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Partial Genome Detection, Characterization of TYLCV (MZ546492) Infecting Tomato Plants and siRNA Sequences Detection for Alternative Control Strategy

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TOMATO yellow leaf curl disease (TYLCD) is a significant limitation factor in tomato crops and ranks among the top 10 plant viruses affecting the production of many crops, particularly tomatoes, leading to considerable economic losses without an efficient control strategy till now. In this study, PCR technique was used to amplify partial sequences (670 bp) of tomato yellow leaf curl virus (TYLCV) genome, specifically spanning the trans-activator protein (C2), replication enhancer protein (C3) genes, as well as partial parts of replication (Rep) and coat protein (CP) genes. These sequences were obtained from naturally infected tomato plants collected from different governorates in Egypt. The DNA sequence analyses of the current Egyptian isolate was annotated and deposited in the GenBank with an accession ID MZ546492, revealing high nucleotide sequence identities (99.3 %) to TYLCV isolates in the GenBank. The transmission and host range assays confirmed that the whitefly is the sole transmitter, and tomatoes, zucchini, cucumber, pepper, jimsonweed, and common bean as a host for TYLCV. To explore alternative control strategies, an *in-silico* approach was employed to generate the possible siRNA-producing sequences that could be used to knock down TYLCV in infected plants. The current findings support the development of a rapid, reliable, and robust molecular detection and identification tool of TYLCV in tomato germplasm to ensure the safe and sustainable production of TYLCV-free tomatoes. Additionally, *in silico* analysis indicated the possibility of using siRNA to control TYLCV.

Keywords: Host range, Phylogeny, Sequencing, siRNA, Tomato yellow leaf curl virus, Virus transmission.

Introduction

Solanum lycopersicum, is a major agricultural crop grown all over the world, with a global production of over 189 million tons in 2021. According to the Food and Agriculture Organization's official website, Egypt ranks the sixth in tomato production, with over 6.2 million tons produced in 2021, following China, India, Turkey, the United States of America, and Italy (FAOSTAT, 2023). Despite its widespread growth, tomatoes are vulnerable to various pathogens such as bacteria, fungi, and viruses. Many viruses are spreading as a result of international agricultural commerce.

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Tomato yellow leaf curl disease (TYLCD) is one of the most damaging plant diseases, destroying tomato crops worldwide. It has spread to many countries around the world (Zhou et al., 2022). TYLCV, belonging to the genus Begomovirus in the family Geminiviridae, poses a global threat to agriculture. TYLCV originated in the Mediterranean region of the Middle East and has spread since to many regions of the world (Ning et al., 2015). Geminiviruses cause significant yield losses in tomato production around the world, including Egypt (Bennett & Agbandje-McKenna, 2020). TYLCV ranks as the third most damaging virus affecting tomato production (Ong et al., 2020). It causes up to 100% quantitative and qualitative yield losses in some tomato cultivars (Loriato et al., 2020)**.** The global spread of TYLCV in tomatoes is highly dependent on virus transmission in a persistent and circulative manner by its sole vector, the whitefly, *Bemisia tabaci* (Tabein, 2021). The TYLCV genome is 2.7kb of single-stranded circular DNA that encodes six open reading frames (ORFs), organized bidirectionally into transcriptional units separated by an Intergenic Region (IR) (Singh et al., 2020). TYLCV symptoms include reduced leaf size, upward leaf curling, yellowing of young leaves, stunted growth, and flower abortion, inward rolling of tomato leaf margins and interveinal yellowing of leaflets (Arasimowicz-Jelonek et al., 2020). TYLCV has a very diverse host range and has been detected in 49 species belonging to 16 families (Prasad et al., 2020).

Several approaches to control TYLCV have been developed including molecular and biological approaches, such as application of physical barriers and chemical insecticides for limiting vector populations. Alternatively, several genetic engineering strategies have been investigated (Tabein, 2021).

Early detection of TYLCV is critical for effective management due to its severity. Traditional TYLCV's detection methods involve sample preparation to identify viral DNA or proteins. Serological techniques based on immunoblotting, conventional PCR, restriction fragment length polymorphisms, loop-mediated isothermal amplification, real-time PCR, and rolling circle amplification have all been developed and used to detect TYLCV-infected tomatoes (Zhou et al., 2022). Among these methods, nucleic acid-based PCR is the most commonly used for plant pathogen detection due to its speed, high specificity, and sensitivity (Zhou et al., 2022).

Disease management for viruses is complicated by the widespread nature and the diverse host range; however, because "prevention is better than cure," agricultural success is dependent on good agricultural practices before, during, and after the growing season. To control any plant disease, including TYLCV, proper integration of conventional methods, such as quarantine regulation and integrated pest management (IPM), as well as nonconventional methods like transgenic approaches, gene editing, and antisense RNA interference (RNAi) technology are required (Prasad et al., 2020). RNAi technology, which involves the expression of viral genomic sequences in a hairpin configuration inside the plant prior to infection, has produced promising outcome. Transgenic tomato plants expressing dsRNA-containing sequences from the IR, V1, and V2 regions of TYLCV-Oman had reduced the viral infection in approximately 75% of transgenic tomato plants. Similar findings were obtained in another study, which demonstrated that resistance was linked to the production of siRNAs specific to transgene-containing viral segments (Ammara et al., 2015). Artificial miRNA (amiRNA)-mediated viral gene silencing and synthetic transacting siRNAs (syn-ta-siRNAs) targeting several viral genes have recently shown to be effective in providing resistance against tomato spotted wilt virus (TSWV) (Carbonell et al., 2019).

