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Full Length Article

Genetic Diversity of Tomato Yellow Leaf Curl Virus Isolates and their Associated Beta-Satellite in Tomato Fields in Egypt

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Abstract

Tomato yellow leaf curl virus (TYLCV) poses a significant threat to tomato production in Egypt. This study investigated the genetic diversity and biological characteristics of TYLCV isolates circulating in Egyptian tomato fields. A combination of visual observations, serological, and molecular techniques were employed to detect and quantify the virus in infected plants. Positive samples were categorized based on symptom severity to explore the variable effects of different TYLCV isolates. Disease Severity Index, incidence, and virus concentration were analyzed to understand the behavior and impact of these isolates. DAS-ELISA, PCR, and multiplex-PCR (mPCR) targeting the partial coat protein (pCP) gene were used for virus detection. Phylogenetic analysis revealed that the isolates were highly related (99%) to the TYLCV-IL group and moderately related (97%) to the TYLCV-mild group. Beta-satellite presence and its association with symptom severity were also investigated. The study highlights the genetic diversity and biological variability of TYLCV isolates in Egypt, leading to variable symptom expression and disease severity. Understanding these characteristics is crucial for developing effective management strategies and breeding for resistance against TYLCV in tomato crops.

Keywords: DAS-ELISA; Disease severity index; Genetic variability; Multiplex-PCR; TYLCV-IL; TYLCV-mild

Introduction

Tomato yellow leaf curl virus (TYLCV) is a major possible agricultural threat worldwide, particularly affecting tomato crops. Its global distribution has expanded rapidly since its first identification in the late 1930s, with notable prevalence in the Mediterranean basin, the Middle East, and various tropical and subtropical regions (Yan *et al.* 2021). The whitefly vector, *Bemisia tabaci*, primarily transmitted the virus, contributing to its widespread dissemination (Fiallo-Olivé *et al.* 2020; Ghosh and Ghanim 2021). TYLCV is transmitted in a persistent circulative, non-propagative manner by whiteflies of the *Bemisia tabaci* species complex (Pakkianathan *et al.* 2015). Papayiannis *et al.* (2011) reported that TYLCV has many different plant hosts belonging to various plant families including vegetables, ornamentals, and wild plants.

TYLCV contains a monopartite single-stranded DNA genome (ssDNA), nearly 2.7 kb in size, bidirectionally transcribed to express six partially overlapping ORFs (Butterbach *et al.* 2014). Two genes, V1 and V2, are encoded by the genome; V1 encodes the coat protein, whereas V2 encodes a movement protein. In addition to these two genes, the complementary viral strand consists of four genes, C1, C2, C3 and C4. C1 codes for a protein related to replication, C2 codes for a transcription activator, C3 codes for a replication enhancer, and C4 codes for a small protein that may act as a symptom determinant. C4 is the most interesting among the viral proteins related to the infection mechanism, which contributes to the induction of the plant defense response and is associated with pathogenicity, virus movement and virus-induced post-transcriptional gene silencing (Kim *et al.* 2016).

The global tomato production (*Solanum lycopersicum*) was approximately 177 million metric tons, with significant variations in yields and quality due to TYLCV infections (FAOSTAT 2020). In Egypt, TYLCV poses a severe challenge to tomato cultivation, significantly impacting yield and quality. The virus is widespread throughout the country, with reports of significant economic losses. Despite efforts to control the virus through various management practices and developing resistant tomato varieties, TYLCV remains a persistent threat to Egyptian agriculture (El-Sappah *et al.* 2022). In recent years, TYLCV has been

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reported in several countries across continents, including the Americas, Africa, and Asia, with strains such as TYLCV-Israel and TYLCV-Mild being widely spread and impactful (Marchant *et al.* 2023).

In Egypt, TYLCV poses a severe challenge to tomato cultivation, a critical crop for domestic consumption and export (Rabie *et al.* 2017). The virus is prevalent throughout the country, significantly affecting the yield and quality of tomato plants. Control measures in Egypt have been complicated by the high population of the whitefly vector and the virus's ability to mutate and adapt, leading to the emergence of new strains (Avedi 2022). Consequently, researchers and agricultural experts are focusing on developing resistant tomato varieties and improving management practices to mitigate the impact of TYLCV on Egyptian agriculture (Rahman *et al.* 2024). Fauquet and Stanley (2005) have demonstrated that the phylogenetic analysis has classified many TYLCV isolates globally based on their origin into two major clades: one convenience called the IL clade is separated from Israel, and the second called the Mild strain for the Mild clade. Antignus and Cohen (1994) have suggested the initial description of TYLCV-Mild by inducing milder symptoms than the TYLCV. Beta-satellites have been associated with monopartite *begomoviruses* as described by Lozano *et al.* (2016). Beta-satellites are circular ssDNAs, nearly 1,350 nt in size, that utilize monopartite *begomoviruses* as helper viruses, contributing to the induction of disease symptoms, and are prevalent mainly in eastern Asia. Beta-satellites rely on the helper *begomoviruses* for replication, encapsidation and spread within the host (Gelbart *et al.* 2020).

Multiplex PCR (Polymerase Chain Reaction) is an advanced molecular technique that enables the simultaneous amplification of multiple target DNA sequences within a single reaction. This method is precisely valuable for detecting various pathogens, including viruses like the Tomato yellow leaf curl virus (TYLCV). Using specific primer sets designed for each target, multiplex PCR allows researchers to efficiently identify and quantify multiple viral infections from a single sample, significantly reducing the time and resources required compared to traditional methods. The genetic distances between isolates were calculated based on the number of nucleotide substitutions per site. This comprehensive approach, integrating field observations with laboratory techniques, facilitated a deeper understanding of TYLCV diversity and its impact on tomato production in Egypt (Letunic and Bork 2021). This study aimed to investigate the diversity of Tomato yellow leaf curl virus (TYLCV) isolates and the association of the beta satellite with the symptom's severity in tomato fields under Egyptian conditions.

Materials and Methods

Collecting TYLCV samples from tomato plants

Tomato plants exhibiting symptoms of TYLCV were collected from different agricultural governorates in Egypt, including 83 from Giza, 180 from Qalyoubia, 120 from Menoufia, 100 from Ismailia, 130 from Fayoum, 82 from Benisuef, 78 from El Sharkia, 65 from Alexandria and 76 from El Beheira governorates, as shown in (Fig. 1). Samples were selected based on visual symptoms, including leaf curling, yellowing, stunted growth, and reduced fruit production. The incidence of TYLCV-infected tomato plants was studied in 914 tomato plants collected from the open field and protective greenhouse in the tomato cultivated (Fig. 2) seasons of 2022, 2023 and 2024. Samples were collected from symptomatic plants using sterile tools and labeled for future analysis. Geographical positioning system (GPS) coordinates were also recorded to track the locations of the samples. TYLCV samples were stored in a cool and dry place, preferably at low temperatures to maintain the integrity of the virus.

The incidence of TYLCV

The incidence of TYLCV-infected tomato plants was studied in 914 suspected samples of tomato plants. Regular testing using double antibody sandwich enzyme-linked immune-sorbent assay (DAS-ELISA) to confirm the presence of the virus in the collected samples as described by El-Dougdoug *et al.* (2010), then the positive samples are tested specifically by polymerase chain reaction (PCR) (Kumar *et al.* 2023).

Biological characterization of TYLCV

Whiteflies (*Bemisia tabaci*) were collected from naturally infected tomato plants that developed TYLCV typical symptoms and were used as a virus source for virus transmission experiments. Whiteflies were collected from the tomato plants in the Qalyoubia governorate. The collected whiteflies were then reared on white mulberry plants in insect-proof cages for over one generation to confirm the elimination of any virus residues. The non-viruliferous whiteflies were allowed to feed on infected tomato plants for an acquisition access period (AAP) of 48 h using 15–20 insects per plant. Subsequently, viruliferous insects were transferred onto healthy tomato seedlings cv. 206 and differential hosts using 48 h inoculation access feeding periods. Insects were then killed with an Actelic 50 EC, Syngenta crop protection. The inoculated plants were kept under greenhouse conditions. The developed symptoms were observed daily at 25 days post-inoculation and confirmed by DAS-ELISA and PCR (Faisal *et al.* 2019).

Serological Testing

DAS-ELISA (Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay) from BIOREBA, Switzerland, was used to confirm the presence of TYLCV in a total of 914 samples collected. The absorbance values were measured at 405 nm using a microplate reader to determine the virus concentration (Clark and Adams 1977).

