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Molecular validation for impossible transmission of TYLCV through mechanical route

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Abstract
Tomato yellow leaf curl disease is a major limitation in agriculture across the world, affecting the production of many crops, resulting in significant economic losses. In Egypt, the incidence of infection is very high, with visual estimates of TYLCV infection reaching 100 % in some areas. Here in the mechanical transmission ability of TYLCV is assessed in different plant species well known to be hosts for the TYLCV. The current finding revealed the failness of transmission through mechanical route. None of the mechanically infected plants developed TYLC disease symptoms. Furthermore, the absence of TYLCV genome within mechanically transmitted plants was also validated. Alignment of the amplified partial TYLCV genome from the naturally infected plants from Qalyubia governorate, show 98.99% similarity and 100% query coverage to the TYLCV in the GenBank. In conclusion, the TYLCV can't transmit through mechanical route this may be a result of failing its genome to be establish within plant genome.

Keywords: Tomato, Gene sequencing, Host range, Geminivirus.

1. Introduction

Tomato (*Solanum lycopersicum*) rank sixth among tropical and subtropical crops [1].Tomatoes are one of the most extensively produced crops in the world, but their susceptibility to virus disease causes yield decline as a crop, a result of the high mutation rate, which promotes genetic strain divergence, plant virus management continues to be a significant agricultural challenge [2].

Tomato yellow leaf curl virus (TYLCV), family Geminiviridae, genus Begomovirus, is a severe agricultural hazard worldwide. TYLCV suddenly appeared in the Middle Eastern Mediterranean area and has expanded rapidly over the globe [3]. Monopartite begomoviruses encode six Open Reading Frames ORFs, each of which encodes a protein with a specific function. The coat protein and pre-coat protein genes are present in V1 and V2 whereas C1, C2, C3, and C4 are in the complementary sense strand (minus) viral strand, replication-associated protein, transcriptional activator protein, replication enhancer protein, and pathogenicity-determining protein [2].

Tomatoes diseased in the initial phases of growth have severely stunted leaflets that are reduced in size and abnormally shaped, such as leaf curling, crimple, yellowing, plant dwarfing, and wilting. Early-forming leaves are cupped downward, but later-forming leaves are chlorotic and distorted, with leaf edges folded upward and curling in between veins. The effect on fruit is determined by the age of the plant at the time of infection, with the lowest yields happening when plants are infected during the early stages of development one to five leaves, With output losses ranging from 20 to 100 percent depending on plant's growth stage at the time of infection, resulting in severe economic losses in the tomato production areas [4].

The whitefly *B. tabaci* is fully responsible for the virus's sustained and circulative transmission into tomatoes [5]. Only mechanical transfer of TYLCV to tomato plants has been demonstrated in *Datura stramonium*, *Nicotiana glutinosa* and *Lycopersicon pennellii* plants at a relatively low level. Abdel-Salam achieved Only a few occurrences of mechanical transfer from tomato to tomato occurred in 1990 [6].

Most TYLCV control strategies is focused on infection prevention and genetic resistance. When developing new TYLCV control techniques, The relative relevance of the three elements involved in viral infection (vector, host plant, and their interactions) must be considered. In the control of this viral illness, actions that diminish vector populations by chemical treatment or biological control that affects transmission efficiency by denying vector access to the crop through physical barriers have been applied [6].

The purpose of this research collected TYLCV symptomatic tomato plant leaf samples from Egypt's Qalyubia governorate. The presence of the TYLCV genome within the naturally infected plants was confirmed by PCR technique. The ability of the TYLCV to be transmitted mechanically was also evaluated.

2.Material and procedures

Tomato Samples

10 *Solanum lycopersicum* plants with infected leaves were collected from commercial fields in Egypt's Qalyubia (Qaha) governorate in July (2019). Leaf samples displaying classic geminivirus signs such as upward leaf curling, yellowing, and stunting were kept at 4 ^{o}C until analyzed, according to [7].

Molecular detection of TYLCV genome by PCR

Total genomic DNA was extracted from healthy, naturally infected, and mechanically infected samples. The leaf samples were frozen using liquid nitrogen with a mortar and pestle, grind to a fine powder. According to the manufacturer's procedure, the ZR plant/seed DNA Miniprep TM Zymo research was used to extract and purify the DNA. The extracted DNA was utilized as a template to amplify a portion of the TYLCV genomic sequence using Red DNA polymerase master mix (Cosmo Willofort: WF-1020201) in 25 µl reaction volume according to [8].