This research aimed to develop a rapid, robust molecular-based method for detecting TYLCV in tomato plants without the need to extract and purify the viral genome. In addition, the potential of TYLCV to infect many plant species was evaluated. Finally, an *in-silico* approach was used to generate multiple potential siRNA sequences targeting the viral genome. These sequences could be synthesized in the laboratory and utilized as an effective control tool.

Materials and Methods

Samples collection

Leaf samples showing typical symptoms of geminiviruses suspected to be TYLCV were collected from open fields at different tomato growing areas in Egypt during July, October, and November (2019). Tomato leaf samples were

collected from different location in Egypt as follows: Abo Ghaleb; Giza (30.273816, 30.939546) cultivar (cv N123 and 77), Beni Mazar, El-Minia (28.364057400836607, 30.851805751527937) (cv 55), Qaha, Qalyubi (30.280578, 31.196420) (cv 77), agriculture research center; El-Beharia (30.893040844397852, 30.63803777976037) (cv 77) and agriculture research center; Ismailia (30.619085498534236, 32.236586081623685) (cv 447). The locations of the collected samples were determined using Google earth maps (Fig. 1). Leaf samples were stored at -20°C until used for analysis.

DNA extraction and PCR detection

Leaf samples with and without (control) viral symptoms were dipped in liquid nitrogen and DNA was isolated according to the manufacturer's protocol of ZR plant/seed DNA Miniprep (TM Zymo research Cat no. D6020, Lot no. ZRC184951).

A pair of oligonucleotide primers specific to TYLCV genome template that has no targets in *Solanum lycopersicum* genome were designed using NCBI primer tool. TY7-F (5' AGGCAGCCAAGTACGAGAAC 3') and TY7-R (5' CTTCGTCACCCTCTACGAGC 3') were used to amplify 670 bp of the viral DNA fragments starting at base position 964 and ending at 1633. The amplification reactions were performed in Thermal Cycler (Biometra, Germany) using Cosmo Red DNA polymerase master mix (Cosmo Willofort: WF-1020201, UK) in 25µL reaction volume according to the manufacture instructions. The PCR reaction mixture consists of 2µL of the extracted DNA, 2.5µL of each primer (10 pmol), 1× PCR red buffer and 1 U of Taq DNA polymerase. The PCR thermal cycling was carried out as follow: initial denaturation of DNA was performed at 95°C for 5 minutes followed by 35 cycles of amplification with denaturation at 95°C for 15sec, annealing at 60°C for 15sec, extension at 72°C for 20sec, followed by a final extension cycle at 72°C for 10min. Finally, the amplification reaction was hold at 4°C. The PCR products were separated on 1.5% agarose gel prepared in 1× TAE (Tris-acetate-ethylene diamine tetra acetic acid) buffer containing 0.5µg/mL ethidium bromide. Ethidium bromide-stained gel was visualized and imaged using UV-transilluminator (Vilber Lourmat-Germany).

Fig. 1. Geographical locations of the collected naturally infected tomato plants showing typical symptoms of TYLCV [The sampling areas are labelled in red icons as follows: Giza, El-Minia, Qalyubia, El-Beharia and Ismailia]

Sequence analysis

The PCR products were purified using PCR-M clean up system (VIOGENE cat# PF1001) according to manufacturer's protocol. The purified samples were sequenced by GATC company using ABI 3730xl DNA sequencer (Germany) and TY7-F primer to further confirm the sequences. The obtained nucleotide sequences were aligned to the total nucleotide collection of NCBI using basic local alignment search tool for nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree based on the obtained sequences was constructed using UPGMA tree build method in Geneious 8.1.9 software (Kearse et al., 2012).

Host range and mode of transmission

Under greenhouse conditions at (25-28ºC), one month old of each plant species (in triplicates) belonging to 7 families (see Table 2) were inoculated by two methods: mechanical and whitefly infection. For mechanical infection: cell sap was extracted by grinding leaf tissue from naturally TYLCV infected plants that showed positive results by PCR detection confirmation. The extracted sap was injected into the phloem of one month old healthy plants with an equivalent amount of 0.1 M Phosphate buffer pH 8 to prevent cells rupturing (Ajlan et al., 2007). The injections were done with a 1.0mL syringe at three points on the stem. In control plants, cell sap of healthy plants was used instead of infected plants. For whitefly infection: Whiteflies were collected from tomato plants growing in open areas and identified by the Agricultural Research Center. Virus-free whiteflies were used as vectors in transmission experiment. Insects are allowed to feed on infected tomatoes in insect-proof cages after they have matured on non-hosted plants. The insects were enabled to feed for 72h on healthy hosts after a 24h acquisition access period then the viruliferous whiteflies were removed by spraying the tomato plants with insecticides and left for symptoms development. The inoculated and the control plants were kept in a greenhouse and symptoms were observed twice a week for up to 4 weeks post transmission. TYLCV infection was determined visually by developing the typical symptoms.

