 73.3% were multi-infected. Regarding cv. Sultani, 82.2% of the tested samples were infected and 57.1% of them were mixed infection. The lowest infection

rate was recorded with cv. Aswed Diyala (74%) with the highest mixed infection rate (88%).

DISCUSSION

In this study, symptoms of FMD were observed on fig leaves in all surveyed cultivars and regions in Iraq. A range of symptoms was observed on the same old or young leaf and on the same tree, showing the complexity of the disease etiology.

The survey showed that six viruses out of seven studied in this work were present in the main Iraqi fig-growing areas, with levels of infections that were substantially higher than those reported from other countries, with few exceptions as described below.

The high incidence rate of FMV in Iraq (37%) is not surprising since this virus is the most widespread virus infecting fig plants in the world (Preising et al. 2021). The infection rate is in line with those reported from other countries such as Tunisia (34.4%) (Elair et al. 2015), Montenegro (43%) (Perović et al. 2016), China (44.4%) (Mitij et al. 2017) and Syria (56.6%) (Elbeaino et al. 2012b). FMV is closely associated with mosaic symptoms and the high incidence is probably due to its transmission by the eriophyid mite *Aceria ficus*, as the transmission rate reaches 70% (Caglayan et al. 2012).

The closterovirus FLMaV-1 was detected in 39% of Iraqi tested trees. The infection rate is considered high compared to the Global FLMaV-1 infection (22%) (Preising et al. 2021). The incidence of FLMaV-1 was very high in the Mediterranean and the Balkan regions, i.e. Italy (64.9%) (Elbeaino et al. 2006), Bosnia and Herzegovina (51%) and Montenegro (57%) (Delić et al. 2017), which are the traditional countries of fig tree cultivation. FLMaV-1 is vectored by the wax scale insect *Ceroplastes rusci* (Yorganci and Açikgoz 2019), the large presence of this putative vector in Iraqi fig orchards can explain the high incidence of this virus.

Surprisingly, the infection rate of FCV reached 45%, compared to the global virus infection (9%) (Preising et al. 2021) and the average infection rate in the Mediterranean region (18.5%) (Elbeaino et al. 2011b). The presence of this virus was low in other countries such as Tunisia (9.9%) (Elair et al. 2015) and Iran (4.5%)

(Ale-Agha and Rakhshandehoo 2014). FCV was found in mosaic diseased figs and is not mechanically transmitted and no vector was identified (Elbeaino et al. 2011b). However, plant material should be tested for FCV before planting to avoid the introduction of this virus in newly planted area.

The incidence of FFkaV (16%) was comparable to the global FFkaV infection (19%) (Preising et al. 2021) and to those from Tunisia (10.3%) (Elair et al. 2015) and Turkey (9.2%) (Elçi et al. 2017). In contrast, a relatively high infection rates were found in China (44%) (Mijit et al. 2017), Syria (36.7%) (Elbeaino et al. 2012b) and Palestine (33%) (Jamous et al. 2020). This virus is transmitted only by vegetative propagation material and has been found in mosaic affected figs in many countries.

The infection rate of the closterovirus FLMaV-2 (5.7%) is lower than the global virus infection (9%) (Preising et al. 2021). The incidence of this virus in Iraq is in line with those from Tunisia and Turkey (Caglar et al. 2011; Elair et al. 2015), but lower than those recorded in Middle-East countries, Lebanon (24.9%) (Elbeaino et al. 2007), Syria (31.1%) (Elbeaino et al. 2012b) and Palestine (61.5%) (Jamous et al. 2020).

The incidence of FMMAV (28%) was the highest in the world till now. This virus was detected in many countries and the incidence was relatively low, i.e. Syria (12.2%) (Elbeaino et al. 2012b), Iran (11%) (Alishiri et al. 2018), Tunisia (10.7%) (Elair et al. 2015), Montenegro (10%) (Perović et al. 2016) and China (0.4%) (Mijit et al. 2017). The high incidence of viruses associated to this disease in Iraqi fig orchards, can be explained by the use of infected plant propagation material and the presence of vectors especially for FMV transmitted by

the eriophyd *Aceria ficus* and FLMaV-1 transmitted by *Ceroplastes rusci*.

A study on the prevalence of viruses associated with fig mosaic disease in other Iraqi regions is underway and preliminary results show the presence of these viruses with rates similar to those found in the present study.

