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Multiple Important Plant Viruses are Present on Vegetable Crops in Kuwait

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Abstract

Plant virus diseases are wide-spread in Kuwait and cause significant economic losses in many crops. Four plant families, including Cucurbitaceae (cucumber, squash, melon, zucchini), Solanaceae (tomato, potato, pepper, eggplant), Liliaceae (onion) and Leguminosaea (bean) were surveyed for viruses by ELISA from December 2007 through May 2008. The survey was conducted on six farms located in the two main production areas, Wafra and Abdally agricultural districts. Each farm was visited four times during the production season. Eighteen plant viruses were detected, including *Cucumber mosaic virus*, *Garlic common latent virus*, *Iris yellow spot virus*, *Onion yellow dwarf virus, Melon necrotic spot virus*, *Papaya ringspot virus*, *Pepino mosaic virus*, *Pepper mild mottle virus*, *Potato moptop virus*, *Potato virus M, Potato virus X*, *Potato virus Y, Squash mosaic virus*, *Tomato mosaic virus, Tomato spotted wilt virus, Tomato yellow leaf curl virus*, *Watermelon mosaic virus*, and *Zucchini yellow mosaic virus*. Virus incidence was close to 100% on some crops, including cucurbit and onions and double or triple infections were common. Several important viruses were not found, including *Potato virus A*, *Potato virus S*, *Potato leafroll virus*, and *Potato virus V*.

Keywords: Enzyme Linked Immunosorbant Assay; Cucurbitaceae; Solanaceae; Liliaceae; Leguminosaea

Introduction

Kuwait imports the majority of its food, but despite limited water and agricultural land, it aims to become partially self-sufficient in food production. Over the past few decades, Kuwaitis have made substantial investments in vegetable production in protected environments, such as net houses and high tunnels. The plant protection infrastructure to require supporting profitable and vegetable production in protected environments is still in its infancy in the Gulf States and as a result, significant losses have occurred due to pests and diseases. Surveys of the diseases present in vegetable production are required to begin to develop control strategies.

The rapid spread of viral diseases into new geographical regions, usually due to vector movement and the transport of infected planting materials, lead to severe yield losses. Most viruses are seed-transmission for some important viruses that are excluded from true seed during plant development. However, they are easily transported in planting material of vegetative crops, such as onion [1,2] or in vegetable transplants. A recent example of this is the large losses in solanaceous vegetables caused by the recent spread of Tomato Yellow Leaf Curl Virus (TYLCV) into the Middle East.

Farmers must rely on preventive measures and sanitation in order to prevent, reduce the incidence or delay viral infections. Once infections occur in the field, normally there is no remedy; the only possibility is to take measure to delay the spread.

Virus diseases of plants are best managed by an integrated approach that includes planting healthy seed, plant resistance, isolation, sanitation, elimination of plant reservoirs of viruses such as weeds or volunteer plants, cross protection, crop rotation, virus or vector avoidance by alternating planting or harvesting time, host-free periods, control of insect virus vectors through pesticides, sticky traps, netting, trap or border crops, or reflective mulches, and rouging. Control of virus spread is required since once infected, there is no way to cure greenhouse or field grown plants of viruses. Accurate diagnosis of the viruses present in a region is required for developing appropriate integrated management of these diseases.

Plant virus diseases are widespread in Kuwait and cause significant economic losses in the main greenhouse and field vegetable crops of Kuwait, including cucumber, pumpkin, squash, melons, onions, tomato, potato, eggplant and pepper. Identification of the major viruses present is required to make control recommendations, such as vector control and resistant varieties. Virus identification will also aid in determining which viruses are not yet widespread and that therefore may be excluded through quarantines from Kuwait agriculture. Fortunately, ELISA and PCR-based assays are available for nearly all important viruses that infect vegetable crops.

To date, only three plant viruses have been reported in Kuwait, including TYLCV, Cucumber Mosaic Virus (CMV), and Watermelon Mosaic Virus (WMV) [3]. The objective of this work was to identify plant viruses commonly found in major Kuwait vegetable crops as a first step toward control of these viruses and quarantine of viruses not yet present in Kuwait.

Materials and Methods

Sample collection

Fresh plant leaf samples were collected from green houses and open fields (symptomatic and non symptomatic). Four samples from each farm were collected on monthly bases throughout the growing season (first month of planting, before flowering, flowering time, fruiting time). Six farms in two main production areas, Wafra and Abdally agricultural districts, were surveyed four times per year from December 2007 through May 2008. Each farm was visited four times during the growing season. Tomato samples were collected from November 2007

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to February 2008. Pepper, potato, and onion samples were collected from December to March 2008. Bean samples were collected from August 2008 to October 2008. Eggplant samples were collected from February 2008 to May 2008. A total of 720 plant samples were collected, with three leaves collected per sample. All samples were stored at -80°C. Viral symptoms were documented with a digital camera.