siRNA Design, scoring, and selection steps

SiDirect (Ui-Tei et al., 2008), i-SCORE Designer (Ichihara et al., 2007), OligoWalk (Lu & Mathews, 2008), and DSIR (Vert et al., 2006) were used to build all possible siRNAs for incomplete cds of TYLCV (MZ546492). The Huesken dataset was used as our primary filtration (Huesken et al., 2005). Then, the most effective siRNAs were chosen based on those that exceeded all previous thresholds. It was observed that the off-target effect is closely related to the thermodynamic stability or Tm of the target duplex angle of the seed, which forms between nucleotides positioned 2–8 of the 5' end of the siRNA guide strand and its target sequence (Henschel et al., 2004). In a second step, to avoid the off-target effect, the Tm of the target duplex was calculated using the nearest neighbor model and the thermodynamic parameters of RNA duplex formation (Henschel et al., 2004). The formula for calculating Tm is: Tm = {(1000 x ΔH) / (A + ΔS + R ln (CT / 4))} - 273.15 + 16.6 log [Na +], where ΔH (kcal / mol) is the sum of the nearest change in adjacent enthalpy, A is the helix initiation constant (-10.8), ΔS is the sum of the nearest neighbor entropy change (Freier et al., 1986), R is the gas constant (1.987 kcal/degree/mol), and CT is the total molecular concentration of the ropes (100 µM). [+Na] is fixed at 100mm. the calculated Tm of 21.5°C may be a criterion for distinguishing nearly non-target seed sequences from positive off-target sequences.

Thermodynamics and in Silico Target Accessibility

The thermodynamic stability of the duplex generated between the seed region of the siRNA guide strand and its target mRNA is substantially connected with the capacity of siRNAs to induce seed-dependent off-target impact (Ui-Tei et al., 2008) A positive connection was seen between the melting temperature (Tm) of the seed-target duplex and the production of seed-dependent off-target effects. The results indicated that the Tm of 21.5ºC may serve as the standard for distinguishing almost off-target-free seed sequences from off-target-positive sequences. Selecting siRNAs with a low Tm of the seed-target duplex should therefore reduce seed-dependent off-target silencing.

In the case of i- SCORE Designer, thermodynamics are calculated using the RNA mfold technique. Subsequently, the best siRNAs with dG values between 35 and 27 kcal/mol were chosen (Matveeva et al., 2010) .

Results

Field survey and molecular detection of TYLCV

The field survey of specific locations at five different governments in Egypt; Giza, El-Minia, Qalyubia, El-Beharia and Ismailia for TYLCV revealed the presence of typical symptoms of the virus infection (for specific locations see materials and methods section). The main symptoms noticed are sever leaf curling, leaf crinkle with marginal yellowing, twisted and stunted leaves (Fig. 2).

To find a quick robust method for further confirming the TYLCV infection, pair of novel primers was designed to amplify a partial genome fragment of the virus from the infected plants using conventional PCR technique with no targets in the tomato genome. The forward primer was located at nucleotide position 964 of the terminal part of the coat protein (CP) gene while the reverse primer was at the nucleotide position 1633. Both primers spanning the full length of the trans-activator protein (C2), replication enhancer protein (C3) genes, partial parts of replication (Rep) and CP genes. The TYLCV genome organization and the location of the designed primers within the TYLCV genome was indicated in Fig. 3.

Applying this approach for all collected samples that showed the typical symptoms of TYLCV infection has revealed the amplification of the expected fragment length of 670 bp in comparison to no amplification from the control healthy plants which confirms the primer specificity to TYLCV genome (Fig. 4).

The primers successfully amplified the expected TYLCV fragment from all collected samples, five samples from Ismailia, four from El-Mina, six from El-Behera, ten from Qalyubia, and nine from Giza.

Partial genome annotation and phylogenetic analysis of the TYLCV Egyptian isolate

The amplified PCR product from the TYLCV naturally infected plants was purified and sequenced for verification. The megablast genome alignment to the NCBI genome nucleotide collection revealed 99.3% pairwise identity with 100% query coverage to TYLCV isolates (YN6557, YN6579, YN6583 and others as indicated in the constructed UPGMA phylogenetic tree (Fig. 5).

