Molecular Detection of Tomato yellow leaf curl virus in Tomato Crops in Kuwait

KISR
Kuwait Institute for Scientific Research

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Presented By:
Ebtisam Al-Ali

Introduction

- In the last few years, vegetable crops in Kuwait have frequently been infected with viruses, causing heavy losses and a dramatic reduction in cropping area.
- A major virus responsible for these losses is the tomato yellow leaf curl virus (TYLCV)

- During a survey in 2013, the Kuwait Institute for Scientific Research (KISR) observed several symptoms suggesting the presence of more than one whitefly-transmitted virus species affecting tomato crops in Kuwait, with losses exceeding 90%
- 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

- The aim of this study was to adapt and optimize a very sensitive, rapid method for the detection of tomato yellow leaf curl virus (TYLCV) and other whitefly-transmitted viruses in the tissues of tomato plants and their vectors.

Methodology

Sampling:

- Tomato leaf samples were collected monthly for two tomato-growing seasons: October 2012 to January 2013 (first period), and November 2013 to February 2014 (second period).

- The collections were made from greenhouse farms in Wafra and Abdally.

- **Kuwait Map showing two agricultural areas.**
- **Al- Wafra**
- **Al-Abdally**

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Detection of TYLCV in Tomato using PCR

DNA Extraction:

DNA was extracted from 200 infected tomato leaf samples using Dellaporta method for total genomic extraction (Dellaporta et al.,1983).

PCR Amplification

Two pairs of primers were used in the PCR protocol to detect several genes in the TYLCV spp (replicase genes, the intergenic region, and partially-coat protein genes): TY1(+) and TY2(-) (Accotto et al., 2000), and TYC1R and TYC1F

Rolling Circle Amplification (RCA)

- Rolling circle amplification (RCA) was carried out using TempliPhi Kit (GE Healthcare, formerly Amersham) following the manufacturer's protocol.

Enzyme Digestion

- Five samples from the above were digested with ten restriction enzymes. Aliquots of 500 ng nucleic acid in a total volume of 20 µl were digested by *Bam* HI, *Hae* II, *Sac* I, *Cla* I, *Xba* I, *Pst* I, *Nco* I, *Eco* RI, *Xho* I, and *Spe* I (New England Biolabs) for 2 hrs following the manufacturer recommendations.
- Restriction products were run at 1% agarose gel for 1 hr at 100V followed by staining in ethidum bromide for 20 min. and finally destaining the gel in distilled water for 5 min.
- The fragment with size of ~2800 nt was purified using a gel purification kit (Qiagen Sciences, Germantown, MD).

- and cloned into the *Xba* I site of pBluescript® KS (-) (Stratagene, La Jolla, CA) and sequenced by the dideoxynucleotide chain-termination method.

Sequencing

- Sequence was assembled using Sequencher 5.2.4 (Gene Codes Corporation, Ann Arbor, MI 48108, USA) and analysed using Vector NTI Advance® 11.5.3 (Life Technologies, Grand Island, NY 14072, USA).

Blast

- Assembled sequence was compared with other related sequences available in the GenBank database using BLASTn .
- Full- length TYLCV sequence (tomato yellow leaf curl virus – KISR) was selected for further comparisons.

Gen Bank Submission

- Full- length sequence of the positive TYLCV was submitted to the genbank.

Results

Symptomatology

- A high incidence of severe viral symptoms were observed in greenhouse tomato plants; yield loss exceeded 90% in Wafra.
- Viral symptoms included stunting, upward cupping, leaf deformation, leaf curling, and leaf yellowing (Plates 1 to 5). Since the virus is transmitted to tomato plants by whiteflies (Plate 6), the symptoms would be expected to appear on plants in the wake of any whitefly infestations.
- Field-grown tomatoes were exposed to high whitefly infestation and showed severe viral symptoms.
- A viral symptom previously unreported in tomato plants was observed: leaves turned purple, with the whole plant becoming as purple as an ornamental plant (Plates 7 and 8).

