Research Article

# Detection and Molecular Characterization of Tomato Yellow Leaf Curl Virus in the Local and Hybrid Tomato Varieties in District Mansehra, Pakistan Accession Number (BankIt2535530 Sample OM100018)

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**Abstract** | Tomato Yellow Leaf Curl Virus (TYLCV) is one of the most devastating threats to tomato crop in Pakistan as well as in many of the tropical and subtropical regions of the world. Research was conducted to evaluate the presence of TYLCV in six different local tomato varieties/hybrids (Larica, Picasso, 1225-F1, Yaqui, 2202 and 1554), grown in different areas of district Mansehra of Khyber Pakhtunkhwa Pakistan. In this research, coat protein genes of TYLCV from six symptomatic tomato varieties were amplified and analyzed by sequencing the amplified PCR product. Four primer pairs (PRA, PAL, BEG and WTG) were used, where WTG primer pair produced positive results in two *i.e.,* Larica and Picasso. The purified PCR products were sent to MACROGENE Korea, for sequencing. The resultant sequences were bioinformatically analyzed, using online available tools. The sequence was submitted to GenBank and accession number (BankIt2535530 Sample OM100018) was allotted accordingly. The results showed that Mansehra Sample 1 (MS1) was 96% similar with Tobacco Curly Shoot Virus isolate (KM383753.1), followed by Curly Shoot Virus tomato leaf curl karnataka virus isolate begomovirus`genome assembly (KM383757.1) with 94% similarity, Tobacco Curly Shoot Virus KM383754.1(92%), Tomato Leaf Curl Virus (ToLCV) isolate from India JX987088.1 (91%), Papaya Leaf Curl Virus (PLCV) strain from India Y15934.1 (91%), Tobacco Curly Shoot Virus (TbCSV) JQ733557.1 isolate from India (91%), Tomato Leaf Curl Virus (ToLCV) KM383758.1 (90 %), Radish LCV EU19494.2 (90 %), Papaya LCV JN135233.1 (89%), Tomato LCV HG969206.1 (88 %), Crotton Yellow Vein Mosaic Virus isolate CYVMV-Del-Cr from India JN817516.1 (87%), Crotton Yellow Vein Mosaic Virus isolate CYVMV-Del-Turnip KF888655.1 (87%), Tomato Leaf Curl Virus (ToLCV) KM383765.1 (87%), Euphorbia Leaf Curl Virus (EuLCV) KC852148.1 isolate in passion fruit from China and Taiwan (87%) and Chili Leaf Curl Virus Ahmedabad virus KM880103.1 (86 %) similarity respectively. The phylogenetic analysis of sixteen species with MS1 grouped them into four clusters. Our findings clearly show the presence of a new TYLCV gene with maximum 96% and minimum 86% similarity to the online gene bank and this is the first report of presence of TYLC viruses in local tomato crop of Mansehra areas that are not reported by other studies.

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## Introduction

Tomato (*Solanum lycopersicum*) is an economical important vegetable crop that is consumed globally belongs to the family *Solanaceae* (Ahringer, 2006; Li *et al*., 2022; Wang *et al*., 2024). The cultivated tomato and its wild relative are diploid (2n=24) with similar chromosomes number of 12 (Dhaliwala *et al*., 2020). Tomato is a model plant for research on fruit developmental processes because of its short lifespan, succulent fruit and moderate genome size 900 Mb has been sequenced (Sato *et al*., 2012; Shahwar *et al*., 2023; Shirasawa and Ariizumi, 2024). It is an important commercial vegetable crop, grown in tropical, subtropical and temperate regions of the world, ranking second after potato in area and production worldwide (Lucier *et al*., 2000; Minfal, 2007; Gerhardt *et al*., 2009; Bakht and Khan, 2014; Iftikhar *et al*., 2021).

In Pakistan, it is grown both as daily based use vegetable and as commercial crop (Ayaz *et al*., 2015). Due to the natural variations in climatic conditions of different ecological zones of Pakistan, the cultivation and production of tomato spread over nearly the whole year in the country. Thus, its fresh fruit remains available in the market throughout the year. Moreover, it is a major source of health beneficial antioxidants such as vitamin C, vitamin E, carotenoids, lycopene, ascorbic acid and polyphenolic compounds (Zhu *et al*., 2018). These compounds play a vital role in human nutrition and contribute significantly to the prevention of diseases such as cancer, diabetics, cardiovascular and neurodegenerative diseases (Giovannucci, 1999; Shakoor *et al*., 2010).

In Pakistan, tomato was cultivated on an area of 68863 hectares with a production of 762736 tons and average yield of 11.076 tons per hectare (FAOSTAT, 2023). In Pakistan its average per hectare yield (11.076 ton) is very low as compare to other developed countries (Arooj *et al*., 2019). The major causes for this low yield include fluctuations in climatic conditions and diseases, as it is pruned to more than 200 diseases including viral, bacterial, fungal and nematode (Iquebal *et al*., 2013; Junaid *et al*., 2021; Khan *et al*., 2023; Lata *et al*., 2024). Tomato Yellow Leaf Curl Virus (TYLCV) is a member of the genus Begomovirus family Geminiviridae (Kumar *et al*., 2013; Xie *et al*., 2013). This TYLCV is one of the most severe pathogen causing overwhelming damage to tomato crop in tropical and subtropical regions of the world (Li *et al*., 2022). Due to fast spreading of this pathogen, the disease is now a serious problem in more than 30 countries of the world and the major affecting regions are Mediterranean basin, Southern Asia, Africa and South, Central and North America (Glick *et al*., 2009; Yan *et al*., 2021).