Fig. 2. Natural disease symptoms (chlorotic leaflets and curling of the leaves) within tomato plants attributed to Tomato yellow leaf curl virus (TYLCV) [A. non-infected healthy tomato plants. B, C, D. Natural TYLCV infected plants show disease symptoms]

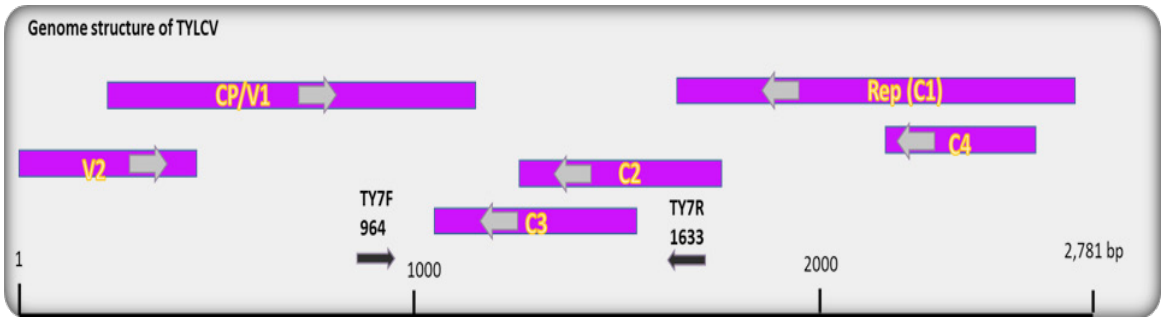

Fig. 3. The TYLCV genome composition and the position of the primers used in the current study (black arrows) [The forward primer (TY7F) is located at 964 bp and the reverse primer (TY7R) at 1633 bp. The scale bar refers to the full genome sequence of the TYLCV genome]

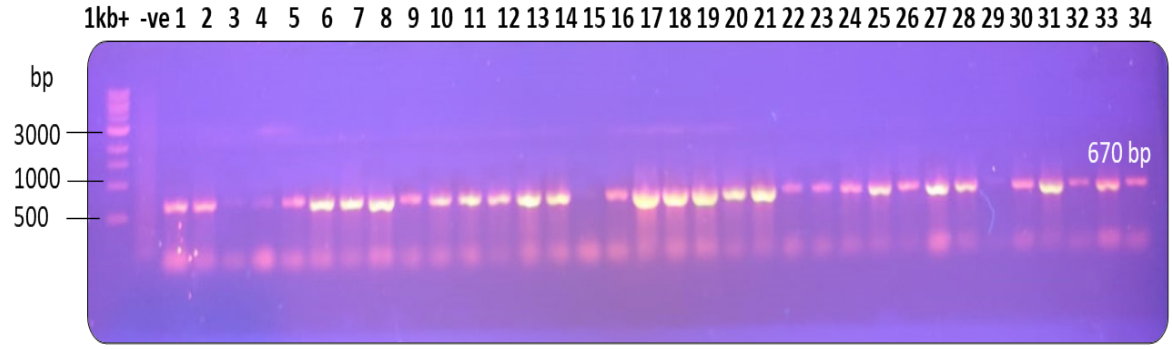

Fig. 4. TYLCV detection from tomato leaf samples of 34 DNA samples by PCR using TYLCV-specific primer set. 1kb+, DNA ladder, -ve, PCR amplification from healthy tomato plants no products confirm the primer specificity to TYLCV genome [Lanes 1-34 refer to PCR amplification from TYLCV naturally infected tomato plants collected from different governorate at Egypt as follow; 1-5 (Ismailia), 6-9 (El-Mina), 10-15 (El-Behera), 16-25 (Qalyubia), 26:34 (Giza)]

