Phylogenetic analysis of TYLCV on tomato plants in Kuwait

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Kuwait Institute for Scientific Research

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Introduction

- Recent studies have shown that diseases caused by viruses cause remarkable economic losses in many vegetable crops in Kuwait.
- Tomato growers have reported economic losses as high as 90-95% for the last several years.

- The virus mainly responsible for these losses is tomato yellow leaf virus (TYLCV). TYLCV has been reported as a major tomato pest, but it has not been fully characterized at the molecular level.
- Whiteflies are the main vector for TYLCV. During the survey, several symptoms were observed that suggested the presence of more than one whitefly-transmitted viral species in tomato plants in Kuwait; losses exceeded 90% in some greenhouses.

Objective

- Molecular characterization of Tomato Yellow Leaf Curl Virus (TYLCV) isolates from different tomato-growing regions in Kuwait and their comparison with virus species detected in other countries.

Methodology

Sampling:

- One hundred tomato leaf samples were collected for two tomato-growing seasons: September to December 2015 and January to April 2016.
- The collections were made from greenhouse farms (Abdally-North Kuwait)

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

Sampling

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Detection of TYLCV in Tomato using PCR

DNA Extraction:

DNA was extracted from 100 infected tomato leaf samples using Dellaporta method for total genomic extraction (Dellaporta et al.,1983).

PCR Amplification

Polymerase chain reaction (PCR) detection was performed on 50 infected samples using the degenerate primer pair, TY1/ TY2 (Accotto et al., 2000)

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.

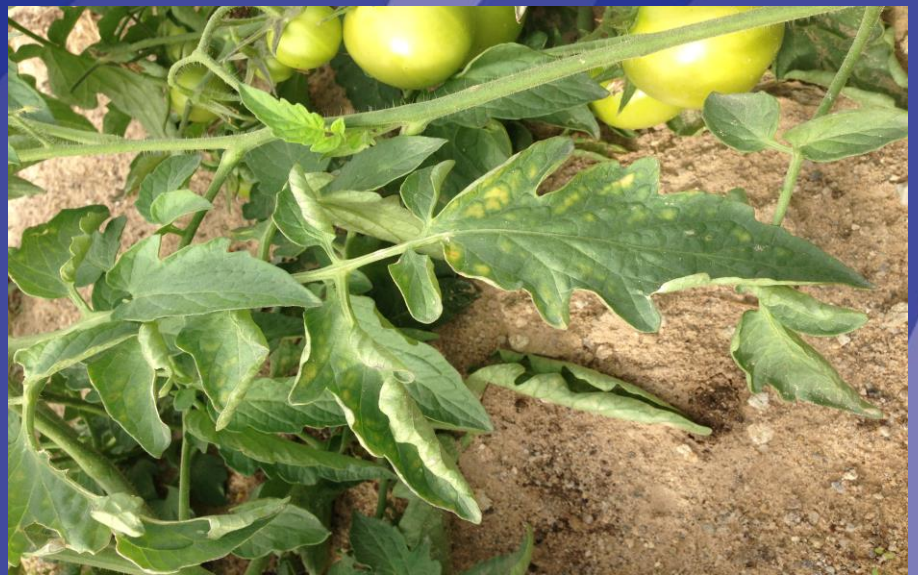

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Plate 5. Leaf yellowing in a tomato plant.

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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.