These begomoviruses are among the genera possessing both mono-partite (DNA-A) and dipartite (DNA-A and DNA-B) genome organization that infect most dicotyledonous plant species including tomato (Gronenbor, 2007; Briddon *et al*., 2008; Devendran *et al*., 2022). Both the mono-partite and dipartite are mainly composed of single-stranded (ss) DNA genome (Miozzi *et al*., 2014). Their genome size ranges from 2.5 to 3 kb and encodes viral factors essential for viral replication in the nuclei of the infected cells (Varma and Malathi, 2003; Baltes *et al*., 2014; Fiallo-Olivé et al. 2020). Its genome encodes 6-7 open reading frames and one of them codes for Coat Protein (CP) which indicates structure mass of viral particle (Glick *et al*., 2009). TYLCV is transmitted by a vector insects called as whitefly (*Bemisia tabaci*) and silver leaf whitefly (*Bemisia argentifolii*).

Early studies of diagnosing geminiviruses were mostly based on observations of symptoms which have been of limited use as symptoms vary greatly as function of soil, growth conditions and climate. However, the advent of DNA sequences has proved to be a tool of choice, for robust and accurate identification of viruses, their evaluation and relationship with other strains of TYLCV isolates (Kheyr-Pour *et al*., 1991; Noris *et al*., 1994; Rochester *et al*., 1994; Crespi *et al*., 1995; Hong *et al*., 1995). These high-throughput technologies allow studying the changes in gene expression upon virus infection at the genome level and further proved as powerful tools in evaluating the functions of these genes during infections (Sato *et al*., 2010).

The objective of this research was to identify the type and problem of virus occurring in local tomato and hybrid varieties in District Mansehra through high-throughput technology and find the solution. As result of this research, a new strain of virus was isolated and identified as TYLCV and named as Mansehra sample 1 (MS1) through analyzing the nucleotide sequences of TYLCV in tomato crop, grown in Mansehra Pakistan, and is reported for the first time.

## Materials and Methods

*Study area and samples collection*

Leaf samples were collected from six tomato varieties and hybrids showing typical tomato yellow leaf curly virus (TYLCV) symptom, grown in five different areas of district Mansehra Khyber Pakhtunkhwa Pakistan which include Baffa, Bajna, Murghuzar, Shinkiari and Gandia. The tomato varieties and hybrids were named as Larica, Picasso, 1225-F1, Yaqui, 2202 and 1554 (Table 1).

**Table 1:** *Names and distributors of tomato varieties used in this experiment.*

| Variety name | Distributors in Pakistan | Seed Source |
|---|---|---|
| Larica | Kisan agro, Saadat Zari | syngenta |
| Picasso | Mehar Mohd. Din and sons | Haris Morin |
| 1225 F1 | IcI Pakistan | Advanta |
| Yaqui | Mehar Mohd. Din and sons | Seminis |
| 2202 | Phool Zari Mansehra | unknown |
| 1554 | Akora seeds | Agritip Germany |

The samples were brought to laboratory of the Department of Genetics, Hazara University, Mansehra and stored at −80 °C until further processing to be carried out in the same laboratory.

*DNA extraction and amplification of TYLCV gene through PCR*

DNA was extracted from the collected samples using CTAB method (Porebski *et al.*, 1997). About 100 mg of leaf of each sample was taken. The leaf samples were crushed in 500 µl 10% CTAB extraction buffer. After crushing the mixture was centrifuged for 5 minutes at 14000 rpm. The pellet was discarded and the supernatant was taken with 50 µl of sodium acetate with final concentration of 0.3M and 500 µl isopropanol, the tubes were inverted several times. The mixture was then centrifuged at 14000 rpm for 10 minutes. The supernatant was discarded and 500 µl of ethanol (80% v/v) was added to the pellet and then centrifuged for 3 minutes at 14000 rpm. Then the pellet was air- dried over night and was dissolved in 50 µl of TE buffer. The DNA quality was checked on 1% agarose gel.

Four sets of specific primers including PAR (Bela-ong and Bajet, 2007), PAL (Bela-ong and Bajet, 2007), BEG (Wyatt and Brown, 1996) and WTG (Bhyan *et al.*, 2007) were used to amplify the TYLCV. For simplicity purpose, the six samples studied in the present study were named as Mansehra sample 1 (MS1), Mansehra sample 2 (MS2), Mansehra sample 3 (MS3), Mansehra sample 4 (MS4), Mansehra sample 5 (MS5) and Mansehra sample 6 (MS6). Where MS1 is Larica, MS2 is Picasso, MS3 is 1225 F1, MS4 is Yaqui, MS5 is 2202 and MS6 is 1554 tomato genotype.

The PCR products were analyzed on 1% agarose gel. Briefly, 3µl loading dye was mixed with 5µl of PCR product for each sample and then the samples were loaded in the wells of gel. A DNA ladder was also run along with the samples. The gel was run for a period of 40 minutes at 70V and then visualized under UV using gel doc system.

*Cleaning and sequencing of the PCR product*

TAINGEL kit was used for cleaning of PCR product as per instructions given in the kit manual. Briefly, band from gel was cut and 200 µl PN buffer was added and kept 50 min. Once completely dissolved, it was centrifuged for two minutes at 8000 rpm. The supernatant was discarded and the spin column was washed two times with 500 µl PW buffer. The empty column was centrifuged for two minutes at 8000 rpm and then dried for 20 minutes at room temperature. The cleaned eppendorf tubes were properly labeled and the columns were placed in them along with 30 µl EB buffer and stored for 10 minutes at room temperature and then centrifuged for two minutes at 8000 rpm. The cleaned PCR products of these six samples were sent to the sequencing company, MACROGEN Inc. Korea. The first 30 nucleotides were excluded from analysis because of unclear peaks. The nucleotide sequence of clear peaks up to 430 nucleotides were considered for further analysis and the sequence after 430 nucleotides was removed by trimming.

*Analysis of the gene sequences*

To check the similarity and differences among the virus strains the results of TYLCV sequencing were analyzed using bioinformatics tools. The nucleotide sequences were identified with online available BLASTn program and online ClustalW2 tool was used for multiple sequence alignments.