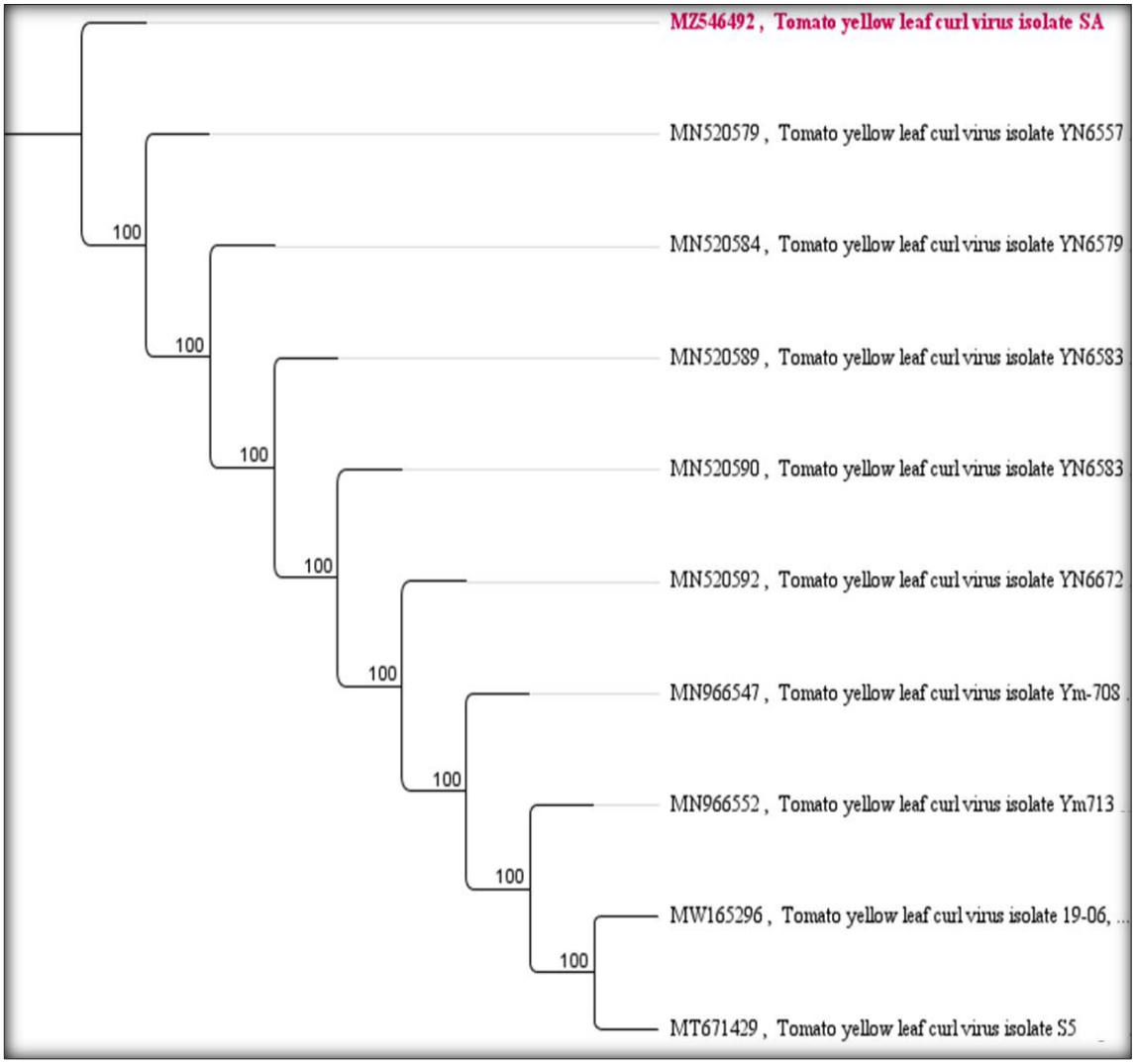

Fig. 5. Phylogenetic tree generated using UPGMA tree build method of Geneious software based on partial TYLCV genome sequencing of the current isolate (red bold font) in relation to the most-closest accessions in the NCBI database [The number on the branches indicates the percent of 100 bootstrapping]

The obtained nucleotide sequence of the current isolate that named (SA) isolate was annotated and deposited in the NCBI database and acquired an accession number MZ546492. The annotation analysis for the trimmed and submitted sequences to the NCBI database showed that the amplified fragment spans four different genes, C1, C2, C3 and V1 of the TYLCV genome (Table 1).

Host range and mode of transmission

To determine the host range of TYLCV infection we screened 11 plant species belonging to 7 families (Table 2). All plant species were infected using mechanical and whiteflies. The infected plants were observed to develop the typical symptoms of TYLCV on the plants. Positively infected plants developed symptoms up to 4 weeks post transmission. Interestingly, none of the syringe infected plants developed symptoms. While whitefly infected plants developed symptoms that depend on plant species. *C. sativus, C.pepo, C. annum cv Chilli, Datura stramonium* and *Phaseolus vulgaris* belong to Cucurbitaceae, Fabaceae and Solanaceae families were the only ones that developed symptoms post whitefly transmission. Leaf curling with marginal yellowing was first developed after 2-3 weeks till it gives cup shape to leaves, stem twisted, and severe stunting after 5-6 weeks under greenhouse condition (Table 2).

siRNA Design, scoring, and target accessibility.

The most potent predicated siRNA that could silence the TYLCV genome based on siDirect 2.0 tool, were selected, and shown in Supplementary Tables. The thermodynamic stability analysis has showed that the duplex stability (Tm) of 21°C will prevent off target silencing. Therefore, these sequences were only shown in Table 3.

TABLE 1. Partial genome annotation of the of the TYLCV Egyptian isolate (SA) (MZ546492) using NCBI database, showing gene position within the sequence, gene product and product ID

	Gene position and orientation within the sequence/ gene ID	Gene product	Protein ID
1.	1 > 54/gene="V1"	Coat protein	UXX22718.1
2.	Complement (51 > 455) /gene="C3"	Replication enhancer protein	UXX22719.1
3.	Complement (196 >590) /gene="C2"	Trans-activator protein"	UXX22721.1
4.	Complement (512 > 590) /gene="C1"	Replication protein	UXX22720.1

TABLE 2. Host range analysis of TYLCV transmission by mechanical method (syringe injection) and whitefly transmission to various plant species belonging to different plant families

Host plants	Mechanical transmission symptoms	Whitefly transmission symptoms
Cucurbitaceae		
C. sativus	NS	LC, Y
C. pepo	NS	LM, LK
Chenopodiaceae		
Chenopodium amaranticlor	NS	NS
Cruciferae		
Brassica oleracea var. *capitate*	NS	NS
Solanaceae		
Solanum melongena	NS	NS
C. annum cv Chilli	NS	Y, LK, S
Datura stramonium	NS	LC, E, S
Lamiaceae		
Mentha sp.	NS	NS
Compositae		
L.sativa	NS	NS
Fabaceae		
Vicia faba	NS	NS
Phaseolus vulgaris	NS	Lk, S, N

NS= no symptoms, LC = leaf curl, LM=leaf malformation, LK=leaf crinkle, N= necrosis, S=stunting, E= epinasty, Y = yellowing.