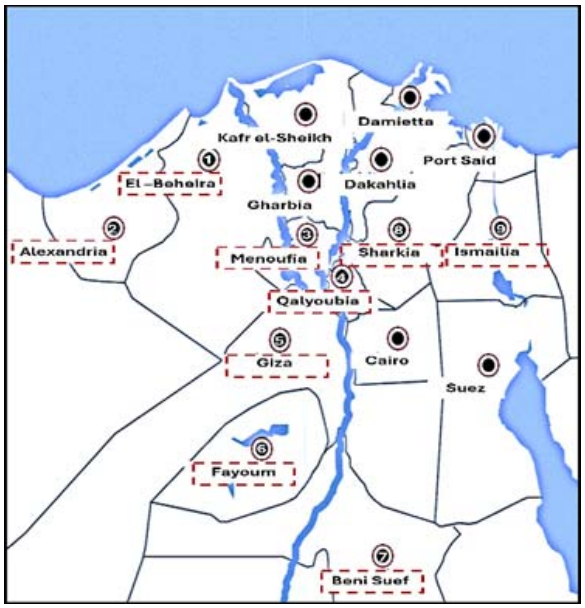

Fig. 1: Map for naturally infected tomato plant locations with TYLCV in different governorates in Egypt, marking regions of: 1. El Beheira, 2. Alexandria, 3. Menoufia, 4. Qalyoubia, 5. Giza, 6. Fayoum, 7. BaniSuef, 8. Sharkia and 9. Ismailia

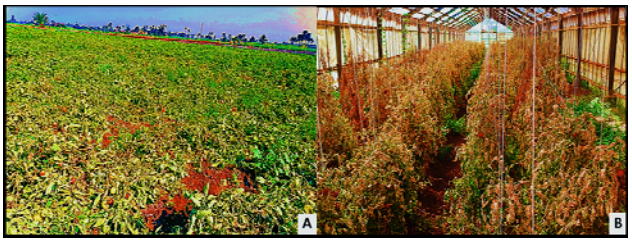

Fig. 2: (**A**) Naturally TYLCV-infected tomato plants in the open field, (**B**) TYLCV-infected tomato plants in a greenhouse in Qalyoubia governorate

Determination of pathogenicity

The Disease Severity Index (DSI) was used to assess the infection level in the sampled populations. The numbers of infected plants per unit area were calculated for the index. Symptoms were classified into two categories based on visual observation and a rating scale: severe stunting and leaf curling, mottling, and yellow pattern. The DSI values were computed using the formula proposed by Raupach *et al.* (1996):

$$\text{DSI}(\%) = \frac{\Sigma(\text{disease grade} \times \text{No. of plants in each grade})}{(\text{total No. of plants} \times \text{highest disease grade})} \times 100$$

Virus concentration was determined as antigen at 405 nm by DAS-ELISA.

Host range and symptomology

Different host plants were inoculated with whiteflies, as described by Legarrea *et al.* (2020). Symptoms were monitored daily for 25 days, during which viral transmission was confirmed through DAS-ELISA and PCR techniques. All experiments involving virus transmission and whiteflies were conducted in insect-proof cages, maintaining optimal temperatures between 26 and 32ºC to ensure the integrity of the study conditions.

Molecular characterization for TYLCV

Detecting and analysis of TYLCV: Total DNA was extracted for both symptomatic and healthy tomato plants using the Genomic DNA Mini Kit (Plant) from Geneaid (Cat. No. GP100, Taiwan), following the manufacturer's recommended protocol. The quality and quantity of the extracted DNA were assessed using a NanoDrop spectrophotometer (260*50 ng μL^{-1}, Thermo Scientific, One Microvolume UV-Vis, India). The total nucleic acids served as templates for PCR amplification in a reaction volume of 25 μL, which included 12.5 μL of GoTaq® long PCR master mix (Cat. No. M4021, Promega), 1 μL of each primer (5 nmol mL^{-1}) and 1 μL of the DNA sample. The PCR conditions consisted of an initial denaturation step at 94ºC for 3 min, followed by 40 cycles of denaturation at 94ºC for 30 s annealing as specified in Table 1 and extension at 72ºC for 1 min, concluding with a final extension at 72ºC for 10 min. The PCR products were analyzed *via* electrophoresis on 1.0, 1.5 and 2.0% agarose gels according to the expected band size and visualized under UV illumination using a gel documentation system (Gel Doc Chemidoc+, Bio-Rad, USA). The PCR products were purified using the FavoPrep Gel Purification Mini Kit (50 preps FAVORGEN, China), and sequencing was performed by Macrogen, Inc., Seoul, Republic of Korea. The partial nucleotide sequences of the core CP gene fragments from TYLCV isolates collected from various governorates were submitted to the GenBank database and compared with published sequences using the NCBI BLAST program. A phylogenetic tree was constructed using DNAMAN version 7.0.2 software by aligning the nucleotide sequences from the nine governorates involved in the study.

A specialized primer set was designed to differentiate between Tomato yellow leaf curl virus (TYLCV) isolates based on variations in the C1 and C4 regions (Fig. 3). The forward primer TC1C4-F and reverse primer TC1C4-R were developed to target specific sequences associated with Israeli and mild strains (Table 1).

Table 1 provides the primer sequences, expected amplicon sizes, annealing temperatures, and references for various PCR and multiplex PCR (mPCR) assays used to detect and differentiate TYLCV isolates. Primers were designed to target different regions of the TYLCV genome, including the coat protein (CP) gene, the intergenic region (IR), C4, as well as the beta-satellite. Additional reverse primers were designed at the core C4 gene to distinguish the genome of the TYLCV-IL from TYLCV-mild and were

Table 1: Nucleotide sequences of the primers used for TYLCV detection

Set	Primer name		Nucleotide sequence (5'-3')	Expected size (bp)	Annealing temp.	References
	PCR	mPCR				
1	AV-Core (F)	-	GCCHATRTAYAGRAAGCCMAGRAT	~550	60°C/1 min	Abdel-Salam *et al.* (2005)
	AC-Core (R)		GGRTTDGARGCATGHGTACANGCC			
2	TY-1 (F)	-	GCCCATGTAYCGRAAGCC	580	60°C/40 s	Accotto *et al.* (2000)
	TY-2 (R)		GGRTTAGARGCATGMGTAC			
3	Ty-78(R)	Ty-78(R)	GCAATTTGATTGGTTGAYAGTSACR	mPCR and PCR:	60°C/1 min	Belabess *et al.* (2015)
	IL-2629(F)	IL-2629(F)	GGTGTCCCTCAAAGCTCTAWG	IL: 230		
	Ty-78(R)	Mld-2277(F)	GCAATTTGATTGGTTGAYAGTSACR	Mild: 592		
	Mld-2277(F)		CTSWCCCCARTCGABGGTG			
4	Beta01	-	GGTACCACTACGCTACGCAGCAGCC	~1320	60°C/1.5 min	Gelbart *et al.* (2020)
	Beta02		GGTACCTACCCTCCCAGGGGTACAC			
5	TC1C4 (F)	-	AGRGCCTCKGAYTTACTGCCTGA	~240	55°C/1 min	Current Study
	TC1C4 (R)		RAAYCARCGRTTCTTCGACCTGG			
	TC1C4 (IL-R1)	-	GAACCAACGGTTCTTCGACCTGG			
	TC1C4 (MLD-R2)		AAATCAGCGATTCTTCGACCTGG			

NB: R: Purine (A/G) Y: Pyrimidine (C/T) H:(A/C/T) W:(A/T) D:(A/G/T) N: Any nucleotide (A/C/G/T) B:(C/G/T) K:(G/T)

used with the TC1C4-F. The mPCR assays allow for the simultaneous detection of multiple TYLCV variants and can be used to distinguish between the IL and mild isolates.

Nucleotide sequences of the core CP regions: PCR products of the core CP regions of the collected samples from different governorates were sequenced at Macrogen Company in Korea to verify the presence of the specified DNA. The sequences were aligned using DNAMAN version 7.0.2. software program and were submitted to and assigned to GenBank with accession numbers PQ206352, PQ304521 to PQ304528 representing Giza, Alexandria, El Beheira, Sharkia, Fayoum, Benisuef, Ismailia, Menoufia, and Qalyoubia Governorates.