## Results

*Symptoms of TYLCV in diseased tomato*

Leaf samples of six tomatoes varieties and hybrids

collected from five locations in district Mansehra of Pakistan showed typical TYLCV symptoms (Figure 1A-F). The samples of Larica and Picasso varieties (Figure 1 A-B) showed upward curling and uniform chlorosis of the leaves that eventually resulted in color change from green to yellow of the leaves. Other tomato varieties (Figure 1 C-F) showed symptoms of puckering, chlorotic leaf margins and also reduction in leaf size. The severely affected plants remained stunted with growth retardation and produced no fruit or very small sized fruits.


**Figure 1:** *Symptomatic of tomato leaves of Larica (A), Picasso (B), 1225 (C), Yaqui (D), 2202 (E) and 1554 (F) infected by TYLCV selectedfrom local fields of Mansehra.*

*Cleaning of coat protein gene of TYLCV and amplification of PCR*

Figures 2 and 3 show PCR amplification products resolved in agarose gel. Among the 4 specific primers pairs, WTG primers pair showed positive results in two tomato samples of tomato i.e. MS1 and MS2 (Table 2) showed clear bands of size 1.5 kb (Figure 2), while no band was observed for rest of the samples *i.e.,* MS3, MS4, MS5 and MS6 (Figure 2).The PCR amplified DNA fragments of the respective samples were cut under UV light from the gel. The gel electrophoresis showed clear band for the respective sizes of 1.5 kb (Figure 3). The rest of the tomato samples (MS3, MS4, MS5 and MS6) did not produce any PCR product with any of the 4 sets of specific primers, revealing the absence of WTGs in these samples.

**Table 2:** *Molecular detection of TYLCV using different PCR primers*

| Primer | Locus identified by PCR in the samples | | | | | |
|---|---|---|---|---|---|---|
| | Larica | Picasso | 1225 | Yaqui | 2202 | 1554 |
| PAR | -ve | -ve | -ve | -ve | -ve | -ve |
| PAL | -ve | -ve | -ve | -ve | -ve | -ve |
| BEG | -ve | -ve | -ve | -ve | -ve | -ve |
| WTG | +ve | +ve | -ve | -ve | -ve | -ve |

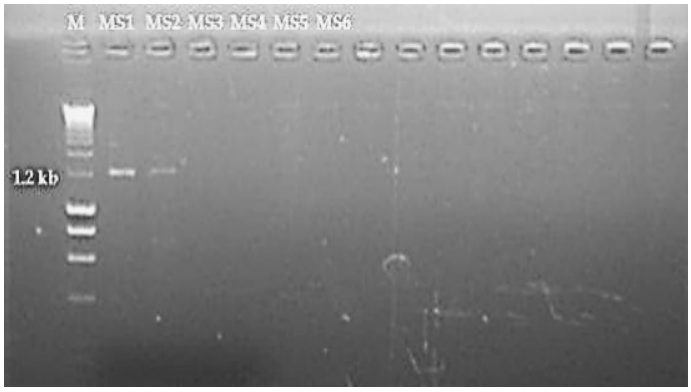


**Figure 2:** *PCR amplification of DNA fragment of TYLCV using WTG pair of primers identifying 1.2 kb fragment among the samples of MS1 and MS2. M=1 kb DNA ladder.*

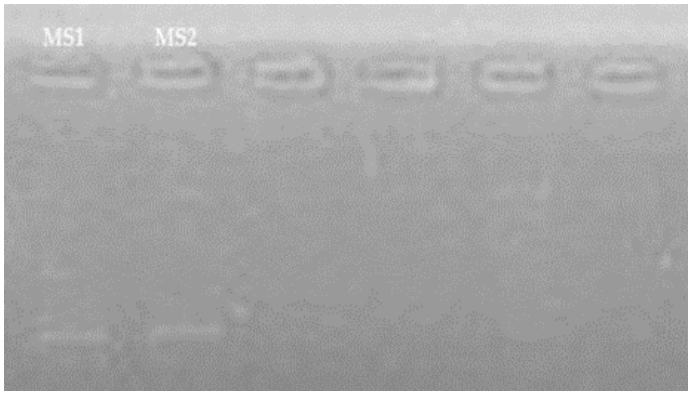


**Figure 3:** *Agarose gel electrophoresis of PCR amplified DNA fragment after gel clean.*

*equence analysis of TYLCV gene*

MS1 sample: The sequencing results and subsequent alignment results of MS1 are shown in Figures 4, 5. Eztaxon (online search tool) provided the nucleotides with best peaks. The resulted new sequence was awarded accession number (BankIt2535530 sample OM100018) by the GenBank. The sequence of *TYLCV* gene showed high similarity index with a class of viruses which infect tomato and other plant species (Table 3). The highest similarity of 96% was observed with Tobacco curly shoot virus isolate TbSCVCN (KM383753.1) isolated from Bangladesh Chittagong. This was followed by tomato leaf curl Karnataka virus isolate (KM383757.1) isolated from Bangladesh, 94%, Tobacco curly shoot virus