TABLE 3. A typical output of siDirect 2.0: siRNAs targeting

Target position	Target sequence	RNA oligo, guide	Passenger	Duplex stability (Tm) guide
115-137	TTGTGTTTTCAAGTACATCATAC	AUGAUGUACUUGAAAACACAA	GUGUUUUCAAGUACAUCAUAC	21.5
156-178	CTGATTACATTGTTAATGGAAAT	UUCCAUUAACAAUGUAAUCAG	GAUUACAUUGUUAAUGGAAAU	21.2
306-328	CTGATGTTGTGGTTGAATCTTAT	AAGAUUCAACCACAACAUCAG	GAUGUUGUGGUUGAAUCUUAU	20.4
314-336	GTGGTTGAATCTTATCTGAATGG	AUUCAGAUAAGAUUCAACCAC	GGUUGAAUCUUAUCUGAAUGG	20.4
414-436	CAGATAAAAACGCCATTCTCTGC	AGAGAAUGGCGUUUUUAUCUG	GAUAAAAACGCCAUUCUCUGC	20.4
17-39	CCCAGTGTATGCAACTATGAAAA	UUCAUAGUUGCAUACACUGGG	CAGUGUAUGCAACUAUGAAAA	20.3
246-268	GGCTGTAATGTCGTCCAAATTCG	AAUUUGGACGACAUUACAGCC	CUGUAAUGUCGUCCAAAUUCG	20.1
322-344	ATCTTATCTGAATGGAAATGATG	UCAUUUCCAUUCAGAUAAGAU	CUUAUCUGAAUGGAAAUGAUG	20.1
101-123	TTCTGTTACATTTATTGTGTTTT	AACACAAUAAAUGUAACAGAA	CUGUUACAUUUAUUGUGUUUU	19.3
103-125	CTGTTACATTTATTGTGTTTTCA	AAAACACAAUAAAUGUAACAG	GUUACAUUUAUUGUGUUUUCA	19.3
256-278	TCGTCCAAATTCGGAAGTTGAGA	UCAACUUCCGAAUUUGGGACGA	GUCCAAAUUCGGAAGUUGAGA	19.2
534-556	ACGCCTTATTGGTTTCTTCTTGG	AAGAAGAAACCAAUAAGGCGU	GCCUUAUUGGUUUCUUCUUGG	19.1
172-194	TGGAAATTACACCAAGACTATCT	AUAGUCUUGGUGUAAUUUCCA	GAAAUUACACCAAGACUAUCU	18.9
209-231	TTGATATCTAAATACTCTTAAGA	UUAAGAGUAUUUAGAUAUCAA	GAUAUCUAAAUACUCUUAAGA	18.9

Discussion

In several locations of the world, TYLCV causes significant yield losses in tomato crop; TYLCV continues to be the most frequent and economically significant virus for tomato crop (Arasimowicz-Jelonek et al., 2020). Detecting and diagnosing TYLCV as early as possible is crucial for preventing the spread of infectious disease and limiting financial damages. Management of TYLCV, which does not rely solely on the visual observation of symptoms, requires the implementation of sensitive and accurate diagnostic tools. To select the appropriate resistance strategy, it is also crucial to properly identify the viral species infecting plants. Several methods have been developed to detect TYLCV, including immunological assays that identify the presence of viral coat protein and (PCR) assays that detect TYLCV DNA. TYLCV infection can be confirmed by DNA sequencing following amplification of TYLCV DNA fragments using suitable primers (Li et al., 2022).

To confirm the natural acquisition of TYLCV, the current study has successfully constructed a new PCR primer combination that was used to detect TYLCV infection in tomato plants. Previously, Kil et al. (2016) used the PCR approach to validate that TYLCV is a seed-transmissible virus in tomato plants, and they also demonstrated that the amount of the viral genome within the plant is a measure of its infectivity. Comparatively, fewer PCR products are generated from TYLCV-tolerant tomato plants than from susceptible tomato plants. Similarly, Li et al. (2012). designed a pair of TYLCV-specific primers to molecularly identify the presence of TYLCV by amplifying a 543 bp fragment from tomato plants infected with the virus. Zhang and his co-author (2016) used the same primers to detect the impact of *Isaria fumosorosea* inoculation on TYLCV accumulation in tomato plants and *Bemisia tabaci* (Zhang et al., 2016). In 2020, a group of Spanish scientists present clear evidence based on PCR that TYLCV is seedborne but not seed transmitted in tomato or tobacco (*N. benthamiana*), suggesting that seed transmission is not a general characteristic of TYLCV (Pérez-Padilla et al., 2020). This protocol may provide a rapid, dependable, and sensitive method for the molecular detection and identification of TYLCV in tomato germplasm to ensure the safe and sustainable production of TYLCV-free

tomatoes. Development of as many tested primers for detecting TYLCV infection is essential for establishing a broad platform of alternatives that can be used by all scientists to minimize time, cost, and effort required for detection.