Results

Collecting TYLCV samples from tomato plants

A total of 914 samples were collected and tested from different Egyptian-infected regions. The collection of TYLCV samples involves identifying tomato fields with symptoms like leaf curling, yellowing and stunted growth (Fig. 4).

There was a substantial difference in infection rates among the different governorates. Some regions have significantly higher infection rates compared to others as shown in Table 2. Qalyoubia, Sharkia, and Ismailia reported the highest infection rates, with percentages exceeding 80%. This suggests a concentrated outbreak or endemic situation in these areas while Menoufia, Giza, Benisuef, and Fayoum Governorates exhibited moderate infection rates, ranging between 50% and 70%, while El-Beheira and Alexandria recorded relatively lower infection rates (Fig. 5). The infection rates exhibit significant fluctuations across different governorates over the three years. Some governorates show a consistent increase in infection rates, while others experience a relatively stable increase (Fig. 6). We observed an increasing trend in the samples tested. There's a noticeable spike in infections between 2022 and

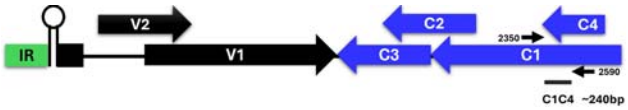

Fig. 3: Diagram represents the development of a specialized primer for differentiation of TYLCV isolates based on C1 and C4 region variations

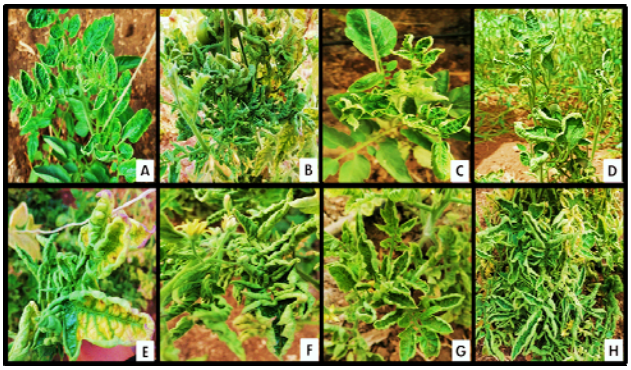

Fig. 4: Phenotypic expression symptoms to classify naturally infected tomato plants from different fields from **A** to **H** present the key symptoms of TYLCV observed in infected tomato plants from various fields

2023, followed by a slight decrease in 2024. This suggests that factors influencing the infection rate may be complex and subject to fluctuations.

Insect transmission

Tomato plants inoculated with TYLCV isolates exhibited distinct phenotypic expressions in insect-proof cages, including mottling, leaf curling, yellow patterns, and notable stunting. The study involved inoculating the plants with TYLCV-IL and TYLCV-Mild of infectious sap, each associated with specific symptoms, resulting in varying levels of symptom severity (Wu *et al.* 2022). According to visual symptoms, TYLCV-IL isolate was linked to leaf

Table 2: Total % of infection in different governorates in years (2022, 2023 and 2024)

Governorates	No. of Tested Samples			No. of Infected Samples			Percentage of TYLCV infected samples		
	2022	2023	2024	2022	2023	2024	2022	2023	2024
Qalyoubia/Qaha	50	60	70	40	50	60	80.00	83.33	85.71[a]
Sharkia	20	25	33	10	19	30	50.00	76.00	90.91[a]
Menoufia	40	41	45	20	33	35	50.00	80.49	77.78[b]
Ismailia	30	33	37	25	29	30	83.33	87.88	81.08[a]
Giza	25	30	28	19	26	21	76.00	86.67	75.00[b]
Fayoum	40	42	48	25	37	40	62.50	88.10	83.33[a]
El Beheira	20	25	31	5	18	28	25.00	72.00	90.32[a]
Alexandria	20	25	20	5	10	10	25.00	40.00	50.00[b]
Benisuef	25	27	30	15	22	15	60.00	81.48	50.00[b]
Total	270	308	342	164	244	269			

Statistical analysis. The mean values in each of the columns that begin with the same letter are not statistically different ($P \leq 0.05$). The data analyzed using ANOVA test

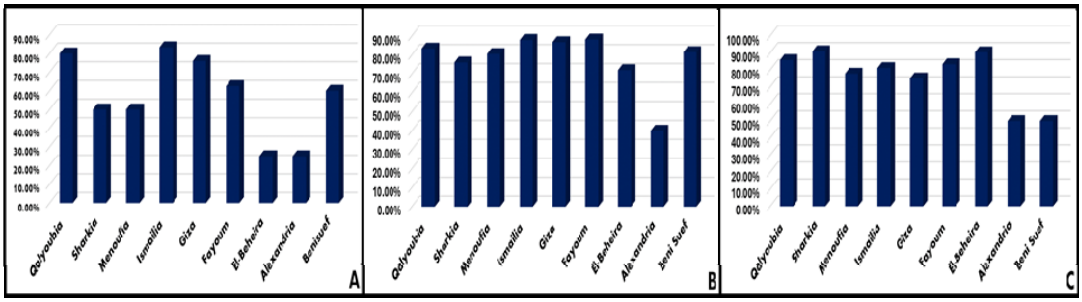

Fig. 5: Total % of infection in (**A**) 2022, (**B**) 2023 and (**C**) 2024

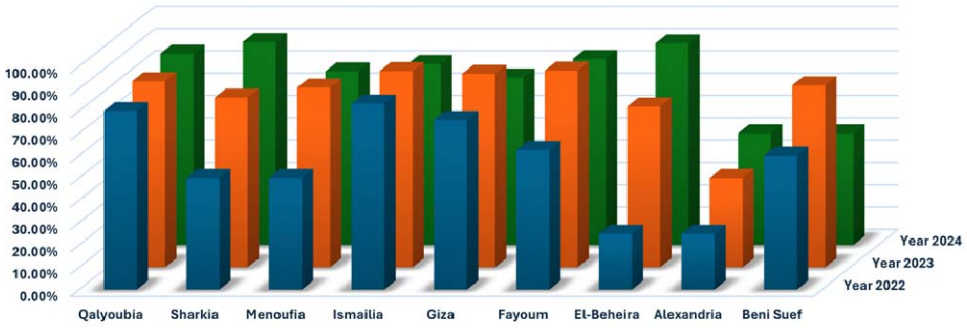

Fig. 6: The provided bar graph depicts the percentage of infections in different governorates of Egypt over three years (2022, 2023 and 2024)

curling, severe stunting, and pronounced curling, while the TYLCV-Mild isolate was associated with mottling, yellow patterns, stunting, and curling in the infected plants. These symptoms manifested on different days post-inoculation (dpi), specifically at 16 dpi for TYLCV-Mild, 20 dpi for a combination of symptoms and 22 dpi for TYLCV-IL (Fig. 7 and 8).

Serological testing

The serological results for the TYLCV isolates were as follows: TYLCV-Mild had an OD value of 0.331 and a "++" reaction, indicating a moderately positive reaction. This isolate was associated with mottling and yellow-pattern symptoms. TYLCV-IL had an OD value of 0.355 and a "+++" reaction, suggesting a strong positive reaction, and was associated with severe stunting symptoms. The positive control has an OD value of 0.523 and a "+++" reaction, indicating a strong positive reaction. In contrast, the negative control had an OD value of 0.015 and a "-" reaction, suggesting a negative reaction (Table 3).

Determination of pathogenicity

The study of the disease severity of TYLCV has revealed significant insights into the virus's infection and associated symptoms in inoculated tomato plants. A notable observation was the high infectivity of the TYLCV isolate, with 90% of the inoculated plants exhibiting symptoms. The degree of symptoms index highlighted that the predominant symptoms included leaf crinkling and rigidity (LCR), followed by leaf curl and narrowness (LCN).