Enzyme-linked immunosorbant assay (ELISA)

ELISA kits were purchased from BioReba [4] and Agdia [5] and assays were performed following the manufacturers' directions. Results were recorded after visual observation or spectrophotometrically by measuring absorbance at A_{405} nm.

Results

Viruses infecting cucurbitaceae plants

Severe virus symptoms were observed on cucurbits grown in agricultural areas, Wafra and abdally, with incidence reaching nearly 100% in most cases (Table 1). The high incidence of virus symptoms observed on cucurbit plants during the survey was confirmed by ELISA. Most samples had mixed infections by two or three viruses. Most cucumber samples had double infection with WMV and MNSV. The third most commonly found virus was ZYMV. The viruses CMV, PRSV and SqMV were each present in less than 10% of the samples. Most samples of melon, squash and zucchini had mixed infections with three viruses, ZYMV, MNSV and WMV, with incidences ranging between 93-100%. Zucchini was also infected by PRSV, with an incidence of 45%. CMV was not detected in melon and SqMV was not detected in squash or zucchini.

Viruses infecting Solanaceae plants

Tomato, potato, pepper and eggplant were surveyed for fourteen viruses, including CMV, PVY, PVX, PVA, PVS, PVM, PLRV, PVV, PMTV, PMMoV, ToMV, TSWV, and TYLCV by ELISA. In Kuwait, the high incidence and severity of symptoms observed on tomato were attributed to infection by four viruses, including PepMV (83%), ToMV (19%), TYLCV (13.3%), CMV (6.6%) and PVY (3.3%) (Table 2).

In potato the most important viruses were PVM (18%) and PVY (13%) (Table 3). PVX (3%) and PMTV (1%) were also found. CMV, PVA, PVS, PLRV, PVV, PMMoV, ToMV, TSWV, and TYLCV were not found on potato. These results differ from those reported in the Middle East and Europe, where PVY is the predominant virus. A high background was observed while using the ELISA kits for PVY and PVX, which led us to consider all samples with an A_{405} reading double the negative control as positive. Virus incidence on pepper was low, with only three viruses detected, including PVY (2%), PMMoV (1%) and ToMV (1%). And eggplants showed negative results.

Viruses infecting Liliaceae plants

A high incidence and severity of symptoms were observed in onion fields. Samples were tested with ELISA for three viruses, including IYSV, OYDV and GarCLV. IYSV was the most common virus, with an incidence of 100% in all fields tested (Table 4), and followed by GarCLV (2%). OYDV was not found.

Viruses infecting Leguminosae plants, (TSWV and AMV)

Concerning (Leguminosae) beans only was tested and most of the requested ELISA kits were not made available, therefore tests were

Crop	Viral Disease						No. of Infections per Sample			
	WMV	ZYMV	CMV	MNSV	PRSV	SqMV	Single	Double	Triple	> 3
Cucumber	88	20	8	90	8	5	1	63	16	10
Melon	90	90	0	90	7	3	0	0	81	9
Squash	86	88	11	89	3	0	0	6	71	13
Zucchini	84	90	6	90	41	0	0	4	45	41

A total number of 90 samples were tested.

Table 1: Survey of Viruses Affecting Cucurbit in Kuwait: Relative Frequencies of Samples Infection with CMV, MNSV, PRSV, SqMV, WMV, and/or ZYMV, as Determined by DAS-ELISA.

Crop	Viral Disease					No. of Infection per Sample			
	CMV	PVY	TYLCV	PepMV	ToMV	Single	Double	Triple	4
Tomato	6	3	12	75	17	56	24	2	0

A total of 90 samples were tested

Table 2: Survey of Viruses Affecting Tomatoes in Kuwait: Relative Frequencies of Infection with CMV, PVY, TYLCV, PepMV and/or ToMV, as determined by DAS-ELISA.

Crop	Viral Disease										
	TSWV	ToMV	PMTV	PVX	PVA	PVS	PVV	PLRV	PVY	PMMoV	PVM
Potato	0	0	1	3	0	0	0	0	12	0	15
Pepper	0	1	0	0	0	0	0	0	2	1	0
Eggplant									0	0	0

A total number of 90 samples were tested per crop

Table 3: Survey of Viruses Affecting Other Solanaceae (i.e., Potato, Pepper and Eggplant) in Kuwait: Relative Frequencies Infection with TSWV, ToMV, PMTV, PVX, PVA, PVS, PVV, PLRV, PVY, PMMoV and PVM, as Determined by DAS-ELISA.