The positive PCR samples were successfully identified by sequencing the amplified TYLCV DNA fragment and determining its phylogenetic relationship to the most relevant isolates in the database. Homology blast identified a 99.3% similarity to TYLCV isolates (YN6557, YN6579, and others) with a 100% query coverage. The detected genetic variation between the isolate in the present study and the closely related isolate in the database may be a result of an ecological influence that triggers mutation and recombination events, thereby generating new variants with unique biological and ecological characteristics to survive in the environment (Belabess et al., 2015; Lefeuvre & Moriones, 2015). Phylogenetic analysis proved indispensable for tracking changes in the viral population concurrent with a viral pandemic. For phylogenetic analysis, TYLCV-specific PCR products from TYLCV-resistant tomatoes in Spain were sequenced. The analysis revealed that the detected Spanish isolates belonged to two groups, one related to early isolate of TYLCV TYLCV-IL isolates (Group 1) and the other closely related to El Jadida (Morocco) isolates TYLCV-IS76, (Group 2), indicating a recent introduction. The *in vitro* analysis has supported the co-occurrence of both types of isolates. This phylogenetic research revealed the spread of novel mutations in the populations of viruses associated with TYLCD in Spain, as well as the possibility of complex interactions across viral types (Torre et al., 2018).

The PCR revealed 100% of TYLCV infection for the naturally infected samples that were collected from specific locations at five different Egyptian governorates; Giza, El-Minia, Qalyubia, El-Beharia and Ismailia. This result was consistent with a study conducted on the same governorate in 2016 (Rabie et al., 2017). In which the PCR detected the presence of TYLCV-IL genome from the naturally collected samples from these governorates (Rabie et al., 2017). Compiling the results obtained in 2016 and the current result may highlight the persistence of TYLCV genome in these governorates which suggested its transmission through seeds to new generation. This phenomenon was confirmed by Kil et al. (2016). Changes in band intensity among different samples (Fig. 4) may be a result of different concentration of viral DNA in each sample, which may reflect the symptoms severity.

Bemisia tabaci (commonly known as whitefly) of the order Hemiptera attacks over 600 plant species, and TYLCV transmission to plant hosts is caused by virulent whiteflies establishing a feeding site in phloem sieve elements (Prasad et al., 2020). The persistence of any pathogen in different hosts is a major challenge in controlling it. TYLCV has a wide host range, having been found in 49 different species from 16 different families (Prasad et al., 2020). Table 2 summarized the results of the host range study and the symptoms associated with hosts in this study. The results demonstrated that the mechanical inoculation's is a non-transmitter while the whitefly-transmission nature of TYLCV. Studies related to TYLCV transmission considered the whitefly as a sole transmitter however, Kil and his co-authors have proved that the TYLCV could be transmitted to the new generation of tomatoes through seeds produced from TYLCV infected plants (Kil et al., 2016).

The host range of TYLCV included members within the families Cucurbitaceae, Chenopodiaceae, Cruciferae, Solanaceae, Lamiaceae, Compositae and Fabaceae. No symptoms were developed when tomato and other crops were inoculated mechanically using the sap of naturally infected plants with 0.1 M phosphate buffer that agrees with (Ajlan et al., 2007). Whitefly carries TYLCV successfully transmit TYLCV to the infected plants in which some developed obvious disease symptoms, while others are susceptible to symptomless virus carriers. *C. sativus*, *C.pepo*, *C. annum* cv Chilli, *Datura stramonium* and *Phaseolus vulgaris,* all developed TYLCV disease symptoms so they may serve as a natural reservoir for TYLCV. While *Chenopodium amaranticlor*, *Solanum melongena*, *Vicia faba*, *Brassica oleracea* var. capitate, *Mentha sativa* and *L. sativa* showed no symptoms and did not react systemically with TYLCV. The transmission capability of TYLCV to the host plant is mainly dependent on their interactions together and their genome composition (Yang et al., 2019). The noncoding intergenic region (IR) of the TYLCV has a 25-nt region that is nearly completely complementary to a long noncoding RNA (lncRNA, identified as

SlLNR1) in TYLCV-susceptible tomato cultivars, but is absent in resistant cultivars due to a 14-nt deletion within the 25-nt area. Therefore, the viral infection to TYLCV-sensitive tomato cultivars stimulate the synthesis of small-interfering RNAs (siRNAs) produced from the 25-nt IR sequence triggers silencing of SlLNR1 in susceptible tomato plants that is associated with stunted and curled leaf phenotypes indicative of TYLCV. Additionally, the over-expression of SlLNR1 in tomato has lowered the accumulation of TYLCV. Silencing of SlLNR1 has led to generation of TYLCV-like leaf phenotypes in tomato plants in the absence of viral infection (Yang et al., 2019).