**Table 3:** *Pairwise correlations of TYLCV amplified coat protein gene MS1 with other reported plant viruses.*

| | NS1 | Tobacco LCV KM 383753.1 | Tomato LCV HG 969206.1 | Crotton YVM JN 817516.1 | Crotton YVM KF 888655.1 | Tomato LCV JX 987088.1 | Tomato LCV KM383758.1 | Tomato LCV KM383765.1 | Tomato CSV KM383757.1 | Papaya LCV Y15934.1 | Papaya LCV HM143914.1 | Tobacco CSV KM383754.1 | Chili CSV KM880103.1 | Radish LCV EU19494.2 | Euphorbia LCV KC852148.1 | Papaya LCV JN135233.1 | Tobacco CSV JQ733557.1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MS1** | | | | | | | | | | | | | | | | | |
| Tobacco LCV KM383753.1 | 96% | | | | | | | | | | | | | | | | |
| Tomato LCV HG969206.1 | 88% | 89% | | | | | | | | | | | | | | | |
| Crotton YVM JN817516.1 | 87% | 88% | 94% | | | | | | | | | | | | | | |
| Crotton YVM KF888655.1 | 87% | 88% | 94% | 98% | | | | | | | | | | | | | |
| Tomato LCV JX987088.1 | 91% | 90% | 98% | 90% | 90% | | | | | | | | | | | | |
| Tomato LCV KM383758.1 | 90% | 91% | 89% | 90% | 90% | 87% | | | | | | | | | | | |
| Tomato LCV KM383765.1 | 87% | 89% | 87% | 88% | 88% | 85% | 97% | | | | | | | | | | |
| Tomato CSV KM383757.1 | 94% | 95% | 90% | 92% | 92% | 88% | 94% | 91% | | | | | | | | | |
| Papaya LCV Y15934.1 | 91% | 92% | 87% | 87% | 86% | 86% | 88% | 86% | 92% | | | | | | | | |
| Papaya LCV HM143914.1 | 87% | 89% | 87% | 87% | 86% | 84% | 87% | 86% | 87% | 94% | | | | | | | |
| Tobacco CSV KM383754.1 | 92% | 94% | 88% | 87% | 86% | 86% | 90% | 88% | 92% | 97% | 92% | | | | | | |
| Chili LCV KM880103.1 | 86% | 89% | 83% | 83% | 83% | 84% | 86% | 84% | 88% | 88% | 86% | 91% | | | | | |
| Radish LCV EU19494.2 | 90% | 91% | 89% | 89% | 90% | 86% | 89% | 87% | 91% | 93% | 90% | 94% | 87% | | | | |
| Euphorbia LCVKC852148.1 | 87% | 90% | 86% | 85% | 86% | 83% | 87% | 86% | 89% | 90% | 88% | 81% | 86% | 92% | | | |
| Papaya LCV JN135233.1 | 89% | 90% | 86% | 85% | 85% | 84% | 87% | 86% | 90% | 94% | 90% | 93% | 87% | 91% | 89% | | |
| Tobacco CSV JQ733557.1 | 91% | 92% | 87% | 86% | 86% | 85% | 89% | 86% | 91% | 91% | 89% | 92% | 87% | 90% | 88% | 89% | |

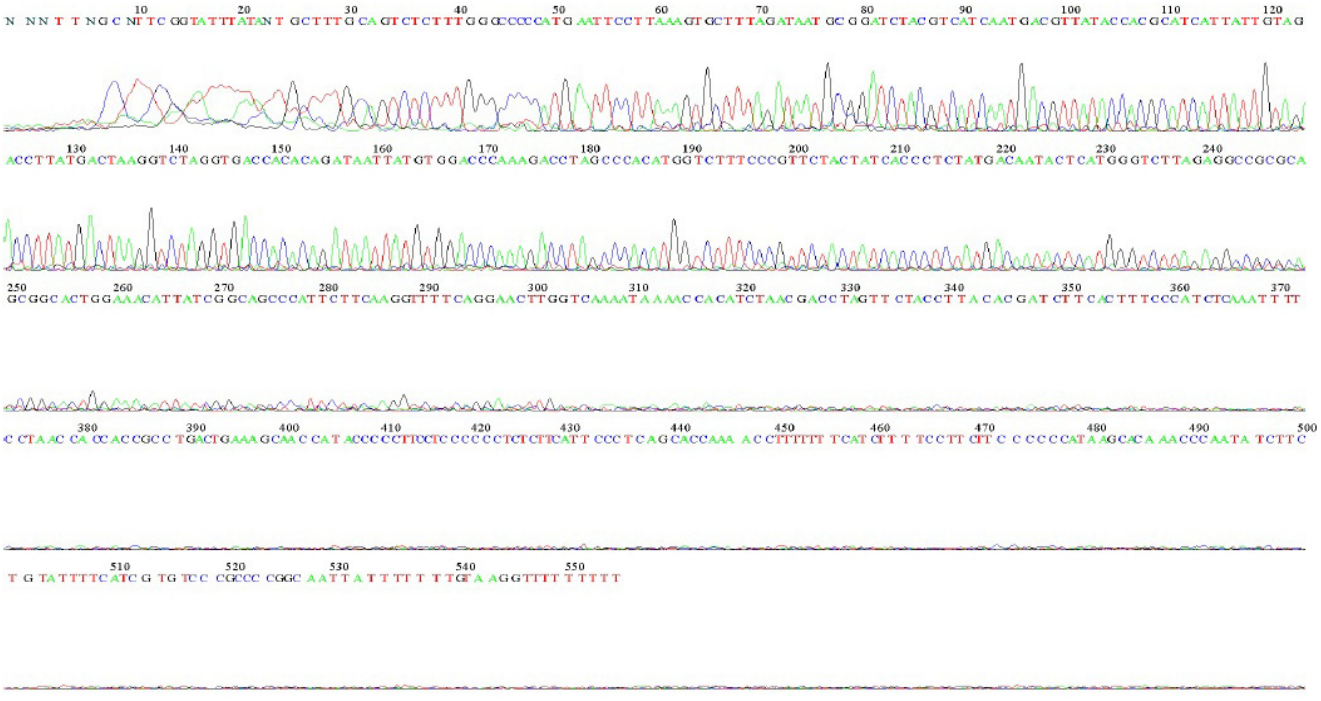

**Figure 4:** *Chromatogram of sample MS1 sequence.*

isolate TbCSV (KM383754.1) 92% isolated from Bangladesh and Tomato leaf curl Karnataka virus isolate (JX987088.1) isolated from Lucknow India, Papaya leaf curl virus AV1, AV2, AC1, AC2, AC3, AC4, AC5 genes and IR region (Y15934.1) isolated from India, Tobacco curly shoot virus isolate FB- 01 segment DNA-A, (JQ733557.1) isolated from India with 91%. Tomato Leaf Curl Virus KM383758.1 and Radish Leaf Curl Virus EU19494.2 (90 %), Papaya LCV JN135233.1 (89%), Tomato leaf curl Oman