Mode of Transmission

Mechanical Infection

Healthy *C. pepo, Solanum lycopersicum, C. annum cv Chilli, Brassica oleracea var. capitata, L. sativa* and *Mentha* plants 21 days ago were injected with syringes containing infected tomato sap [15]. control and Injected plants were maintained in greenhouse settings for up to 60 days, and symptoms were evaluated regularly.

3. Results

Location of the infected area and Egypt's tomato production

The location of the naturally infected samples collected from Qalyubia governorate is indicated in Fig 1A. From 1961 to 2021, the distribution of tomato production in Egypt was sharply increased according to FAOSTAT. The production in 1961 was 869.135 million, while the production in 2021 was 6.245.787 million (fig. 1B) [9].

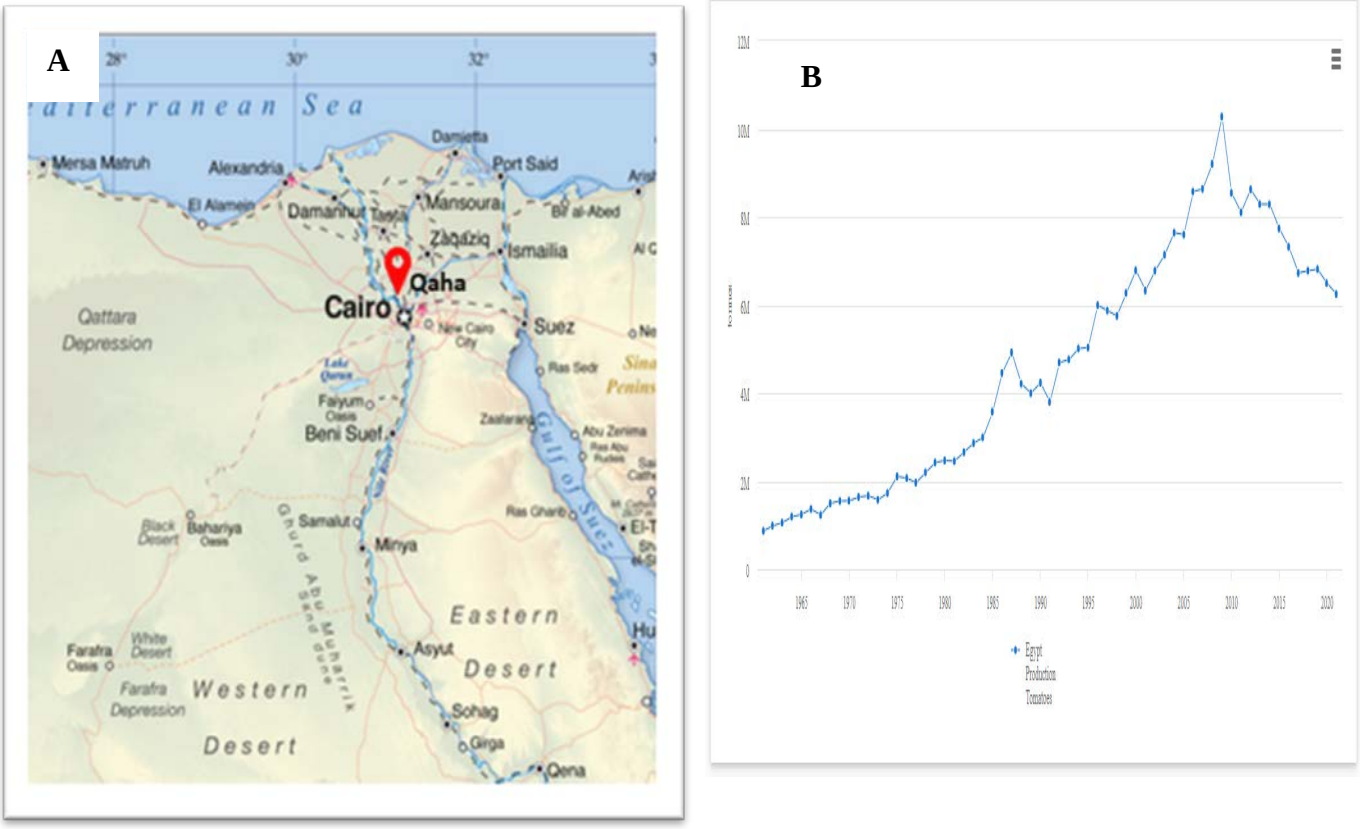

Figure 1 A. Geographical distribution of naturally infected tomato plants displaying TYLCV symptoms. Qalyubia (Qaha) is the location of the sampling.
B. Tomatoes production in Egypt from 1961 to 2021 according to FAOSTAT.

