

Plant Diseases

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Introduction

- Farmers complained to the Kuwait Institute for Scientific Research (KISR) about diseases affecting their vegetable crops, leading to subsequent heavy economical losses.
- Rapid spread of viral diseases due to long distance transportation and increase volume of free trade in plants has lead to sever losses.



- Most viruses are excluded from true seed during plant development. However, they are easily transported in planting material of vegetative crops, such as onion 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.
- The first step for successful management depend on proper identification of pests. However ,the confirmation is usually based on the virus identification using molecular detection methods.



Objective

- **Equipping the existing laboratory to accomplish the work.**
- **Transferring technology for the diagnosis of major viruses infecting vegetable crops in Kuwait, using serological methods ELISA technique.**
- **Ten vegetable crops were selected from 4 families:**
 1. **Cucurbitaceae** (cucumber, squash, melon, and zucchini).
 2. **Solanaceae** (tomato, potato, pepper and eggplant).
 3. **Liliaceae** (onion).
 4. **Leguminosae** (bean).

Methodology

Sampling:

- 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, south and north agricultural districts, were surveyed four times per year.

- **Kuwait Map showing two agricultural areas.**

- **Al- Wafra** ★

- **Al-Abdalli** ★





Enzyme-linked immunosorbent assay (ELISA)

- Double sandwich ELISA kits (DAS-ELISA) were purchased from BioReba and Agdia and assays were performed following the manufacturers' directions. Results were recorded after visual observation or spectrophotometrically by measuring absorbance at A_{405} nm.



**The survey focused on: Thirteen viruses for Solanaceae
CMV, PVY, PVX, PVA, PVS,PVM, PLRV, PVV, PMTV,
PMMoV, ToMV, TSWV, TYLCV.**

- CMV = Cucumber mosaic virus
- PVY = Potato virus Y
- PVX = Potato virus X
- PVA = Potato virus A
- PVS = Potato virus S
- PVM = Potato virus M
- PLRV = Potato leafroll virus
- PVV = Potato virus V
- PMTV = Potato mop-top virus
- PMMoV = Pepper mild mottle virus
- ToMV = Tomato mosaic virus
- TSWV = Tomato spotted wilt virus
- TYLCV = Tomato yellow leaf curl virus

Six Viruses for Cucurbitaceae: CMV, MNSV, PRSV, SqMV, ZYMV, and WMV).

- CMV = Cucumber mosaic virus
- MNSV = Melon necrotic spot virus
- PRSV = Papaya ring spot virus
- SqMV = Squash mosaic virus
- ZyMV = Zucchini yellow mosaic virus
- WMV = Watermelon mosaic virus

Three viruses for Liliaceae: OYDV, IYSV, and GarCLV

- OYDV = Onion yellow dwarf virus
- IYSV = Iris yellow spot virus
- GarCLV = Garlic common latent virus

Two viruses for Leguminosae: TSWV and AMV viruses.

- TSWV = Tomato spotted wilt virus
- AMV = Alfalfa mosaic virus

Detection of TYLCV in Tomato using PCR

Dellaporta DNA extraction (Dellaporta, 1983) and Accotto PCR detection methods (Accotto, 2000) were used to detect TYLCV in tomato plants, in which two degenerated oligonucleotide primers were designed for amplification of an approximate 500 bp fragment of DNA.



Results

Symptomatology

- Symptoms on **Solanaceous crops** included mosaicking, mottling, malformation, yellowing, stunting, interveinal chlorosis, bronzing and silvering of the leaves, as well as fruit malformation and necrotic ring spot.