virus complete sequence. clone Tom 80 Tomato Leaf Curl Virus HG969206.1 (88 %), Croton yellow vein mosaic virus isolate CrYVMV_Del-Cr from India JN817516.1 (87%), Croton yellow vein mosaic virus isolate CYVMV-Del-Turnip, complete genome (KF888655.1) 87%, tomato leaf curl Bangladesh virus ToLCBV-[BD:Cox:01:15:Tom:06], complete genome (KM383765.1) 87% and Euphorbia leaf curl isolates Shandong. (KC852148.1) 87% similarity**.** However, the least similarity was observed with Chili leaf curl Ahmedabad Virus-India (KM880103.1) isolated from India with 86% similarity.

*Phylogenetic analysis of MS1 gene*

To assess the genetic variability of MS1 gene, seventeen isolates of different types of plant viruses on the basis of similarity (16 selected from NCBI and MS1 from our samples) were used in the phylogenetic analysis. The analysis was conducted by using full length nucleotide sequence of each isolate of plant viruses. The dendrogram obtained from phylogenetic analysis using UGENE revealed 4 clusters (Figure 6).

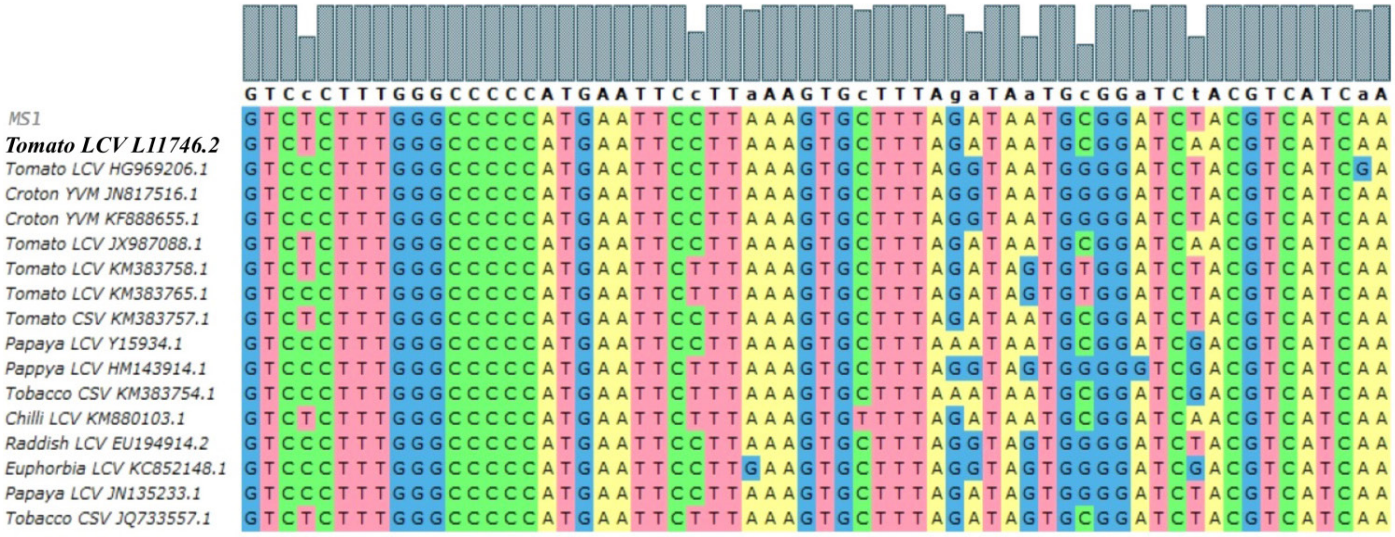


**Figure 5:** *Alignment overview of sample MS1 using UGENE.*

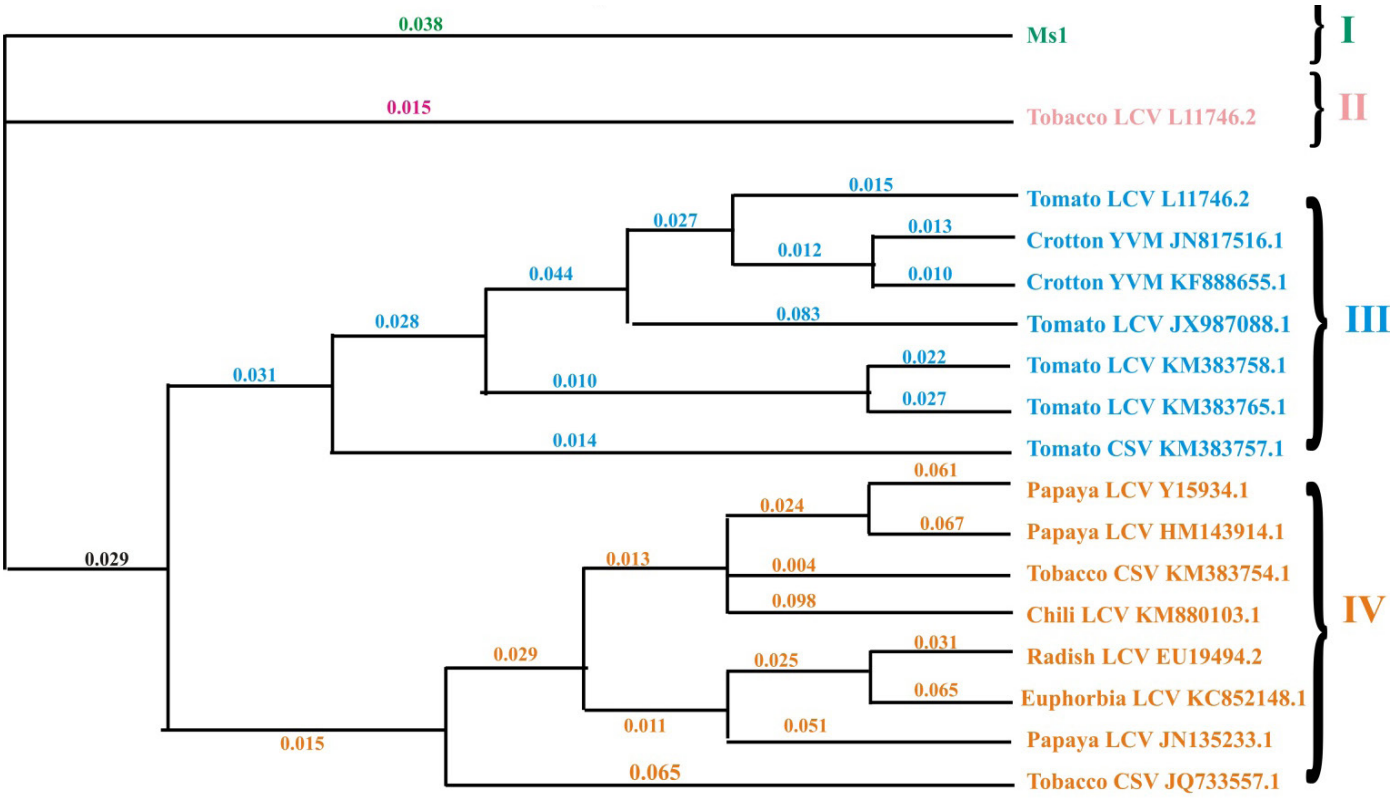


**Figure 6:** *Phylogenetic tree obtained from alignment of nucleotides sequence of TYLCV gene of Pakistani isolate (TYLCV-MS1) and other reported isolates.*

Cluster 1 showed single isolate of *TYLCV* gene which was obtained during the present study. In cluster 2 a single isolate of Tobacco curly shoot virus isolate TbSCV- (KM383753.1) isolated from Bangladesh Chittagong showed 96% similarity with that of TYLCV isolated in this study. Cluster 3 comprised of seven isolates of viruses