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Plate 1. Stunted growth in a tomato plant.

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Plate 2. Cupping of the upper leaves on a tomato plant.

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Plate 3. Tomato leaf deformation.

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Plate 4. Leaf curling in a tomato plant.

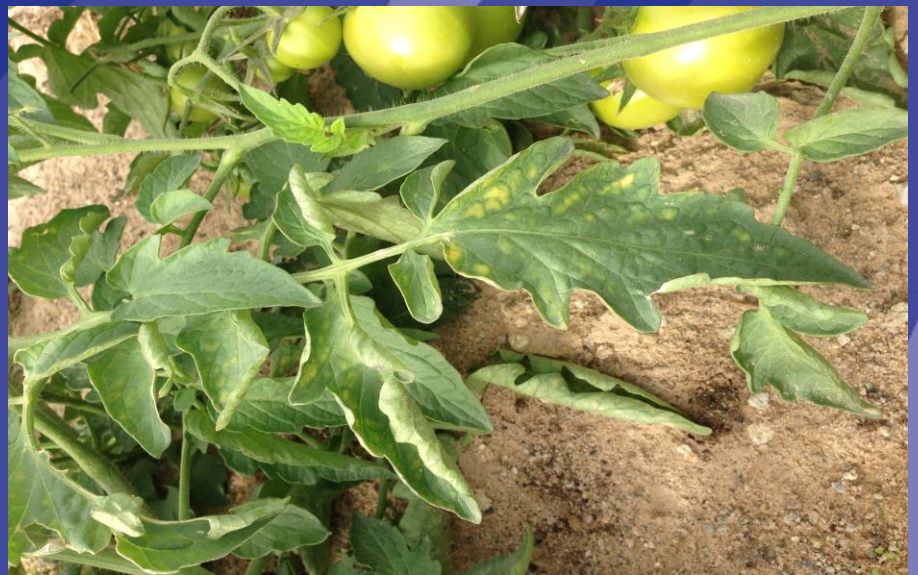

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Plate 5. Leaf yellowing in a tomato plant.

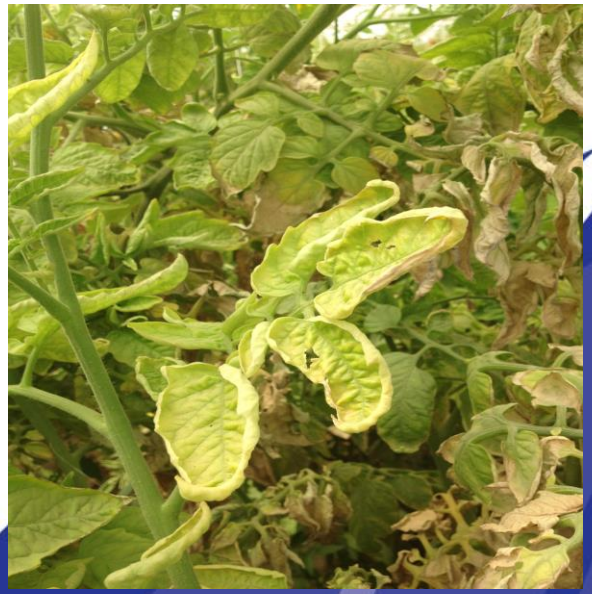

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Plate 6. High population of whiteflies on a tomato plant.

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Plate 7. Tomato plant displaying symptoms of a purpling disorder.