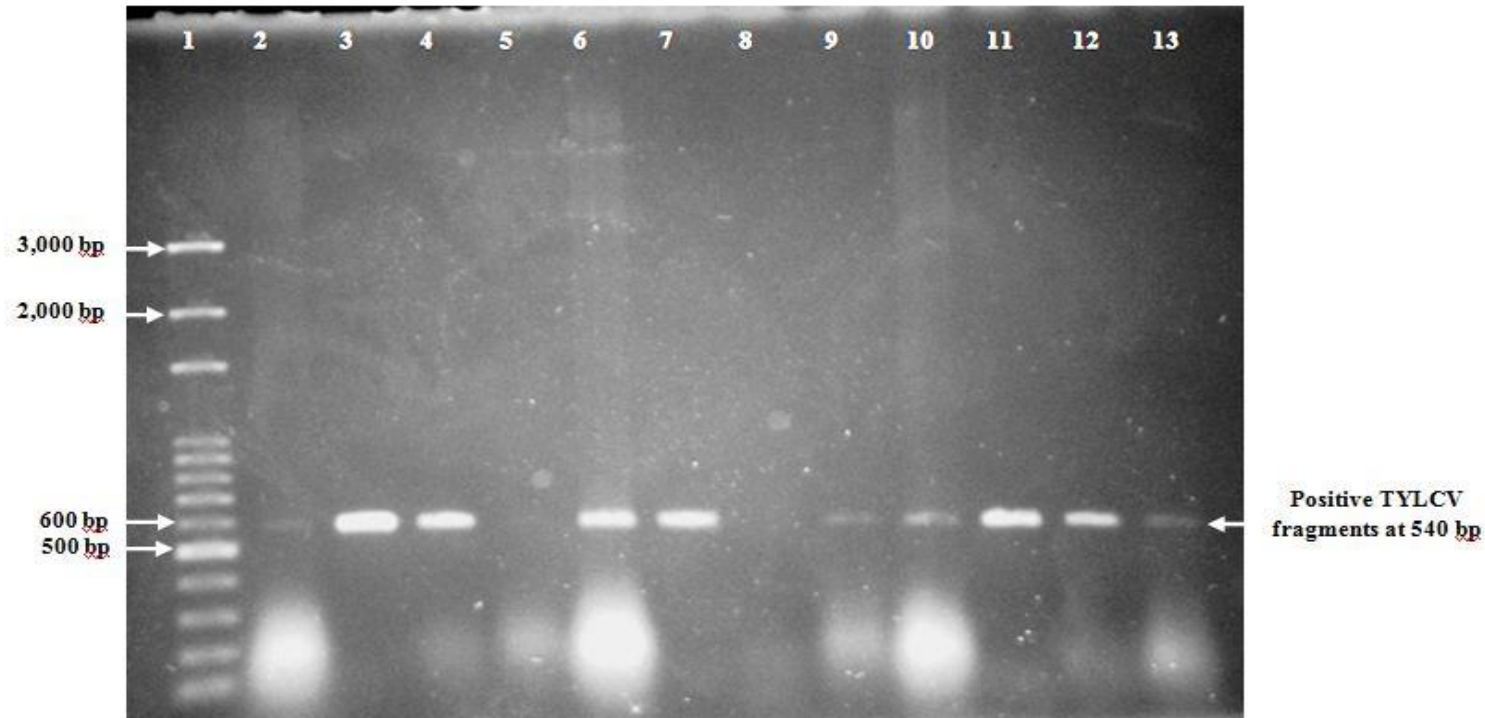
The symptoms on **cucurbits** included mosaicking, mottling, malformation, lesions, stunting, yellowing, and curling of leaves, as well as bumps or deformation of fruits.



Symptoms in **Liliaceae (onion)** plants consisted mainly of lesions, sometimes oval in shape on leaves. Most onion fields contained many weeds, which can act as a source of viruses. The incidence of severe symptoms was close to 100% in most fields. Symptoms in **Leguminosae (bean)** plants included yellowing.



Detection of TYLCV using PCR



1% agarose gel of amplified PCR products. Lane 1: 100 bp marker, Lanes 2-6 and 9-13: amplified PCR products. lane 7: positive control lane 8: negative control.

A partial sequencing was done for those positive unknown band fragments and it is suggested to carry out a thorough search on the results obtained with these primers in other studies then sequencing the full length genome to identify the nature of these unknown fragments is recommended.

1	CCCTGCTCGA	TATCGTCGAA	GTGCCCATCG	TAAAGTCCAG	TCTTATGAGC	AACGGGATGC
	TATTAAAGGC 70					
71	ATGCTGGTAT	TGTTTCGTTGT	GTTAGTGATG	TTACACGTGG	ATCCGGAATT	ACCCACAGAG
	TGGGTAAGAG 140					
141	GTTCTGTGTT	AAATCCATAT	ATTTTTTAGG	TAAAGTCTGG	ATGGATGAAA	ATATCAAGAA
	GCAGAATCAC 210					
211	ACTAATCAGG	TCATGTTCTT	CTTGGTCCGT	GATAGAAGGC	CCTATGGAAG	CAGCCCAATG
	GATTTTGGGC 280					
281	AGGTTTTTAA	TATGTTTCGAC	AATGAGCCCA	GTACCGCAAC	CGTGAAGAAT	GATCTCCGTG
	ATAGGTTTCA 350					
351	AGTGATGAGG	AAATTTTCATG	CTACAGTCAT	TGGTGGACCA	TCTGGAATGA	AGGAACAGGC
	TTTAGTTAAG 420					
421	AGATTTTTTA	AAATTAACAG	TCATGTAACT	TATAATCATC	AGGAAGCAGC	CAAGTACGAG
	AACCATACTG 490					
491	AAAACGCCTT GCTATTGTAT ATGG 514					

Conclusion

- This preliminary survey constitutes the first report in Kuwait of 15 of the 18 viruses detected, in Solanaceae, nine viruses were detected out of 14 tested, *Pepino mosaic virus* (PepMV) and *Tomato yellow leaf curl virus* (TYLCV) were the most predominant viruses. in Curbitaceae, all six viruses tested were detected mainly in double or triple infections. In onion, *Iris yellow spot virus*(IYSV) was widespread; in bean, *Alfalfa mosaic virus* (AMV) was detected. A pamphlet entitled “ Integrated Management of Viral Diseases Affecting Vegetable Crops in Kuwait” was issued and circulated to farmers.
- Full length sequence of the partial sequenced genome will be carried on in the next study to characterize and identify the genomic diversity of TYLCV present in Abdally (north) and Wafra (south) of Kuwait.

Thank You