Crop	Viral Disease			No. of Infections per Plant			
	IYSV	GarCLV	OYDV	Single	Double	Triple	> 3
Onion	90	2	0	88	2	0	

A total 90 samples were tested per crop

Table 4: Survey of Viruses Affecting Liliaceae (i.e., Onion) in Kuwait: Relative Frequencies of Infection with IYSV, GarCLV and OYDV, as Determined by DAS-ELISA.

conducted for only two viruses **(TSWV and AMV)**. AMV was detected in one sample.

Discussion

Viruses were found at a high incidence in several crops, indicating a great need for implementation of virus control measures. Several common viruses were not detected, suggesting that with proper quarantine and control methods, these viruses could be kept from causing losses in Kuwaiti agriculture.

ZYMV and WMV were the two most common cucurbit viruses found. They are transmitted both mechanically and non-persistently by aphids. Both viruses have been reported as major cucurbit viruses worldwide. Most of the cucurbit viruses found are not seed borne, including CMV, ZYMV, and WMV, suggesting that they have either long been in Kuwait or entered on transplants. The incidence of these viruses may be reduced through improved control of aphids in protected environments and mineral oil sprays, which inhibit aphid transmission of non-persistent viruses. However, the high incidence of the third virus MNSV is quite surprising and calls for further confirmation, since normally it does not infect squash and zucchini and it was never reported at such high incidence in the region. There is a high probability that the procedure and kit used may have lead to a high percentage of false positives.

Tomatoes sampled were infected by six viruses, including PepMV, ToMV, TYLCV, CMV, PVY and TSWV. The detection of PepMV at high incidence is among the first reports in the ME and Gulf area. The high incidence may be attributed to the fact that this virus is transmitted mechanically through handling the plants by workers. How the virus was introduced to Kuwait should be investigated, since many countries consider it as a quarantine pest. There are some reports that this virus may be transmitted by seeds with a small percentage of infested seeds [6]. The low initial rate of speed transmission coupled with frequent handlings of plants in the greenhouse allowed rapid transmission of this virus mechanically. Only certified and treated tomato seeds should be imported and sanitation practices should be strictly followed. Concerning TYLCV an integrated pest management approach relying on the selection of resistant varieties, special care in nurseries to prevent seedling infection, protection during seedling transportation, the use of insect-proof nets and double doors in greenhouses, or floating row covers in open fields, silver mulch, sticky yellow traps, insecticides, timing of planting, weed control and several other practices mentioned in the literature review are important factors to ensure a healthy crop. Big commercial tomato production companies are following a close monitoring of the vector and GIS system for timing of transplanting in specific geographical regions to ensure a successful tomato production in open fields [7].

TSWV is another dreadful virus that may lead to great economic losses due to its wide host range, due to the severe symptoms it induces, due to its transmission by thrips which are resistant to many insecticides and due to the presence of TSWV mutants that can overcome resistance genes. [8] developed an IPM program that was adopted by tomato farmers in Florida. The IPM program is based on the timing of planting, the use of silver mulch in combination with careful selection of the appropriate insecticide sprays to control thrips, coupled with sprays of chemical products that improve the host resistance (SAR inducing chemicals like acibenzolar-S- methyl).

Concerning TMV, most commercial tomato varieties carry resistance to this virus. However, attention should be paid to the emergence of new strains of TMV which may break the resistance or the emergence of necrotic strains of TMV or CMV which may cause significant yield losses.

The presence of other viruses is highly suspected, the occurrence of the violet leaf syndrome indicates the presence of a yet unidentifies virus, similarly the presence of ToMoV was suspected in previous studies at KISR [9]. Yellowing symptoms were also observed on lower leaves of tomato, suggesting the infection by *Tomato Infectious Chlorosis* virus (TICV) or *Tomato chlorosis virus* (ToCV) as was reported in other countries [10].

On potato, four viruses were reported PVM, PVY, PMTV and PVX. For potato, in addition to the preventive control measures, the most important control method is to start with certified potato seed with an appropriate phytosanitary certificate. Since most potato seed in Kuwait is imported, careful selection of supplier is a must and this should be coupled by local testing of representative potato seed samples using ELISA or NA based techniques.

Three viruses were detected in pepper, PVY, PMMoV and ToMV. The presence of other viruses is suspected, especially CMV, for which we went out of stock of the ELISA kit during the testing period. The same recommendation given for tomato viruses is appropriate.