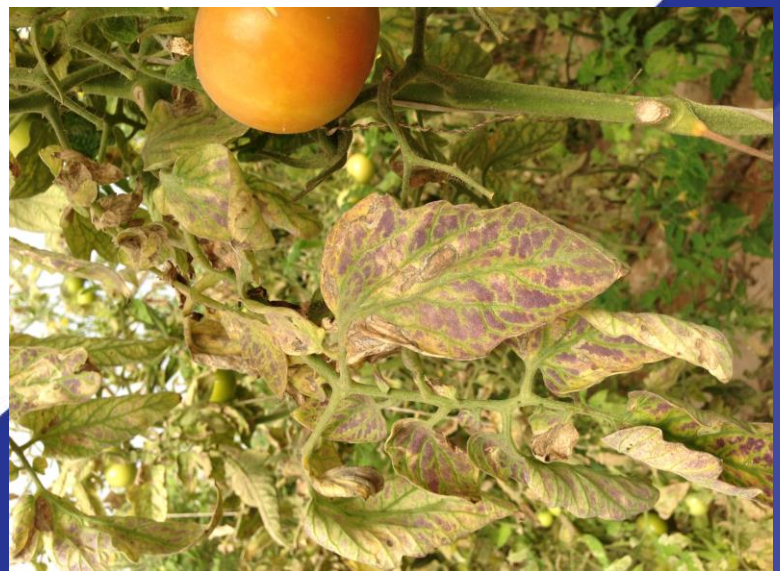

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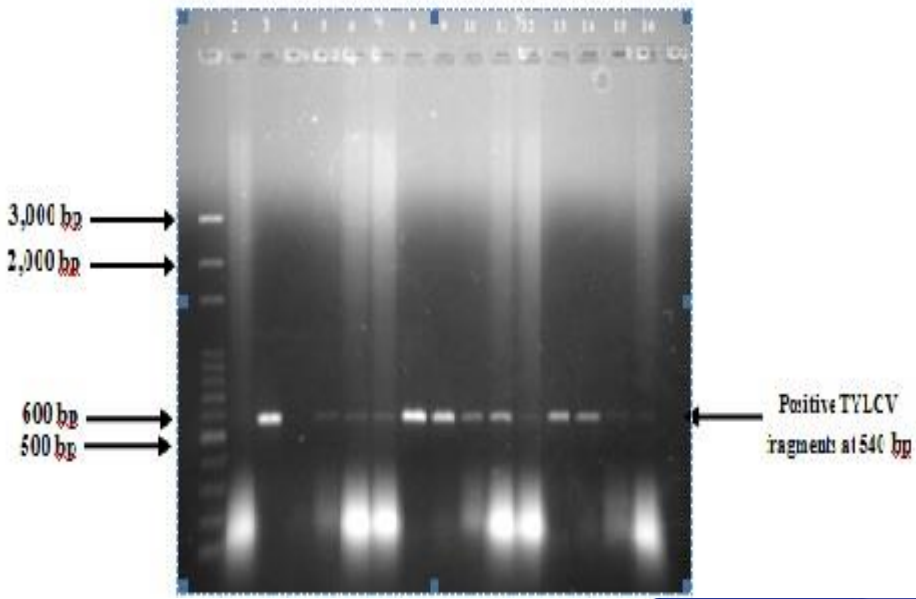
Plate 8. Purpling of a tomato plant.

PCR Gel


3,000 bp
2,000 bp
600 bp
500 bp
Positive TYLCV
fragment at 540 bp

Detection of TYLCV in Tomato using PCR

The data showed that the TY1 and TY2 primer pair was successful in detecting TYLCV in samples collected in January and February.

- Partial sequencing of the positive TYLCV was completed and submitted to the genbank TYLCV-KISR (JF451352).
- The amplicon showed that it was a new species of TYLCV, and that whitefly infestation preceded viral infection.

Conclusion

- The general conclusion of this study is that the Dellapotra method for DNA extraction and the Accotto PCR detection method (Accotto et al., 2000) can be used to consistently and successfully detecting TYLCV. Consistently. If strict hygiene is practiced in greenhouses and adjacent fields, whitefly infestation can be controlled. PCR technique is a rapid, sensitive method, and can be applied to detect viral infections in tomato samples (leaves) from green house grown plants, and therefore it can be offered as a service for the early detection of the infection to save the crops.
- Full length sequence of the partial sequenced genome will be used to characterize and identify the genomic diversity of TYLCV present in Abdally (north) and Wafra (south) of Kuwait.

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Thank You