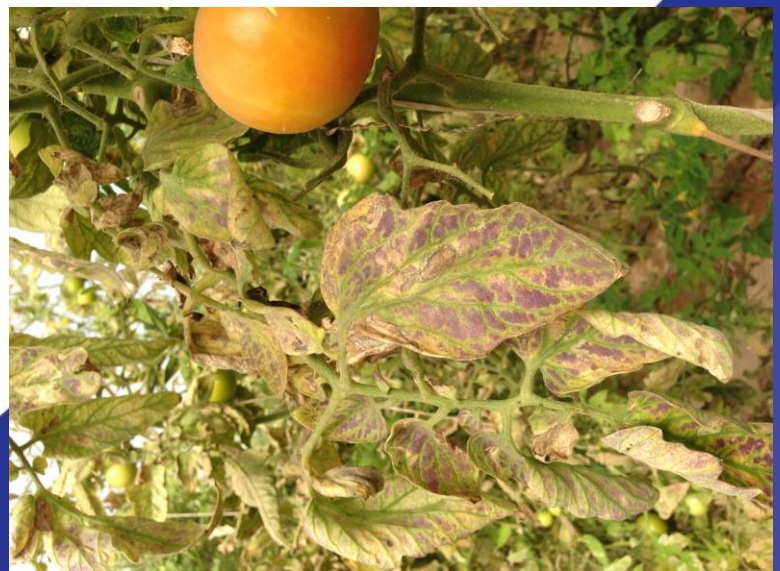

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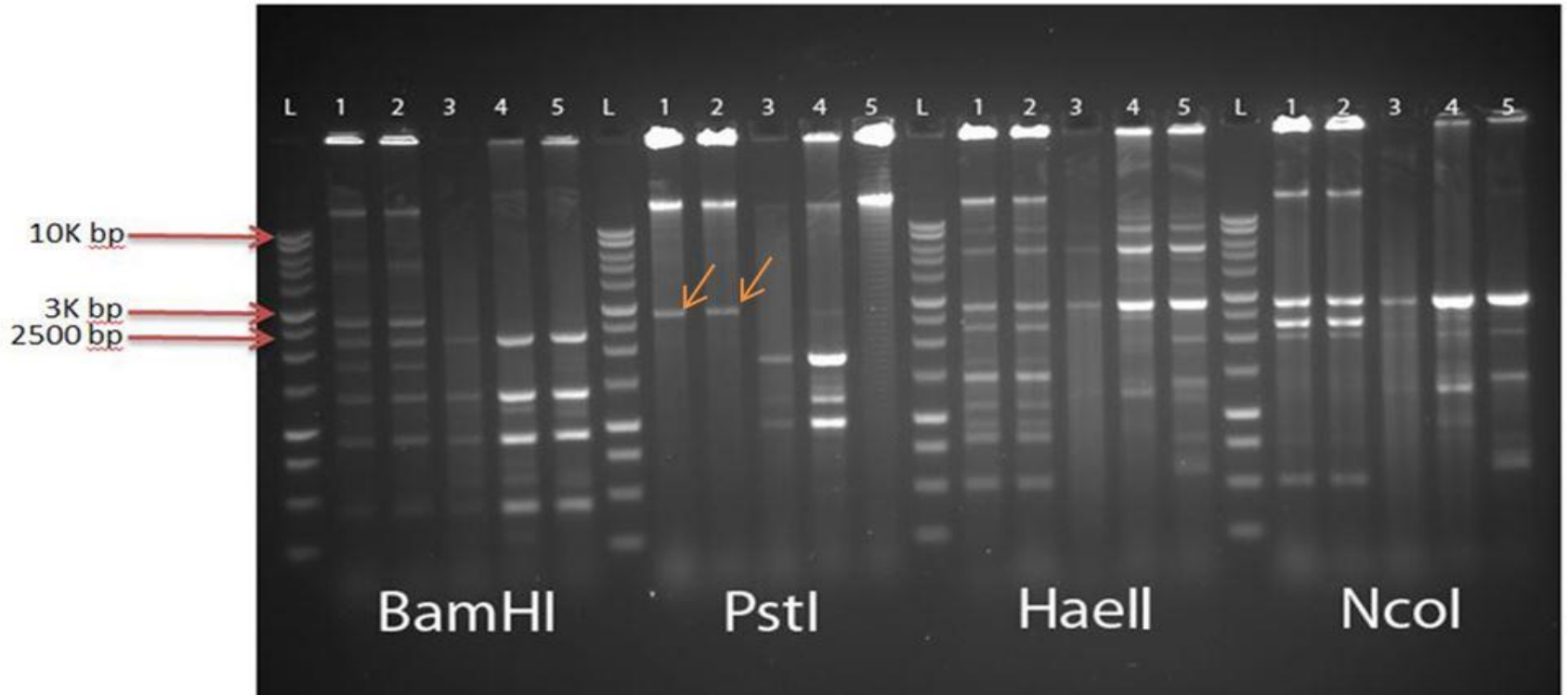
Plate 8. Purpling of a tomato plant.