which showing different similarity with that of TYLCV. This cluster consists of Tomato leaf curl Oman virus complete sequence clone Tom 80 (HG969206.1) 88%. Croton yellow vein mosaic virus isolate CrYVMV_Del-Cr from India, (JN817516.1) 87%, Croton yellow vein mosaic virus isolate CYVMV-Del-Turnip, complete genome (KF888655.1) 87%, Tomato leaf curl Karnataka virus isolate Lucknow segment DNA-A, (JX987088.1) 91% isolated from Lucknow India. Tomato leaf curl Bangladesh virus isolate ToLCBV-[BD:Syl:01:21:Tom:06],(KM383758.1) 87% isolated from Bangladesh. Tomato leaf curl Bangladesh virus isolate ToLCBV-[BD:Cox:01:15:Tom:06], complete genome (KM383765.1) 87% and Tomatoleaf27curl Karnataka virus isolate begomovirus genome assembly, segment: I (KM383757.1) isolated from Bangladesh with 94% similarity with our sample. Cluster 4 is composed of eight isolates of virus which have different similarity level with our amplified sequence of TYLCV. This cluster include Papaya leaf curl virus, such as AV1, AV2, AC1, AC2, AC3, AC4, AC5 genes and IR region (Y15934.1) 91% isolated from India. Papaya leaf curl virus segment DNA-A, (HM143914.1) 87%. Tobacco curly shoot virus isolate TbCSV-CN [BD:Raj:01:24:Tom:10], (KM383754.1) isolated from Bangladesh. 92%, Chili leaf curl Ahmedabad virus–India [India/Ahmedabad/2014], (KM880103.1) 88% isolated from India. Radish leaf curl isolates Pusa Bihar, (EU194914.2) 90%. Euphorbia leaf curl isolates Shandong. (KC852148.1) 87%, Papaya leaf curl isolate Lucknow segment DNAA, (JN135233.1) 89%. Tobacco curly shoot virus isolate FB-01 segment DNA-A, and (JQ733557.1) isolated from India with 91% similarity (Table 3).