Table 3: Different isolates of TYLCV detected by DAS-ELISA were collected based on symptoms

OD value at 405 nm	ELISA test reaction	Symptoms	TYLCV isolates
0.331	++	Mottling and yellow pattern	TYLCV-Mild
0.355	+++	Severe stunting	TYLCV-IL
0.523	+++	-	Positive Control
0.015	-	-	Negative Control

The table shows the reaction of different TYLCV isolates to the antigen measured by the OD value at 405 nm using the DAS-ELISA test

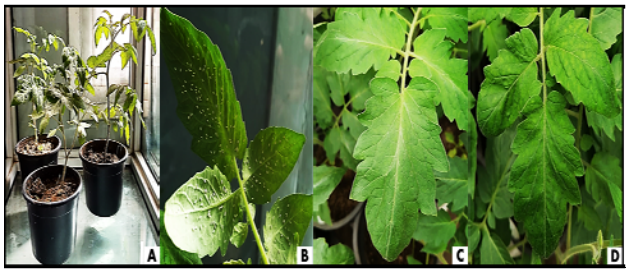

Fig. 7: Tomato plants inoculated with Tomato yellow leaf curl virus (TYLCV) isolates using viruliferous whiteflies collected from the field of infected tomatoes. (**A**)Tomato plants in the cage (**B**) Whitefly populations (viruliferous) in the cage (**C**) Tomato plants before inoculation (**D**) Tomato plants after inoculation by TYLCV by whiteflies

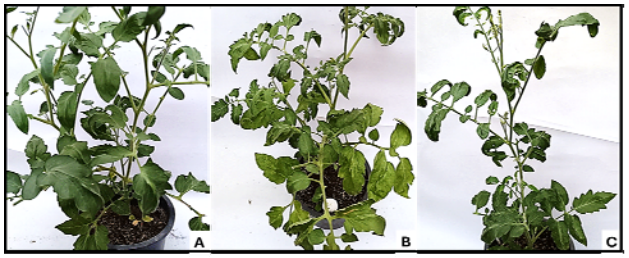

Fig. 8: Viruliferous whiteflies used to infect tomato plants. (**A**) Healthy plant, (**B**) Tomato plants infected by TYLCV-Mild isolate and (**C**) Tomato plants infected by TYLCV-Israel isolate

The virus concentration was relatively low, as measured by a DAS ELISA reading of 0.331. This suggests that TYLCV is particularly effective at inducing symptoms even in low concentrations. The negative control reading of 0.015 provided a baseline, confirming that the observed virus concentration was indeed due to TYLCV rather than background interference. The negative control reading of 0.015 provided a baseline, confirming that the observed virus concentration was indeed due to TYLCV rather than background interference.

Several factors could explain these observations. Strain variation within the TYLCV isolate might account for differences in symptom severity and viral concentration, as different strains can exhibit diverse levels of virulence and replication efficiency. Additionally, plant factors, such as the susceptibility of the specific tomato variety used in the experiment may influence both symptom development and virus concentration. Environmental conditions, particularly temperature and humidity are also critical, as they can significantly affect virus replication and symptom expression. Finally, the complex interactions between the virus and its host plant play a crucial role in determining the outcomes of the infection (Fig. 9).

Host range and symptomology

Certain plant families exhibit distinct patterns of viral symptoms. Plants in the *Cucurbitaceae* family, such as cucumber and squash, are susceptible to viral infections that cause yellowing and leaf curling, with consistently positive PCR results. Common bean plants in the *Fabaceae* family also show leaf curling and yellowing symptoms, and PCR tests are positive for viral infection. In contrast, okra plants in the *Malvaceae* family do not exhibit any viral symptoms, and PCR results are negative, suggesting resistance to the tested virus. Tomato and pepper plants in the *Solanaceae* family are susceptible to viral infections that cause various symptoms, including leaf curling, yellowing, and stunting, with positive PCR results. Chenopodium quinoa plants in the *Chenopodiaceae* family show upward curling symptoms and positive PCR results for viral infection. Plants from the *Moraceae, Apiaceae,* and *Lamiaceae* families do not exhibit any viral symptoms, and PCR results are negative, indicating resistance to the tested virus (Table 4).

Molecular Characterization for TYLCV

PCR analysis for detecting TYLCV and beta-satellite genomes: For TYLCV detection in the collected samples from different governorates, two sets of degenerate primers, universal for geminivirus, was used. TY-1 and TY-2 were able to amplify a fragment of ~580 bp of the core CP gene with infected samples, while AV/AC amplified a fragment of ~550 bp (Fig. 10 and 11).

Differentiation between TYLCV-IL and TYLC-mild: Two methods were used to differentiate between the two groups of TYLCV in the collected samples, detecting the beta-satellite genome and designing primers at the C4 core region that could distinguish the genome of the two groups.

Detection of beta-satellite on tomato plants: For detecting beta-satellite in the extracted DNA from collected samples, beta-satellite universal primer (Beta01/Beta02) was used ~1300 bp which represents its full genome (Fig. 12). Results show that not all infected tomato plants harbor the beta-satellite. Infected tomatoes with TYLCV-IL and mixed infections were positive for beta-satellite, while mild TYLCV was negative for beta-satellite.

Table 4: Different hosts were tested for the infection with TYLCV

Family	Different Hosts	Symptoms	PCR result	ELISA Result
Cucurbitacea	Cucumber (*Cucumis sativus*)	Yellowing	+ ve	++ (0.355)
	Squash (*Cucurbita pepo* L.)	Leaf curling and yellowing	+ ve	+ (0.314)
Fabaceae	Common bean (*Phaseolus vulgaris*)	Leaf curling and yellowing	+ ve	+++ (0.372)
Malvaceae	Okra (*Abelmoschus esculentus)*	NO	- ve	- (0.125)
Solanaceae	Tomato (*Solanum lycopersicum*)	Leaf curling, yellowing and stunting	+ ve	++ (0.367)
	Pepper (*Capsicum annuum*)	Upward curling	+ ve	++ (0.325)
	Eggplant (*Solanum melongena*)	Leaf curling, and mottling	+ ve	+ (0.315)
Chenopodiaceae	Chenopodium (*Chenopodium quinoa*)	Upward curling	+ ve	++ (0.351)
Moraceae	White mulberry (*Morus alba*)	NO	- ve	- (0.115)
Apiaceae	Celery *(Apium graveolens)*	NO	- ve	- (0.125)
Lamiaceae	Basil *(Ocimum basilicum)*	NO	- ve	- (0.105)

(+) = infected (-) = not- infected (NO) = no symptoms

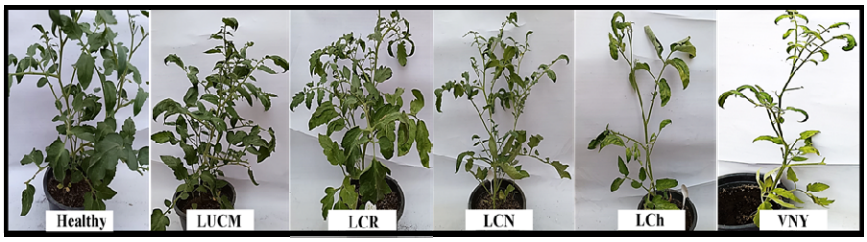

Fig. 9: Tomato leaves inoculated with TYLCV showing degree of Symptoms index (2), 2 = leaf crinkling and rigidity (LCR); 4 = 50% of leaf curl & narrow (LCN), 6 = 50% of leaf chlorosis (LCh), 8 = 100% leaf upward cupping and malformation (LUCM) and 10 = venial necrosis, & yellowing (VNY)

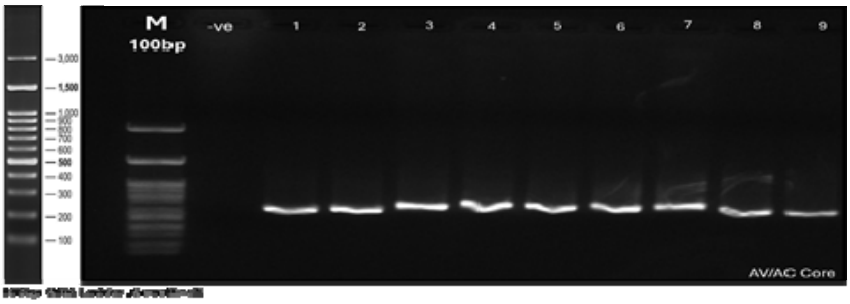

Fig. 10: PCR amplification using AV/AC core degenerate primer giving an expected size of ~550 bp in the collected samples from different governorates as follows: 1. Giza, 2. Menoufia, 3. Alexandria, 4. Ismailia, 5. Fayoum, 6. Sharkia, 7. El-Beheira, 8. Benisuef and 9. Qalyoubia; -ve control and M: 100bp DNA ladder