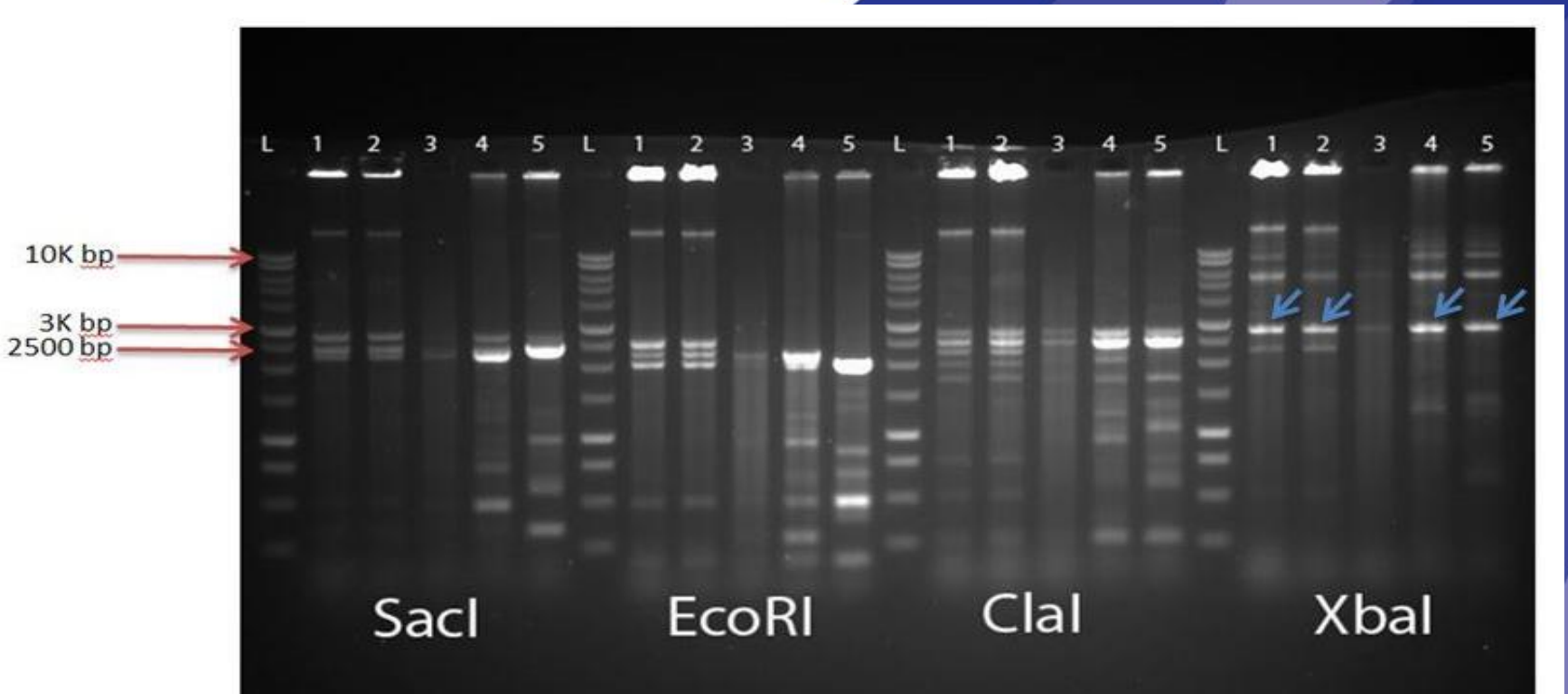
Results of RCA and restriction analysis of selected plant samples.

Digestion of RCA amplified DNA extracted from tomato samples using *BamH*I, *Pst*I, *Hae*II, and *Nco*I restriction enzymes. Lanes with the same number indicate the same sample. Lanes 1 tomato sample no. 1, lanes 2 tomato sample no. 2, lanes 3 tomato sample no. 3, lanes 4 tomato sample no. 4, lanes 5 tomato sample no. 5. Ladder 10Kbp ladder (New England Biolabs). Arrows indicate band of 2800 bp that indicates a full genome of TYLCV.