## Discussion

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop of *Solanaceae* family after potato that is grown throughout the world (Bakht and Khan, 2014; Asif *et al*., 2022; Wang *et al*., 2024). It is used in various forms such as fresh in cooking and salad as well as in processed form such as tomato ketch up (Khan *et al*., 2021). Use of tomato as a diet can also help in preventing several diseases such as diabetes, cancer and cardiovascular diseases as tomato is a main source of antioxidants such as carotenoids, lycopene, vitamins A, C, E, K, and phenolic compounds that play a vital role in human diet and also helps in prevention of these diseases (Shahidi *et al*., 2011; Bacanli *et al*., 2017; Coelho *et al*., 2023). Although, tomato is an important vegetable crop of Pakistan and is widely grown as a daily based used vegetable in most parts of the country, still its yield and fruit quality is greatly influenced by over 200 diseases caused by fungi, bacteria and viruses (Wani, 2011). Among them, viral diseases are the most important biotic constraints that affect fruit quality and reduce yield (Kumar and Kumar 2018). The most noticeable viral diseases that influence tomato crop include *Tomato golden mosaic virus*, *Tomatochlorotic mottle virus*, *Tomato rugose mosaic virus* (Abhary *et al*., 2007), *Tomato spottedwilt virus* (German *et al*., 1992), *Tomato mottle virus* (Simone *et al*., 1990), *Pepino mosaicvirus* (Vander-Vlugt *et al*., 2000). Most of the viral diseases that infect tomato crop belong to the family Geminiviridae. *Tomato yellow leaf curl virus* (TYLCV) also belongs to this family and is responsible for up to 100% yield loss in several tropical and subtropical regions (Pandey *et al*., 2010; Majid *et al*., 2023). Globally tomato yellow leaf curl virus disease is among the common devastating plant disease observed in several countries of the world (Kumar and Kumar 2018). Tomato yellow leaf curl virus is the most damaging virus for global tomato production and has become one of the most studied plant virus due to its vast economic importance (Li *et al*., 2022). This disease was first identified in Israel in 1960 and then became the most notorious disease in most regions of the world (Czosnek and Laterrot, 1997). Naturally, TYLCV is transmitted through whitefly (*Bemisia tabaci* Gennadius Hemiptera: *Aleyrodidae*) with monopartite ssDNA (Moriones and Navas-Castillo, 2000). Therefore, the use of molecular techniques is essential for proper detection and analysis of the viruses and effective management of the disease. Although, virus specific drug development is a costly and time consuming process, but it is the need of the day for effective management of the disease and the first step towards drug development is the *in silico* identification of reliable inhibitors (Prajapat *et al*., 2011; Prasad *et al*., 2012; Shikhi *et al*., 2013).

The present study was conducted with the objective to detect the presence of TYLCV in tomato fields of

Mansehra by analyzing the tomato samples through high-throughput analysis. During this study, the samples were collected on the basis of symptoms like inward curling, uniform chlorosis and yellowing of the leaves, stunted growth and puckering from five locations (Baffa, Bajna, Murghuzar, Shinkiari and Gandia) in Mansehra District of Hazara Division Khyber Pukhtunkhwa Province and evaluated for the presence of TYLCV. The samples of Larica and Picasso (Figure 1A, B) showed the symptoms of upward curling and uniform chlorosis of the leaves and resulted in the yellowing of the leaves as reported by Bela-ong and Bajet. (2007); Zambrano *et al.* (2007); Ghimirey *et al.* (2024). While other tomato plants (Figure 1C-F) showed puckering, chlorotic leaf margins and reduced leaf size. Severely affected plants were stunted due to retardation of growth and produced no fruit, or in some cases produced small size fruits as reported by several other investigators (Ajlan *et al.*, 2006; Zambrano *et al.*, 2007; Petrov *et al.*, 2024). Although all the tomato samples showed the typical symptoms of tomato TYLCV but among the four specific primers pairs, WTG primers pair showed positive results in only two samples of tomato i.e. MS1 and NS2 (Table 2) and showed clear bands of size 1.5 kb (Figure 2) while no band was observed for the other four samples *i.e.,* MS3, MS4, MS5 and MS6 (Figure 2). The rest of the tomato samples (MS3, MS4, MS5 and MS6) did not produce any PCR product with any of the 4 sets of specific primers, revealing the absence of WTGs in these samples or strainal differences in the causal virus due to changes at nucleotide level (Verma *et al.*, 2016). Yet the possibility of leaf curl symptoms typical of geminivirus infection cannot be ignored. The WTGs may be present in the leaves samples but they could not detect by PCR due to contaminants in the total nucleic acids used as templates (Bela-ong and Bajet, 2007).

From the pairwise comparison and correlation analysis, it was observed that tomato TYLCV MS1 has maximum 96% similarity with Tobacco curly shoot virus isolate TbCSV-CN [BD:Chi:02:14:Tom:06] (KM383753.1) isolated from Bangladesh Chittagong (https://www.ncbi.nlm.nih.gov/nuccore/KM383753.1/) followed by 94% similarity with Tobacco curly shoot virus isolate TbCSV-CN[BD:Cox:02:16:Tom:06] segment DNA-A, complete genome accession number (KM383757.1) isolated from Bangladesh submitted to NCBI by Akhond *et al.* (2014) (https://www.ncbi.nlm.nih.gov/nuccore/KM383757.1/) and the minimum similarity of 86% was observed with Chilli LCV(KM880103.1) Ahmedabad-India virus submitted by Chahwala *et al.* (2014) to NCBI (https://www.ncbi.nlm.nih.gov/nuccore/KM880103.1/) (Table 3 and Figure 5).

Our results confirmed the association of a begomovirus with TYLCV. Based on high nucleotide sequence identity (96% for DNA-B and 94% for DNA-A) with the strains of TbCSV-CN and the demarcation criteria in species demarcation (Fauquet *et al.*, 2008), the MS1 sample of Begomovirus isolated from diseased tomato is considered as a variant of TbCSV-CN and we suggest the name Mansehra sample 1 (MS1).

The sequence comparison was also estimated through cluster analysis to observe the difference among different isolates of plant viruses. The same method was also used by Ghanim and Czosnek (2000) studying maize streak viruses coat protein gene in sugarcane. The Clustal W series of programs are mainly used in molecular biology for carrying out automatic multiple alignments of nucleotide or amino acid sequences and for preparing phylogenetic trees (Chenna *et al.*, 2003). The MS1 sample TYLCV was compared with 16 other viruses isolates by sequence alignments. The resulted phylogenetic tree separated the 17 isolates into 4 clusters including our isolate (Figure 6). However, our sample was grouped in a separate cluster (Cluster I, Figure 6) than the other. The clustering in separate cluster of MS1 sample of TYLCV identified during the current study further strengthen the idea that MS1 is a new strain of viruses infecting tomato crop in Pakistan and has not been previously reported by other studies.