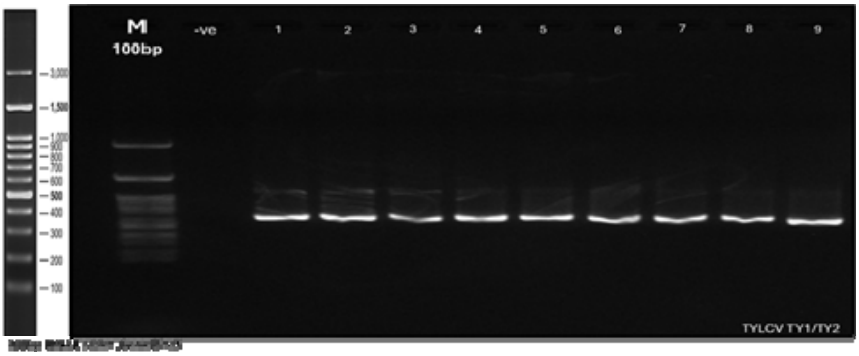

Fig. 11: PCR amplification using TY1/TY2 primer for detecting TYLCV-CP giving an expected size of ~580 bp in the same governorates; M: 100bp DNA ladder

Multiplex PCR: Multiplex PCR (mPCR) reactions were done, for isolate detection and amplicons of the expected sizes for TYLCV-Mild (592 bp) and TYLCV-IL using Ty-78(R) with either IL-2629(F) or Mld-2277(F) primers to amplify a fragment of 230 bp as presented in Table 1 and shown in detail in Fig. 13.

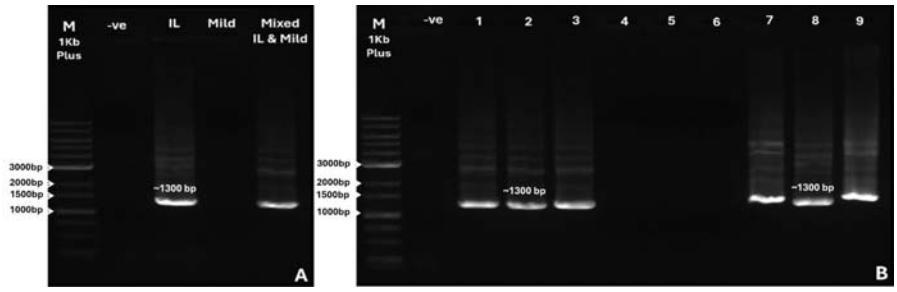

Fig. 12: Detection of β DNAs complete genome ~1300 bp: (**A**) Lane 1: M1 kb plus DNA ladder, Lane 2: - ve control; Lane 3: beta-satellite in TYLCV-IL sample, Lane 4: beta-satellite in TYLCV-Mild sample, Lane 5: Beta-satellite in TYLCV-IL and Mild mixed infection sample (**B**) - ve control; lane1, 2 and 3: Beta-satellite in TYLCV-IL samples which obtained from Sharkia, El-Beheira, Qalyoubia Governorates respectively; lane 4,5 and 6: Beta-satellite in TYLCV-Mild obtained from Menoufia, Alexandria, Benisuef; lanes labeled with numbers 7, 8 and 9 are Giza, Ismailia, Fayoum Beta-satellite in TYLCV-IL and Mild mixed infection samples and M: 1 kb plus DNA ladder

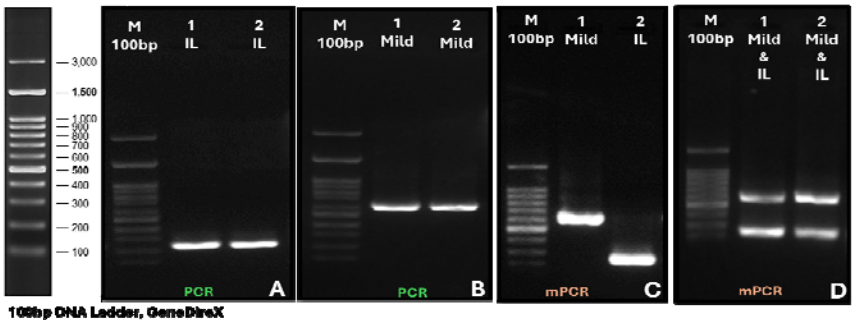

Fig. 13: PCR amplification results for PCR represented in (**A**): ~230 bp band for the TYLCV-IL; (**B**) ~592 bp band for the TYLCV-Mild using the primers individually; (**C**) mPCR for IL and Mild TYLCV isolates ;(**D**) mPCR for mixed samples with IL and Mild TYLCV; M: 100 bp DNA ladder

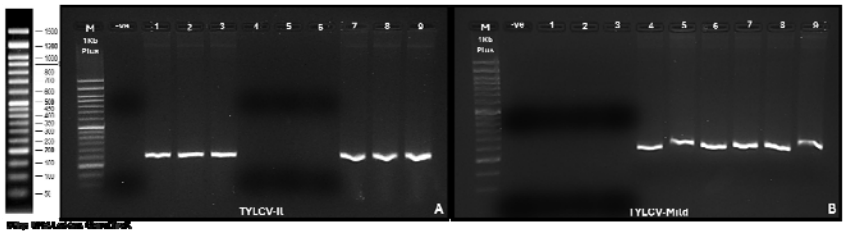

Fig. 14: Detection of C1C4 region ~240 bp (**A**- TYLCV-IL) and (**B**- TYLCV-Mild): - ve control; 1, 2 and 3 lanes: C1C4 region in TYLCV-IL samples obtained from Sharkia, El-Beheira, Qalyoubia governorates respectively, while lanes 4, 5 and 6: C1C4 region in TYLCV-Mild obtained from Menoufia, Alexandria, Benisuef, for 7, 8 and 9 lanes: Giza, Ismailia, Fayoum: C1C4 in TYLCV-IL and Mild mixed infection, and M: 1 kb plus DNA ladder

Differentiation of TYLCV isolates based on C1 and C4 regions: We designed additional primers at the C1C4 region to distinguish the genome of the two isolates: TC1C4 (F) with either TC1C4 (IL-R1) or TC1C4 (Mld-R2). A fragment of ~240 bp specific for the IL genome was presented in infected plants with TYLCV-IL only and a fragment with the same size was obtained with TYLCV-Mild when using its specific primers (Fig. 14).

Viroinformatics analysis

Genetic diversity between nine governorates for TYLCV isolates: A phylogenetic tree was constructed using DNAMAN version 7.0.2. software program by aligning the nucleotide sequences for the 9 governorates showed a degree of identity ~98% for Giza and Alexandria and ~97% for El Beheira, Fayoum, Benisuef and Ismailia governorates, While Menoufia and Qalyoubia governorates showed ~97%. Sharkia governorate showed a ~96% degree of similarity (Fig. 15).

Multiple sequence alignment by Multalin software: Multiple sequence alignment (MSA) of several nucleic acids and amino acids sequences showed a high degree of similarity among the sequences, which suggests that these isolates are likely to share a common ancestor and are closely related (Fig. 16 and 17). This alignment was utilized