Field Survey of TYLCV

TYLCV disease symptoms was naturally observed in tomato plants grown in Qalyubia governorate (Egypt). The plants showed the typical symptoms like those previously described to be caused by TYLCV. Disease symptoms were detected for the leaf, shoot, and stem (Fig. 2).

Figure 3: A, B and C appear TYLCV symptoms on naturally infected tomato plants as yellowing and leaf curling collected from Egypt's Qalyubia (Qaha) governorate.

PCR detection of TYLCV

PCR was used to amplify the entire produced DNA using the oligonucleotides TY-FR and TY-RV as PCR primers. The PCR product's size was calculated by comparing its electrophoretic mobility to that of conventional DNA markers, as demonstrated in (Fig. 2). The amplified DNAs were of the predicted size (~670bp).

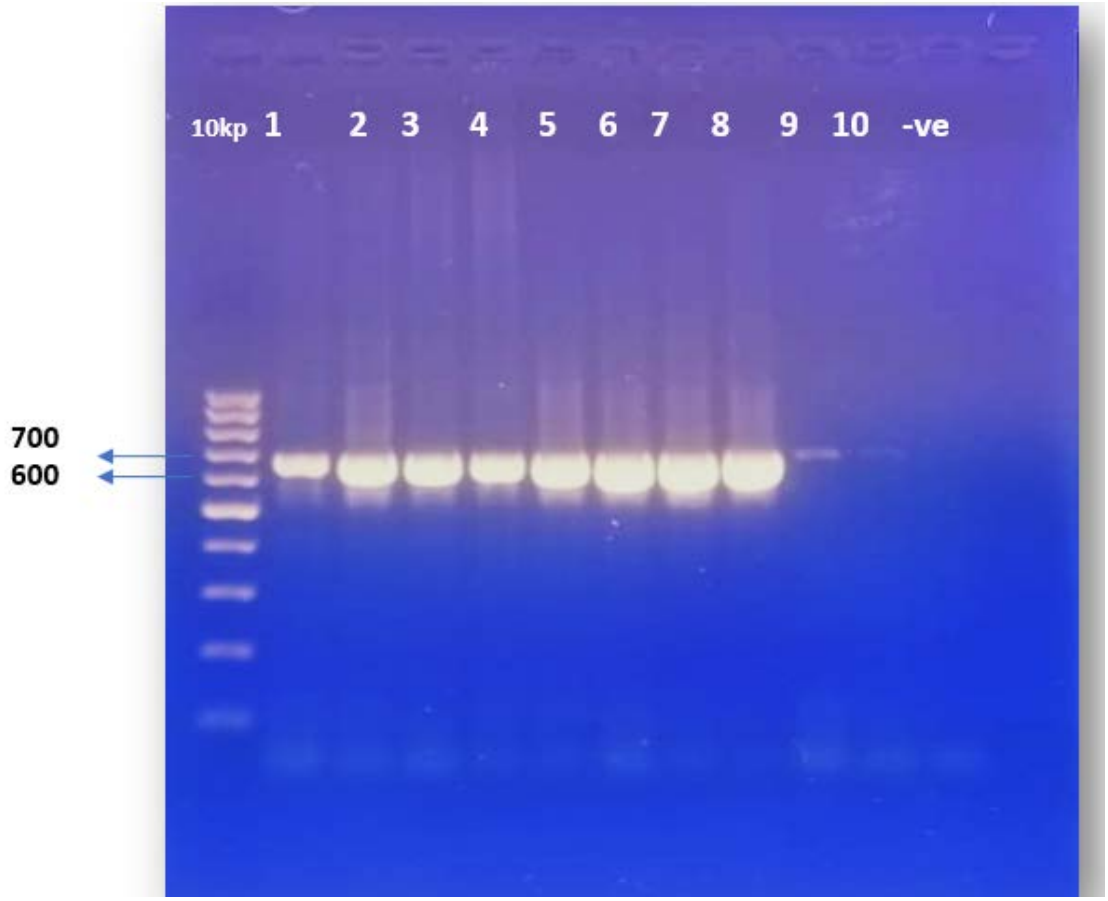

Figure 2: 1.5 percent agarose gel electrophoresis revealed PCR products (670 bp) recovered from naturally infected tomato plants infected with TYLCV. Lane 1: 10kb DNA ladder size marker, 1-10 genomic DNA from collected tomato plants and -ve as a negative control.