One of the recent approaches to control and restrict TYLCV infection is the production of siRNA that target the viral sequences (Prasad et al., 2020). RNA silencing mediated by double-stranded RNA (dsRNA) is a well known mechanism in many eukaryotes that regulates gene expression and protects cells from intrusive nucleic acids, such as viruses. During infection, host Dicer-like enzymes convert virus-derived dsRNA into 21 to 24 nucleotide (nt) virus-derived small interfering RNAs (vsRNAs). These vsRNAs are bound to the argonaute protein to create an RNA-induced silencing complex and direct the destruction of single-stranded viral RNA (Hull, 2014). Hence, the prediction of siRNAs that target the TYLCV genome is crucial for control approach. Yet, the identification of particularly potent short interfering RNAs is the initial step for successful RNAi approaches (siRNAs). Design techniques based on thermodynamic characteristics that impact siRNA activity increase the likelihood of producing effective siRNAs.

In the current investigation, a list of possible oligonucleotides was generated that could be synthesized in the laboratory and transformed into the plant prior to viral infection that would stimulate the plant's resistance. When these plants are infected with TYLCV, they will be able to produce siRNAs that guide TYLCV RNA degradation, thereby alleviating and reducing its severity. These sequences were designed based on the thermodynamic stability of the produced siRNA. Nonetheless, the efficiency of these sequences requires in vivo evaluation.

Conclusion

TYLCV poses a threat to the tomato crop in multiple countries due to its rapid spread and significant production losses. Unfortunately, there are currently no effective control techniques available. Efforts to minimize the prevalence of TYLCV by reducing inoculum sources or regulating vector transmission are generally futile, particularly when whitefly populations are at their height. A deeper understanding of disease epidemiology may be essential for the development of disease control programs. To ensure the safe and sustainable production of TYLCV-free tomatoes, the present work has presented a novel primer sequence for rapid and reliable molecular detection and identification of TYLCV in tomato germplasm. Additionally, the current study, along with prior available studies, found that specific governorates in Egypt, such as Giza, El-Minia, Qalyubia, El-Beharia, and Ismailia, could be referred to as TYLCV settlers, as supported by the current study and previous research (Rabie et al., 2017). An *in-silico* approach successfully generated siRNA sequences that could be used to knock down TYLCV in infected plants.

Abbreviations:

IPM: integrated pest management.

siRNA: small interfering RNA.

TYLCV: tomato yellow leaf curl virus.

TYLCD: tomato yellow leaf curl disease.

vsRNAs: virus-derived small interfering RNAs.

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الكشف والتوصيف الجزيئي لجينوم فيروس تجعد واصفرار الأوراق في الطماطم (TYLCV- MZ546492) وتعيين متواليات siRNA كإستراتيجية بديلة للتحكم في الفيروس

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يعد مرض تجعد وإصفرار الأوراق (TYLCD) عاملاً مقيدًا مهمًا في محاصيل الطماطم ويصنف ضمن أكثر 10 فيروسات نباتية تؤثر على إنتاج العديد من المحاصيل، وخاصة الطماطم، مما يؤدي إلى خسائر اقتصادية كبيرة دون وجود استراتيجية تحكم فعالة حتى الآن. في هذه الدراسة تم استخدام تقنية تفاعل البلمرة المتسلسل لتضاعف التسلسلات الجزيئية (670 قاعدة نيتروجينية) لجينوم فيروس تجعد وإصفرار الأوراق للطماطم (TYLCV) الذي يمتد عبرمنطقة جينات (C2) و (C3) و(Rep) و (CP). في هذه الدراسة تم جمع نباتات طماطم مصابة طبيعياً بالفيروس من محافظات مختلفة في مصر. تم تحليل تسلسل الحمض النووي للعزلة المصرية الحالية وإيداعها في قاعدة البيانات برقم MZ546492. أظهر تحليل تسلسل الحمض النووي تشابه يصل ل (99.3 ٪) لعزلات TYLCV المسجلة في قاعدة البيانات الخاصة ببنك الجينات. أكدت الدراسة أيضا أن الذبابة البيضاء هي الطريقة الوحيده لنقل الفيروس وكانت الطماطم، الكوسة، الخيار، الفلفل، والفاصوليا من النباتات التي تستخدم كعوائل للفيروس. تم استخدام طرق المعلوماتية الحيوية لإنتاج متواليات siRNA المحتملة التي يمكن استخدامها لتقليل أعراضTYLCV في النباتات المصابة. تدعم النتائج الحالية استخدام تفاعل البلمره المتسلسل كأداة كشف وتعريف جزيئي سريعة وموثوقة وقوية لـفيروس تجعد وإصفرارالأوراق للطماطم في الأصول الوراثية للطماطم لضمان الإنتاج الآمن والمستدام للطماطم الخالية من الفيروس. وقدمنا في هذه الدراسه أيضًا بديلاً محتملاً لاستراتيجية التحكم في الفيروس.