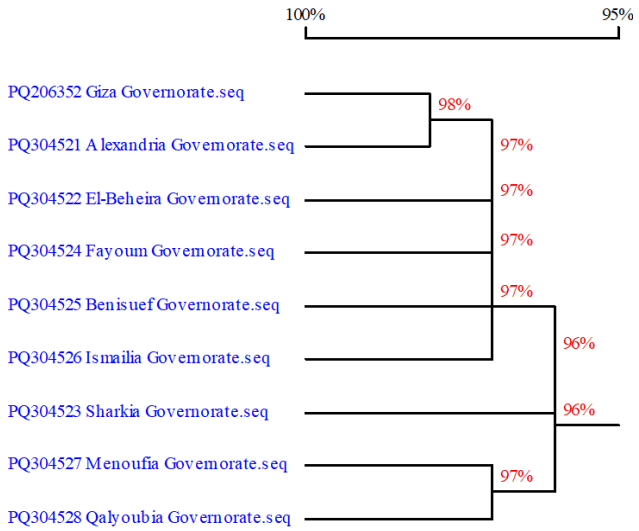

Fig. 15: The homology tree between all the nine governorates' isolates

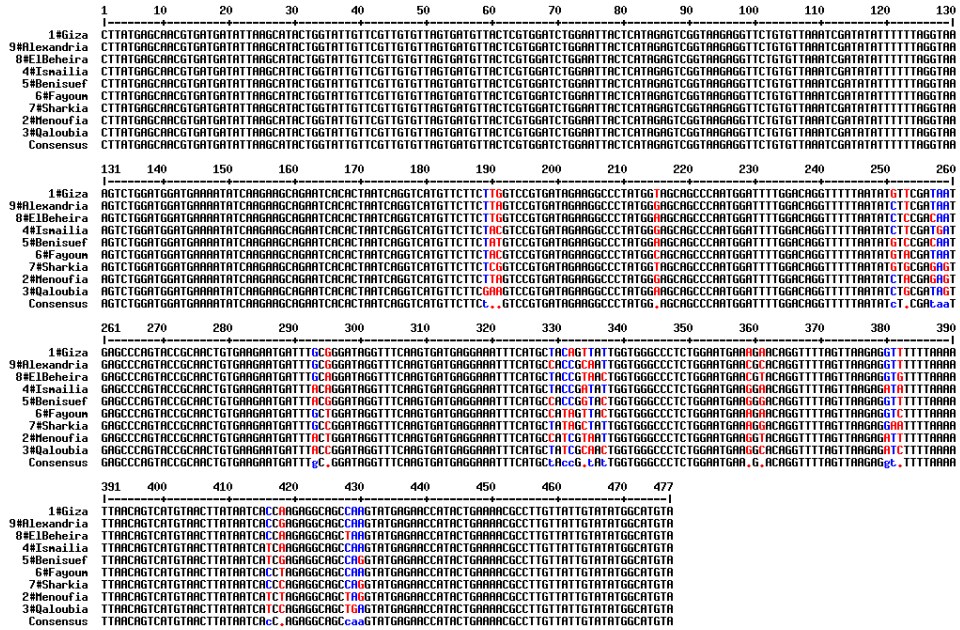

Fig. 16: Studying the different N.A sequence of isolates by multiple sequence alignment with hierarchical clustering using Multalin version 5.4

to construct a phylogenetic tree, which would visually represent the evolutionary relationships among the isolates. The variations observed may have functional consequences, such as affecting protein structure or function. For instance, a single nucleotide polymorphism (SNPs) in a coding region could lead to the incorporation of a different amino acid into the protein, potentially altering its properties. If these isolates are from different geographic locations or periods, the alignment could provide insights into the spread of the organism and potential transmission events.

Genetic diversity between the nine governorates and other TYLCV sequences in the GenBank database: A phylogenetic analysis was conducted to compare our Egyptian Tomato yellow leaf curl virus (TYLCV) isolates deposited in the NCBI GenBank under accession numbers PQ304521 to PQ304528 and PQ206352 with other TYLCV sequences in the GenBank database. The results indicated that the Qalyoubia isolate was closely related (99.0%) to the isolates (AB116631 and AY594174) from Japan and Egypt, respectively, placing it within the TYLCV-IL group. Additionally, the Sharkia isolate demonstrated a sequence similarity of 92.8% and 92.7% with the Tosa isolate from Japan (AB192965) and the Bundaberg isolate from Australia (GU178820), both of which belong to the Israeli isolates. The Beheira isolate also showed a high similarity of 99% within this group (Fig. 18).

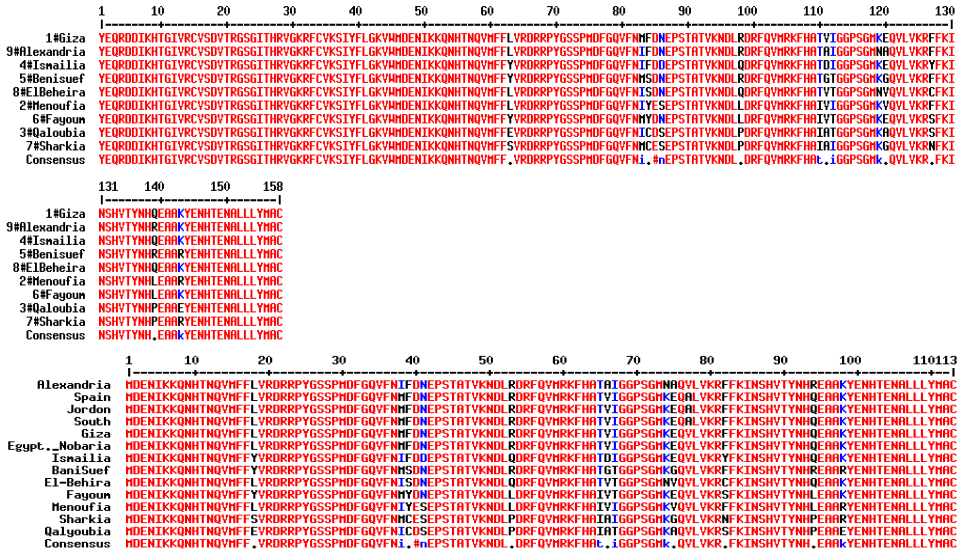

Fig. 17: Studying the different a.a sequences of isolates by multiple sequence alignment with hierarchical clustering using Multalin version 5.4

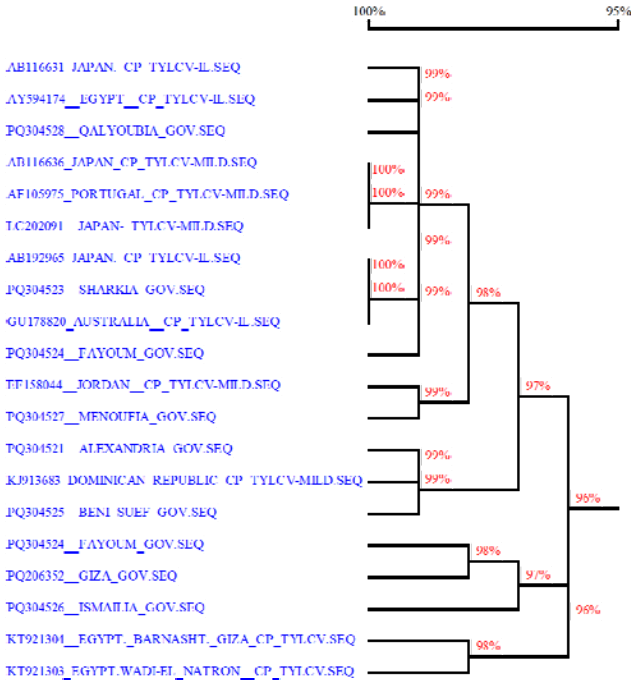

Fig. 18: Phylogenetic analysis of core coat protein gene for infected tomato collected from the 9 governorates and its counterpart in NCBI

Furthermore, the Menoufia isolate exhibited a 99% similarity with a mild Jordanian isolate. Notably, the Alexandria and Benisuef isolates showed a 99% similarity with isolates from the Dominican Republic, both of which belong to the mild isolates. In comparison, the Fayoum and Giza isolates were closely related at 99%, and the Ismailia isolates had a similarity of 97%.

These findings underscore genetic diversity of TYLCV in Egypt and suggest that local environmental factors may influence the evolution of virus. A high degree of similarity between Egyptian isolates and those from other countries indicates ongoing viral movement and potential recombination events, which could lead to the emergence of new variants. This emphasizes the importance of continuous monitoring and research to understand the dynamics of TYLCV and its impact on tomato production in Egypt and beyond.

Discussion

Tomato yellow leaf curl Virus (TYLCV) has become a significant concern in various regions worldwide, particularly in Egypt, where it has been linked to severe economic losses in tomato production, sometimes exceeding 100% (García-Andrés *et al.* 2007; Khan *et al.* 2008; Fazeli *et al.* 2009). Despite Egypt's extensive tomato farming industry, the country continues to face challenges from foreign diseases. Visual inspections during investigations indicated that TYLCV infection prevalence reached as high as 100% in certain areas, as reported by Rabie *et al.* (2017). Infected tomato plants, particularly those affected by whiteflies, displayed severe symptoms, including leaf curling, crinkling with marginal yellowing, and stunted growth. All samples tested positive for TYLCV, confirming their susceptibility to the virus with varying degrees of severity. Notably, the incidence of TYLCV in the governorate was reported at 90.9% (Table 2). This high incidence was attributed to the substantial population of whiteflies that efficiently transmit TYLCV, leading to increased infections in open fields. Through whitefly transmission, TYLCV was isolated and propagated on healthy tomato plants from selected ELISA-positive samples. After 25 days post-infection, typical symptoms such as leaf curling and stunted growth were observed, aligning with findings from other studies (Lestari *et al.* 2023; Idrees *et al.* 2024).