In all four Cucurbitaceae crops surveyed, a very high incidence of mixed infections was observed. Virus incidence reached 100% in many fields. The three major viruses detected were ZYMV, WMV and MNSV; they were followed by PRSV, CMV and SqMV. These results at least concerning the high incidence of ZYMV are in agreement with surveys in Lebanon [11] and in Iran [12]. However the high incidence of MNSV is doubtful and may be caused by false positives.

Many cucurbit samples showed leaf yellowing or interveinal chlorosis such as those shown in plate figures, suggesting infections by viruses inducing yellowing symptoms like CABYV, CYSDV or BPYV [13-16]. Other samples showed leaf curl symptoms suggesting the infection by whitefly transmitted geminiviruses like *Squash Leaf Curl Virus* (SLCV), *Watermelon Chlorotic Stunt Virus* (WmCSV) and several others.

As discussed for tomato, an integrated pest management approach relying on the selection of varieties resistant to the major viruses, special care in nurseries to prevent seedling infection, protection during seedling transportation, the use of insect-proof nets and double doors in greenhouses, or floating row covers or silver mulch in open fields [11,17], sticky yellow traps, insecticides, timing of planting, weed control and several other practices mentioned in the literature review are important factors to ensure a healthy crop. The use of cross-protection (inoculation with a mild strain to protect against a severe strain) was practiced in cucurbits for ZYMV and others [18] or tomato mainly for TMV [19] before the introduction of resistant varieties with relatively good success, However, at present this may not represent a viable approach in most situations due to the presence of mixed infections by two or more viruses and the continuous introduction of new viruses.

In Onion (Liliaceae), the incidence of IYSV reached 100% and GarCLV about 2%. IYSV belongs to the *Tospovirus* genus and is transmitted by thrips, while garCLV belongs to the genus *Carlavirus* and is transmitted mechanically. Management measures in Kuwait should focus therefore on management of IYSV. Since bulbs are used for production of onion, the most important approach would be to start with using healthy bulbs, produced from healthy seeds in isolated areas. This should be coupled with weed control, isolated plantings and vector control.

Concerning bean (Leguminosae), most of the requested ELISA kits were not made available, therefore tests were conducted for only two viruses. AMV was detected in one sample

The most interesting findings in this project were the detection on vegetables crops grown in Kuwait of many viruses. Most of these viruses, except TYLCV, CMV and WMV, are reported for the first time in Kuwait. Therefore, confirmation of their identification should be done using PCR, followed by sequencing to characterize these pathogens at the molecular levels. By adopting this recommendation, convincing and irrefutable evidence will be provided for the scientific community on the proper identification of these viruses, so that publications in international journals will be accepted.

Overall, this survey proved that viral diseases could induce high yield losses of vegetable crops grown in Kuwait, reaching about 90% in some cases. Integrated crop management approaches should be followed in order to reduce their negative impact. It is highly recommended to evaluate several IPM measures under farmers' condition in Kuwait in order to select the most efficient ones. Surveys should be repeated periodically to identify new virus introductions in order to adapt the IPM programs in order to cope with, prevent or reduce damage caused by these newly introduced viruses. Furthermore, legislations should be enacted to prohibit the introduction of planting material that may harbor potentially dangerous pests.

General Recommendations

Based on the preliminary data obtained during this project which revealed the high incidence of vegetable virus diseases in Kuwait, the following recommendations are reiterated:

1. Surveys should be repeated periodically to identify new virus introductions

2. Surveys should not depend solely on serological techniques, many new devastating viruses cannot be detected by these methods; therefore ELISA should be complemented by nucleic acid based methods, mainly PCR.

3. Upon detection of any new virus in Kuwait, confirmation of their identification should be done using biological indexing and/or PCR followed by sequencing to characterize the pathogen at the molecular levels. For publication of a first report of a virus in a country, convincing evidence should be provided for the scientific community, ELISA results alone are usually not enough.

4. Since our ultimate aim is to help the farmer, the survey results should be made useful by studying the epidemiology of the detected viruses and other pests. It is strongly recommended to conduct field research to evaluate the efficacy of the integrated pest management measures locally. This should be followed by demonstration trials to convince farmers to adopt an integrated approach rather than relying only on chemical control using pesticides. Pesticides alone will never give sustained efficient control of virus diseases.

5. For major crops in Kuwait, legislations should be enacted to prohibit the introduction of planting material, plant parts or commodities that may harbor potentially dangerous pests. The red palm weevil is a good recent example. PepMV represents a tangible example on many other virus diseases that could cause significant economic losses upon their introduction to Kuwait, especially those transmitted by whiteflies and thrips. To implement these legislations the plant protection laboratories should be well equipped to conduct the required diagnostic tests. However, the most important factor remains: the dedicated well trained and qualified personnel.

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