FMD constitute a limiting factor for fig production, and our finding

highlight the urgent need of implementation of sanitation program in Iraq for the production of high-quality planting material, which is the efficient way to reduce the risk of infection spreading and enhance high productivity and longevity (Bayouhd et al. 2017; Chiumenti et al. 2013).

RESUME

Al-Kaeath N., Elair M. et Mahfoudhi N. 2024. Prévalence et distribution des virus associés à la maladie de la mosaïque du figuier en Irak. Tunisian Journal of Plant Protection 19 (1): 1-11.

Dans le but d'étudier la prévalence et la distribution des virus associés à la maladie de la mosaïque du figuier en Irak, des prospections ont été réalisées dans les principales régions de production de figuier Al-Hashemiya, Al-Diwaniyah, Al-Samawah et Al-Suwayrah. Cent échantillons ont été collectés au hasard à partir des principales cultivars Aswed Dyala, Waziri et Sultani. Tous les échantillons collectés ont été analysés par des tests moléculaires (RT-PCR) pour la détection du Fig mosaic virus (FMV), Fig leaf mottle associated virus 1 (FLMaV-1), Fig leaf mottle associated virus 2 (FLMaV-2), Fig mild mottle associated virus (FMMaV), Fig cryptic virus (FCV), Fig fleck associated virus (FFkaV) et Fig latent virus 1 (FLV-1). Une panoplie de symptômes foliaires, notamment, des mosaïques, des marbrures chlorotiques, des éclaircissements des nervures, des taches annulaires chlorotiques et des déformations ont été observés sur les feuilles des figuiers. Les analyses moléculaires ont montré que 81% des figuiers testés sont infectés par au moins un virus. Le FCV s'est révélé le virus prédominant avec une incidence de 45%, suivi par FLMaV-1 (39 %), FMV (37 %), FMMaV (28 %), FFkaV (16 %) et FLMaV-2 (10 %). Concernant les cultivars étudiés, le taux d'infection le plus élevé a été enregistré avec cv. Waziri (100%), suivi par cv. Sultani (82,2 %) et finalement cv. Aswed Diyala (74 %). Cette étude représente le premier signalement de la présence du FLMaV-1, FLMaV-2, FMMaV et FFkaV en Irak.

Mots clés: Maladie de la mosaïque du figuier, prévalence, Irak, RT-PCR, virus

ملخص

الغياض، نبيل ومنال العير ونعيمة محفوظي. 2024. دراسة انتشار وتوزيع الفيروسات المرتبطة بمرض تبرقش التين في العراق. Tunisian Journal of Plant Protection 19 (1): 1-11.

لدراسة مدى انتشار وتوزيع الفيروسات المرتبطة بمرض تبرقش التين في العراق، أجريت مسوحات في المناطق الرئيسية لإنتاج التين، الهاشمية والديوانية والسماوة والصويرة. تم جمع مائة عينة بشكل عشوائي من الأصناف الرئيسية أسود ديالا ووزيري وسلطاني. تم تحليل جميع العينات التي تم جمعها بواسطة الاختبارات الجزيئية (RT-PCR) للكشف عن فيروس تبرقش التين (FMV) وفيروس 1 المصاحب لتبقع أوراق التين (FLMaV-1) وفيروس 2 المصاحب لتبقع أوراق التين (FLMaV-2) وفيروس البقع الخفيفة على التين (FMMaV) وفيروس التين المتخفي (FCV) وفيروس بقع التين (FFkaV) وفيروس التين الكامن (FLV-1). وقد لوحظت مجموعة من الأعراض الورقية، بما في ذلك التبرقش، والتبقع الاصفرار، وتفرق الأوردة، والبقع الحلقيّة الصفراء، والتشوهات على أوراق التين. وأظهرت التحاليل الجزيئية أن 81% من أشجار التين التي تم اختبارها كانت مصابة بفيروس واحد على الأقل. كان فيروس FCV هو الأكثر انتشاراً بنسبة 45%، يليه FLMaV-1 بنسبة 39% و FMV بنسبة 37% و FMMaV بنسبة 28% و FFkaV بنسبة 16% وأخيراً FLMaV-2 بنسبة 10%. أما بالنسبة إلى الأصناف، فقد سُجّلت أعلى نسبة إصابة على صنف وزيري (100%)، يليه

الصنف سلطاني (82.2%) وأخيراً أسود ديالي (74%). تمثل هذه الدراسة التقرير الأول عن وجود FLMaV-1 و FFLMaV-2 و FFMaV و FFkaV في العراق.

كلمات مفتاحية: العراق، انتشار، فيروسات، مرض تبرقش التين، RT-PCR

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