High ELISA readings indicated significant viral concentrations in naturally infected plants. The infected plants were classified into two isolates: TYLCV-Mild, associated with mottling and yellow patterns, and TYLCV-Israel, linked to severe stunting and curling. The recorded TYLCV antigen concentrations reflect the severity of the infection and underscore the importance of monitoring viral loads for effective management (Polston and Sherwood 2003).

Host range and symptomology studies revealed that TYLCV-infected species from the *Solanaceae* family and certain species from *Cucurbitaceae*, *Fabaceae*, and *Chenopodiaceae*. However, no symptoms were observed in plants from the *Malvaceae*, *Moraceae*, *Apiaceae* and *Lamiaceae* families. These findings were consistent with those reported by Hai-Xin *et al.* (2021), Lu *et al.* (2023) and Sandra and Mandal (2024) indicating that the first symptoms of TYLCV on tomato plants appear 25 days post-inoculation (dpi) and can fully develop within six weeks. PCR amplification of TYLCV DNA prepared from infected tomato plants was performed using the TYLCV TY1/TY2 primers targeting the coat protein (CP) gene, as detailed by Accotto *et al.* (2000) and Rabie *et al.* (2017).

The successful amplification of the complete beta-satellite genome (~1300 bp) using the Beta01/Beta02 primers in various TYLCV samples indicates the widespread association of beta-satellites with TYLCV infections in Egypt. The presence of beta-satellites can significantly impact the severity of TYLCV-IL-induced symptoms. Voorburg *et al.* (2020) mentioned that TYLCV accumulation increased in the mixed infection of beta-satellites with TYLCV-Il, while those of TYLCV-Mild/beta satellite infection decreased this accumulation in plants, which agreed with our findings where there is a noticed difference between the mild and IL tested samples as well as the severity of symptoms where the IL infected sample was showing very severe curling and rigidity in symptoms, which reveals that beta-satellites, play a pivotal role in TYLCV severity. Therefore, beta-satellites are considered pathogenicity determinants, as reported by Saeed (2010). However, when analyzing samples from tomato Egyptian fields using universal beta-satellite primers. The findings indicated that samples collected from Sharkia, El-Beheira, and Qalyoubia governorates when tested were positive for TYLCV-IL detection primers and exhibited reactions similar to the Israel isolate. In contrast, samples from Menoufia, Alexandria, and Benisuef showed negative results with beta-satellite universal primers but later tested positive when analyzed with TYLCV-Mild detection primers. Additionally, samples from Giza, Ismailia and Fayoum also demonstrated positive reactions. These results suggest a distinct differentiation among the viral isolates present in these regions. TYLCV-encoded C4 is embedded within a larger ORF, C1, in a different reading frame. C4 is a relatively conserved protein that may display diverse biological functions in monopartite and bipartite geminiviruses. In monopartite begomoviruses, tomato leaf curl virus (TYLCV) C4 expression showed virus-like symptoms in transgenic tobacco and tomato plants. Luna and Lazano-Duran (2020) and Padmanabhan *et al.* (2022) have mentioned that there are likely multi-functional roles for TYLCV C4 that would need to be further explored, consequently we have designed a specialized primer set to differentiate between Tomato yellow leaf curl virus (TYLCV) isolates, consisting of the forward primer TC1C4 (F) to be used with TC1C4 (IL-R1) or with TC1C4 (MLD-R2). This design was based on the observed differences in the 3' end of the coat protein region among various isolates. The goal was to create a primer that could effectively distinguish between Israeli and mild strains of TYLCV. When using the forward primer with the reverse primer R1, we found that it specifically amplified isolates from Qalyoubia, which showed a closer resemblance to the Israeli sample. On a global scale, this combination revealed similarities to samples from Japan and Portugal. Conversely, using the forward primer with reverse primer R2 targeted isolates leaning towards the mild strain, successfully detecting samples from Alexandria, Menoufia and Benisuef at the local level. Internationally, this combination indicated a connection to samples from Dominican Republic and Jordan. Importantly, some samples exhibited dual infections. When PCR was performed with both primer sets, the results indicated whether the samples were infected with one or both isolates. This dual detection is crucial for understanding the epidemiology of TYLCV, as mixed

infections can complicate management strategies and influence the severity of symptoms in tomato crops.

A phylogenetic analysis was conducted to investigate the genetic diversity of Tomato yellow leaf curl virus (TYLCV) isolates collected from nine governorates in Egypt. The results revealed a high degree of genetic diversity among the Egyptian isolates, suggesting a long-term presence of the virus within the country. Geographic clustering was observed among some isolates, indicating the influence of local factors on viral evolution. Furthermore, the close phylogenetic relationship between Egyptian and foreign isolates, such as an isolate from Israel and a mild isolate known for causing less severe symptoms, highlights the global nature of TYLCV dissemination. The observed genetic diversity among the TYLCV isolates in Egypt emphasizes the dynamic nature of the virus population and its adaptation to different environmental conditions (Shirazi *et al.* 2014; Zaid *et al.* 2021). In conclusion, the phylogenetic analysis provides insights into the genetic diversity and evolutionary relationships of TYLCV isolates in Egypt. The results highlight the importance of continuous monitoring and characterization of viral populations to understand their epidemiology and develop effective management strategies.

The ability to differentiate between these isolates is vital for effectively monitoring and managing TYLCV, particularly in regions like Egypt where the virus poses a significant threat to tomato production. Understanding the genetic diversity and distribution of TYLCV isolates can help in developing resistant varieties and implementing targeted control measures.

Conclusion

The findings highlight the critical role of TYLCV in tomato production, emphasizing the importance of understanding its infectivity and symptomatology for effective disease management. The interaction of a bipartite begomovirus with a beta-satellite was more complex than just trans-replication by the virus. The data support the need for ongoing surveillance and the exploration of resistant tomato varieties to combat the challenges posed by TYLCV and its co-infections. Since such infections can occur with some regularity in the field, due to co-infection of a bipartite begomoviral and a beta-satellite-associated monopartite begomoviral, they can cause significant additional losses to crop.

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Not Applicable

Author Contributions

NAA, AAA and AKE designed the study; RGE, AKE and NAA performed field work, lab experiments and collected data; RGE, AAA, WSE and NAA analyzed data and drafted manuscript. All the authors read and approved the final manuscript.

Conflicts of Interest

All authors declare no conflicts of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable in this paper

References

Abdel-Salam AM, NA Abdallah, DZR Soliman, AS Rezk (2005). The incidence of squash leaf curl Begomovirus (SqLCV) in Egypt. *Arab J Biotechnol* 9:375–388

Accotto GP, J Navas-Castillo, S Sanchez-Campos, E Noris, D Louro, E Moriones (2000). Natural recombination between Tomato yellow leaf curl virus-Is and Tomato leaf curl virus. *J Gen Virol* 81:2797–2801

Antignus Y, S Cohen (1994). Complete nucleotide sequence of an infectious clone of a mild isolate of Tomato yellow leaf curl virus (TYLCV). *Phytopathology* 84:707–712

Avedi EK (2022). *Distribution and genetic diversity of Tomato yellow leaf curl virus, associated whitefly vectors and the response of selected tomato varieties to the virus*, p:184. University of Nairobi, Faculty of Agriculture & Veterinary Medicine, Nairobi, Kenya

Belabess Z, S Dallot, S El-Montaser, M Granier, M Majde, A Tahiri, A Blenzar, C Urbino, M Peterschmitt (2015). Monitoring the dynamics of emergence of a noncanonical recombinant of Tomato yellow leaf curl virus and displacement of its parental viruses in tomato. *Virology* 486:291–306

Butterbach P, MG Verlaan, A Dullemans, D Lohuis, RG Visser, Y Bai, R Kormelink (2014). Tomato yellow leaf curl virus resistance by Ty-1 involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. *Proc Natl Acad Sci USA* 111:12942–12947

Clark MF, AN Adams (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J Gen Virol* 34:475–483

El-Dougdoug KA, IA Hammad, HS Abd (2010). Detection and identification of Tomato yellow leaf curl virus-EG using molecular technique. *Egypt J Microbiol* 45:95–111