To validate that the amplified PCR fragment is specific for TYLCV genome. The fragment was sequenced, and the obtained sequences was aligned against total nucleotide collection of the GenBank. The alignment of the query cover was 100 percent, and the identity was 98.99 percent (table 1).

Table 1: The sequence alignment of the amplified PCR product from TYLCV infected plant in comparison to the closest accessions in the GenBank.

Description	Scientific Name	Max Score	Total Score	Query Cover	Percentage identity	Acc. Len	Accession
Tomato leaf curl virus isolate SC780, complete genome	Tomato leaf curl virus	1066	1066	100%	98.99	2781	MK559473.1
Tomato yellow leaf curl virus - Poamoho, complete genome	Tomato yellow leaf curl virus – Poamoho	1066	1066	100%	98.99	2781	GU322423.2
Tomato yellow leaf curl virus isolate 19-06, complete genome	Tomato yellow leaf curl virus	1066	1066	100%	98.99	2781	MW165296.1
Tomato yellow leaf curl virus isolate CHN-Hainan-Passiflora edulis-3-2020, complete genome	Tomato yellow leaf curl virus	1061	1061	100%	98.83	2781	MW814908.1
Tomato yellow leaf curl virus isolate CHN-Hainan-Passiflora edulis-48-2020, complete genome	Tomato yellow leaf curl virus	1061	1061	100%	98.83	2781	MW814909.1
Tomato yellow leaf curl virus isolate Guasave segment DNA-A, complete sequence	Tomato yellow leaf curl virus	1066	1066	100%	98.99	2781	FJ012358.1
Tomato yellow leaf curl virus isolate Hwas, complete genome	Tomato yellow leaf curl virus	1066	1066	100%	98.99	2775	GU126513.1
Tomato yellow leaf curl virus isolate S5 segment DNA-A, complete sequence	Tomato yellow leaf curl virus	1066	1066	100%	98.99	2781	MT671429.1

Mode of Transmission

Mechanical Transmission

We screened 6 plant species from 5 families. All plant species were infected mechanically, and none of the syringe-infected plants developed symptoms after 6-8 weeks at 28-30°C under greenhouse conditions (fig. 3). Also, to further confirm that these injected plants are not carrying the TYLCV genome. The DNA was extracted from these plants and served as a template for the PCR. There is no product in PCR is formed from the DNA extracted from the mechanical transmission plant (fig. 4).

Figure 3: Symptomless of TYLCV for the plants that infected by mechanical transmission. A: *C. pepo,* B*: Solanum lycopersicum,* C: *C. annum cv Chilli*, D: *Brassica oleracea var. capitata*, and E: *L. sativa.*

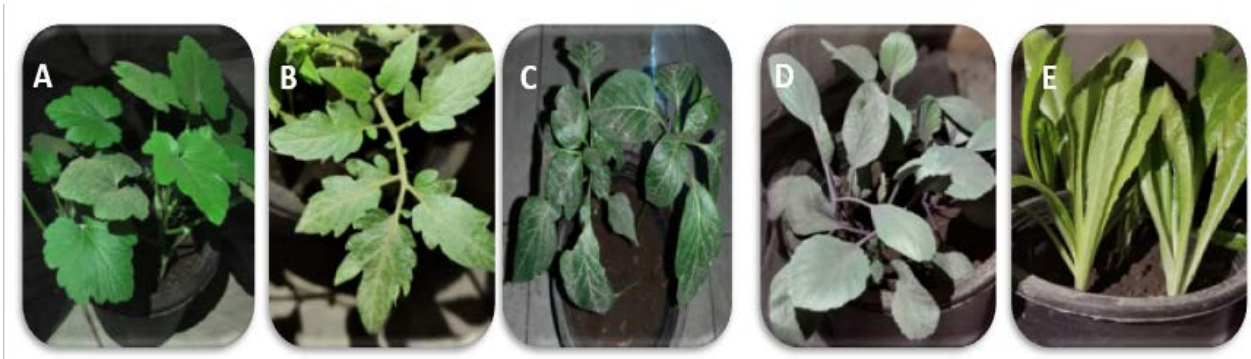

Figure 4: PCR amplification for the DNA extracted from the TYLCV mechanically transmitted plants. 1 *C. pepo*, 2 *Solanum lycopersicum*, 3. *C. annum cv Chilli*, 4. *Brassica oleracea var. capitata*, 5. *L. sativa* and 6. *Mentha*. 10kb DNA ladder size marker and +ve refers to PCR of naturally TYLCV infected plants.