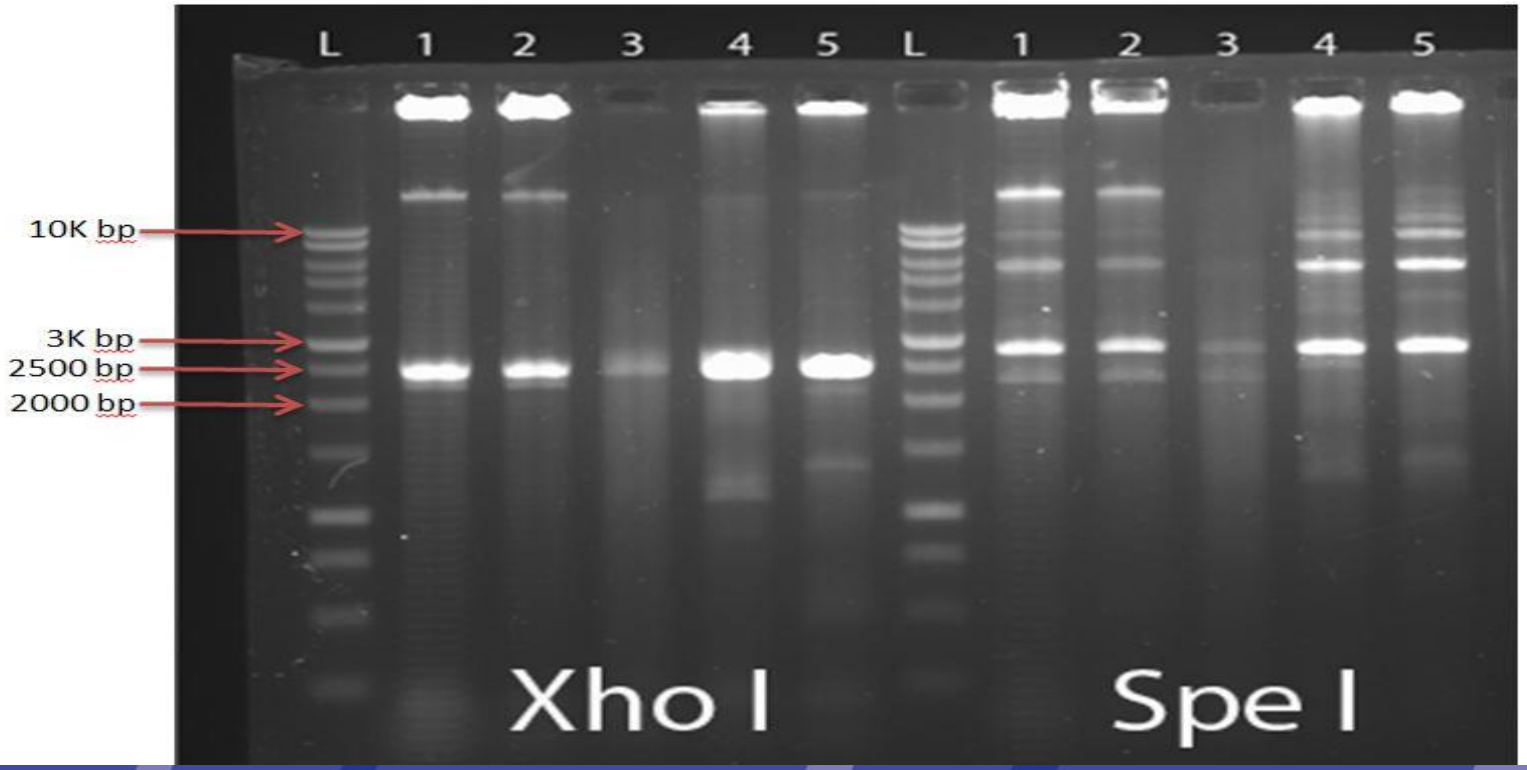
Results of RCA and restriction analysis of selected plant samples.

Digestion of RCA amplified DNA extracted from tomato samples using *Sac*I, *Eco*RI, *Cla*I, and *Xba*I restriction enzymes. Lanes with the same number indicate the same sample. Lanes 1 tomato sample no. 1, lanes 2 tomato sample no. 2, lanes 3 tomato sample no. 3, lanes 4 tomato sample no. 4, lanes 5 tomato sample no. 5. Ladder 10Kbp ladder (New England Biolabs). Samples 1,2, 4, and 5 digested with *Xba*I were cloned and sequenced. Arrows indicate band of 2800 bp that indicates a full genome of TYLCV



Results of RCA and restriction analysis of selected plant samples.

Digestion of RCA amplified DNA extracted from tomato samples using *Xho*I and *Spe*I restriction enzymes. Lanes with the same number indicate the same sample. Lanes 1 tomato sample no. 1, lanes 2 tomato sample no. 2, lanes 3 tomato sample no. 3, lanes 4 tomato sample no. 4, lanes 5 tomato sample no. 5. Ladder 10Kbp ladder (New England Bioloabs).



Detection of TYLCV in Tomato using PCR

TYLCV isolated from severely diseased tomatoes was characterized at the molecular level and the complete genomic sequence was determined. Based on the genome structure and organization and phylogenetic analysis, the begomovirus was found to be a strain of TYLCV.

- One isolate that was characterized in this study had 97% and 95% nucleotide sequence identity with previously characterized Kuwaiti isolate, TYLCV-KISR and the highest sequence identity (95%) was with that of TYLCV-Almeria (Spain) isolate.
- Phylogenetic analysis showed that the Kuwait isolate could be a novel variant of TYLCV, and suggested to be in a different lineage from known TYLCV sequences.

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 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