El-Sappah AH, El-Sappah AH, Qi Shiming, AS Salma, H Qiulan, MS Alaa, Mohammed AS Abourehab, W Lingyun, C Guo-Ting, L Jingyi, I Muhammad, N Zarqa, RM Reyazul, Z Xin, Y Kuan, A Manzar, L Jia (2022). Natural resistance of tomato plants to Tomato yellow leaf curl virus. *Front Plant Sci* 13:1081549

Faisal M, EM Abdel-Salam, AA Alatar, Q Saquib, HA Alwathnani, T Canto (2019). Genetic transformation and siRNA-mediated gene silencing for aphid resistance in tomato. *Agronomy* 9:893

FAOSTAT (2020). *Statistics Division*, FAO, Rome, Italy

Fauquet C, J Stanley (2005). Geminivirus classification and nomenclature: Progress and problems. *Ann Appl Biol* 142:165–189

Fazeli R, J Heydarnejad, H Massumi, M Shaabanian, A Varsani (2009). Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. *Virus Genes* 38:311–319

Fiallo-Olivé E, LL Pan, SS Liu, J Navas-Castillo (2020). Transmission of begomoviruses and other whitefly-borne viruses: Dependence on the vector species. *Phytopathology* 110:10–17

García-Andrés S, GP Accotto, J Navas-Castillo, E Moriones (2007). Founder effect, plant host, and recombination shape the emergent population of begomoviruses that cause the Tomato yellow leaf curl disease in the Mediterranean basin. *Virology* 359:302–312

Gelbart D, L Chen, T Alon, S Dobrinin, I Levin, M Lapidot (2020). The recent association of a DNA beta satellite with Tomato yellow leaf curl virus in Israel-A new threat to tomato production. *Crop Prot* 128:104995

Ghosh S, M Ghanim (2021). Factors determining transmission of persistent viruses by *Bemisia tabaci* and emergence of new virus–vector relationships. *Viruses* 13:1808

Hai-Xin B, W Li, Y Yang, Y Wang, Q Zhang, T Cheung *et al.* (2021). Posttraumatic stress symptoms and attitude toward crisis mental health services among clinically stable patients with COVID-19 in China. *Psychol Med* 51:1052–1053

Idrees MA, A Abbas, B Saddam, MH Bashir, H Naveed, AK Khan, MZN Dara (2024). A comprehensive review: Persistence, circulative transmission of begomovirus by whitefly vectors. *Intl J Trop Ins Sci* 44:405–417

Khan AJ, AM Idris, NA Al-Saady, MS Al-Mahruki, AM Al-Subhi, JK Brown (2008). A divergent isolate of Tomato yellow leaf curl virus from Oman with an associated DNA beta satellite: An evolutionary link between Asian and the Middle Eastern virus-satellite complexes. *Virus Genes* 36:169–176

Kim N, J Kim, B Bang, I Kim, HH Lee, J Park, YS Seo (2016). Comparative analyses of Tomato yellow leaf curl virus C4 protein-interacting host proteins in healthy and infected tomato tissues. *Plant Pathol J* 32:377–387

Kumar M, SR Kavalappara, T McAvoy, S Hutton, AM Simmons, S Bag (2023). Association of Tomato chlorosis virus complicates the management of Tomato yellow leaf curl virus in cultivated tomato (*Solanum lycopersicum*) in the Southern United States. *Horticulturae* 9:948

Legarrea S, A Barman, S Diffie, R Srinivasan (2020). Virus accumulation and whitefly performance modulate the role of alternate host species as inoculum sources of Tomato yellow leaf curl virus. *Plant Dis* 104:2958–2966

Lestari SM, SH Hidayat, P Hidayat, EJ Kil, S Lee, SM Kim, KY Lee (2023). Identification of begomoviruses associated with the insect vector *Bemisia tabaci* and various host plants on Java Island, Indonesia. *Arch Insect Biochem Physiol* 112:21984

Letunic I, P Bork (2021). Interactive Tree of Life (iTOL) V5: An online tool for phylogenetic tree display and annotation. *Nucl Acids Res* 49:293–296

Lozano G, HP Trenado, E Fiallo-Olivé, D Chirinos, F Geraud-Pouey, RW Briddon, J Navas-Castillo (2016). Characterization of non-coding DNA satellites associated with sweepoviruses (Genus *Begomovirus*, *Geminiviridae*) – definition of a distinct class of begomovirus-associated satellites. *Front Microbiol* 7:162

Lu DYH, JY Liao, A Fajar, JB Chen, Y Wei, ZH Zhang, Z Zhang, LM Zheng, XQ Tan, XG Zhou, XB Shi, Y Liu, DY Zhang (2023). Co-infection of TYLCV and ToCV increases cathepsin B and promotes ToCV transmission by *Bemisia tabaci* MED. *Front Microbiol* 14:1107038

Luna AP, R Lazano-Duran (2020). Geminivirus-encoded proteins: Not all positional homologs are made equal. *Front Microbiol* 11:878

Marchant WG, H Mugerwa, S Gautam, H Al-Aqeel, JE Polston, G Rennberger, H Smith, B Turecheck, S Adkins, JK Brown, R Srinivasan (2023). Phylogenomic and population genetics analyses of extant Tomato yellow leaf curl virus strains on a global scale. *Front Virol* 3:1221156

Padmanabhan C, Y Zheng, M Shamimuzzaman, JR Wilson, A Gilliard, Z Fei, KS Ling (2022). The Tomato yellow leaf curl virus C4 protein alters the expression of plant developmental genes correlating to leaf upward cupping phenotype in tomato. *PLoS One* 17:e0257936

Pakkianathan BC, S Kontsedalov, G Lebedev, A Mahadav, M Zeidan, H Czosnek, M Ghanim (2015). Replication of Tomato yellow leaf curl virus in its whitefly vector, *Bemisia tabaci*. *J. Virol* 89:9791–9803

Papayiannis LC, IS Harkoua, YM Markou, CN Demetriou, NI Katis (2011). Rapid discrimination of tomato chlorosis virus, tomato infectious chlorosis virus and co-amplification of plant internal control using real-time RT-PCR. *J Virol Meth* 176:53–59

Polston JE, T Sherwood (2003). Pymetrozine interferes with transmission of Tomato yellow leaf curl virus by the whitefly *Bemisia tabaci*. *Phytoparasitica* 31:490–498

Rabie M, C Ratti, EA Aleem, F Fattouh (2017). Detection and molecular characterization of Tomato yellow leaf curl virus naturally infecting *Lycopersicon esculentum* in Egypt. *Acta Virol* 61:252–263

Rahman HAE, MA Nasr-Eldin, SA Abo-Elmaaty, MA Abdelwahed, M ElHefnawi, AM Elflike, ER Soliman (2024). Partial genome detection, characterization of TYLCV (MZ546492) infecting tomato plants, and siRNA sequences detection for alternative control strategy. *Egypt J Bot* 64:21–23

Raupach GS, L Liu, JF Murphy, T Sadek, WK Joseph (1996). Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). *Plant Dis* 80:891–894

Saeed M (2010). Tomato leaf curl New Delhi virus DNA, a component, and Cotton leaf curl Multan beta satellite can cause mild transient symptoms in cotton. *Acta Virol* 54:317–318

Sandra N, B Mandal (2024). Emerging evidence of seed transmission of begomoviruses: Implications in global circulation and disease outbreak. *Front Plant Sci* 15:1376284

Shirazi M, J Mozafari, F Rakhshandehroo, M Shams-Bakhsh (2014). Genetic diversity, host range, and distribution of Tomato yellow leaf curl virus in Iran. *Acta Virol* 58:128–136

Voorburg CM, Z Yan, M Bergua-Vidal, AMA Wolters, Y Bai, R Kormelink (2020). *Ty-1*, a universal resistance gene against geminiviruses that is compromised by co-replication of a betasatellite. *Mol Plant Pathol* 21:160–172

Wu W, HW Shan, JM Li, CX Zhang, JP Chen, Q Mao (2022). Roles of bacterial symbionts in transmission of plant virus by hemipteran vectors. *Front Microbiol* 13:805352

Yan Z, AMA Wolters, J Navas-Castillo, Y Bai (2021). The global dimension of tomato yellow leaf curl disease: Current status and breeding perspectives. *Microorganisms* 9:740

Zaid N, HA Younis, S Deraz (2021). Molecular studies on Tomato Yellow Leaf Curl Virus in infected tomato and pepper plants. *J Adv Agric Res* 26:213–224