Table 1. Mechanical transmission of TYLCV to various plant species from various plant families

Family	**Compositae**	**Cucurbitaceae**	**Cruciferae**	**Lamiaceae**	**Solanaceae**
Plant species	*L. sativa*	*C. pepo*	Brassica oleracea var. capitate	*Mentha*	*C. annum cv Chilli* *Solanum lycopersicum*
Symptoms	-ve	-ve	-ve	-ve	-ve -ve

Discussion

Tomatoes (*Solanum lycopersicum*) are one of the most extensively produced crops in the world. It is consumed in a variety of ways and has high nutritional value. Because to Geminivirus infection, this crop is currently facing a major threat to its output and survival. TYLCV is one of the Geminivirus species wreaking havoc on tomato crops throughout the world [10] Because it is the most devastating virus to worldwide tomato production, TYLCV is one of the most thoroughly investigated plant viral diseases. To successfully deal with TYLCV outbreaks and worldwide expansion, we must first understand the biology of the virus and then create management techniques [11]. TYLCV is a serious constraint in tomato yields all over the world [5]**.** The virus's extremely invasive nature, combined with a lack of proper control measures, allowed it to become a major global pathogen. The transmission of this virus has been assisted by the dissemination of contaminated planting material and whiteflies. TYLCV is widespread in Australia and China and was most likely brought from East Asia, whereas it was transported to New Caledonia from the Western Mediterranean. The virus has migrated from Australia, East Asia, and the Western Mediterranean to the Americas and the Caribbean. East Asia was most likely brought from the Eastern and Western Mediterranean areas [11]. In Egypt, the incidence of infection is very high, with visual estimates of TYLCV infection reaching 100 % in some areas [12]. TYLCV epidemiology in key tomato growing areas of Iran, notably in the central and southern regions, was found to be 36%, while it was found to be 89 % in tomato fields in Saudi Arabia, which borders Iran's southern region [13]. The unexpected TYLCV outbreak in these areas could be attributed to warm weather conditions that promote vector activity, virus replication, or host susceptibility. It has recently been revealed that The Mediterranean region and the Middle East are the key starting points for worldwide TYLCV movements [13]. Stunting, leaf curving upward, chlorosis, reduced leaf size, flower abortion, stem erect, limited plant growth and loss in tomato production are typical symptoms of TYLCV infection. Accurate TYLCV diagnosis is essential for limiting the spread of infectious illness and minimizing losses. The utilization of accurate and precise detection technologies is crucial for TYLCV management., which does not rely solely on visual observation of symptoms. It is also critical to precisely identify the viral species infecting plants in order to select the best resistance strategy. To detect TYLCV, various methods have been developed, most notably immunological assays that detect the presence of viral coat protein or PCR assays that detect TYLCV DNA [14]. *B. tabaci* of the Hemiptera order spreads TYLCV across plants. Over 600 plant species are attacked by *B. tabaci*, and TYLCV transmission to plant hosts is caused by virulent whiteflies establishing a feeding site in phloem sieve elements [11].

It is critical that the virus remains contained within the infected areas and does not spread to other countries. 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 [11].

The results demonstrated mechanical transmission unable to transfer TYLCV which is agree with [15]. TYLCV was tested on mechanical transmission included members within the families Cucurbitaceae, Cruciferae, Solanaceae , Lamiaceae and Compositae . No symptoms developed when tomato and other crops were injected that agrees with [16]. The sequence alignment of the amplified PCR product from TYLCV infected plant in comparison to the closest accessions in the GenBank and the result shows that the identity was 98.99 %.

Conclusion

TYLCV is endangering tomato crops in a number of nations. It is quickly spreading and wreaking havoc on temperate glasshouse crops. Mechanical transmission was unable of transmitting TYLCV. Unfortunately, there are presently no effective control techniques available. Attempts to minimize TYLCV incidence by removing inoculum sources or restricting vector transmission are generally futile, particularly during peak whitefly populations. More research is needed to increase the effectiveness of these strategies. A greater understanding of disease epidemiology may be critical in the development of disease control initiatives. Breeding for tolerance or resistance appears to be the most appropriate economic or ecological management technique.

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