## Conclusions and Recommendations

To our knowledge, this is the first report of a TYLCV variant infecting tomato crop in Mansehra. Based on the finding of the present study it is concluded that TYLCV genes are present in the tomatoes cultivated in Mansehra area of Khyber Pakhtunkhwa Province of Pakistan. The results obtained from sequencing of our samples and their pairwise comparison with different classes of plant viruses clearly indicates the presence of novel species of TYLCV viral gene different from the existing genes pool of Gemini viruses. From the pairwise sequence comparison and correlation analysis, it was observed that tomato TYLCV MS1

has maximum 96% similarity with Tobacco curly shoot virus isolate TbCSV-CN and is considered as a variant of TbCSV-CN and we suggest the name MS1 (Mansehra sample 1).

The phylogenetic analysis clustered the MS1 gene in a separate cluster in the dendrogram which further strengthen the theory of novelty of this gene. For future perspectives it is recommended on the basis of the results of the present research that more research must be performed to investigate all the species of viruses which cause the TYLCV in tomato and its alternative hosts in Mansehra. The research paper highlights the evolution of new strain of TYLCV through high throughput analysis and also emphasizes on new methods for development of antiviral strategies utilizing the genomic content of infecting virus. The farmers should also be informed about the precautionary measures of this disease and its alternate host before cultivating tomato.

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## Novelty Statement

To the best our knowledge it is the first time that our findings clearly show the presence of a new TYLCV gene with maximum 96% and minimum 86% similarity to the online gene bank and this is the first report of presence of TYLC viruses in local tomato crop of Mansehra areas of Khyber Pakhtunkhwa Pakistan that are not reported by other studies.

## Author's Contribution

**Mohib Shah**: Conceived and designed the experiment, carried out the experiment, recorded and analyzed the data and wrote the paper.
**Noorullah Khan**: Conceived the Idea, designed and supervised the research, helped in analysis, prepared the figures and Tables re-wrote and revised the manuscript and submitted and corresponded the manuscript.
**Aziz ud Din**: Supervised the research as internal supervise, provided technical support at each stage of the study and critically reviewed the manuscript.
**Sajid Ul Ghafoor**: Conceived the idea, helped in recoding and analysis of data, provided technical support at each and every stage of the research and critically review and revised the manuscript.
**Sajjad Ullah Khan**: Helped in carrying out the experiment, recording data, and critically reviewed and revised the manuscript.
**Sabaz Ali Khan**: Helped in molecular analysis and critically reviewed and re-wrote the manuscript.

*Conflict of interest*
The authors have declared no conflict of interest.

## References

Abhary, M., B. Patil and C. Fauquet. 2007. Molecular biodiversity, taxonomy and nomenclature of tomato yellow leaf curl-like viruses. In: Czosnek, Henryk (Ed). Tomato yellow leaf curl virus disease, management, molecular biology, breeding for resistance, p. 85-118. https://doi.org/10.1007/978-1-4020-4769-5_6

Ahringer, J., 2006. Reverse genetics, wormbook, ed. "The C. elegans Research Community", V. Ambros. ed. https://doi.org/10.1895/wormbook.1.47.1

Ajlan A.M., G.A.M. Ghanem and K.S.A. Salam. 2006. Tomato yellow leaf curl virus (TYLCV) in Saudi Arabia: Identification, partial characterization and virus-vector relationship. Arab J. Biotech., 10(1): 179-192.

Akhond, M.A.Y., M.R. Kabir, A. Muqit and V. Mulholland. 2014. Survey and characterization of tomato infecting Geminiviruses in Bangladesh. https://www.ncbi.nlm.nih.gov/nuccore/KM383757.1

Arooj, S., Y. Iftikhar, M. Mubeen, M.I. Ullah, A. Sajid, S. Ali, Q. Shakeel, M. Aatif, W. Raza, I.R. Noorka and H. Qudsia. 2019. Effect of environmental factors on biochemical properties of tomato leaf curl virus infected leaves of tomato. Pak. J. Phytopathol., 31: 105-111. https://doi.org/10.33866/phytopathol.031.01.0467

Asif, M., A.A. Shahid and N. Ahmad. 2022. Ganoderma lucidum as a biocontrol agent for management of Alternaria solani, a pathogen of early blight of tomato. Sarhad J. Agric., 38(2): 734-741. https://doi.org/10.17582/journal.sja/2022/38.2.734.741

Ayaz, M., R. Ahmad, M. Shahzad, N. Khan, M.M. Shah and S.A. Khan. 2015. Drought stress stunt tomato plant growth and up-regulate expression of *SlAREB, SlNCED3*, and *SlERF024* genes. Sci. Hortic., 195: 48–55. https://doi.org/10.1016/j.scienta.2015.08.025

Bacanli, M., N. Başaran and A.M. Başaran. 2017. Lycopene: Is it beneficial to human health as an antioxidant. Turk. J. Pharm. Sci., 14: 311-318. https://doi.org/10.4274/tjps.43043

Bakht, T. and I.A. Khan. 2014. Weed control in tomato (*Lycopersicum esculentum* Mill.) through mulching and herbicides. Pak. J. Bot., 46: 290–293.

Baltes, N.J., J. Gil-Humanes, T. Cermak, P.A. Atkins and D.F. Voytas. 2014. DNA replicons for plant genome engineering. Plant Cell, 26: 151-163. https://doi.org/10.1105/tpc.113.119792

Bela-ong, D.B. and N.B. Bajet. 2007. Molecular detection of whitefly-transmissible geminiviruses (Family *Geminiviridae*, Genus *Begomovirus*) in the Philippines. Philipp. J. Sci., 136(2): 87-101.

Bhyan, S.B., M.A.H. Chowdhury, M.M. Alam, M.S. and M.S. Ali. 2007. Incidence and severity of tomato yellow leaf curl virus under phytopesticidal management. Int. J. Agric. Res., 2: 590-598. https://doi.org/10.3923/ijar.2007.590.598

Briddon, R.W., J.K. Brown, E. Moriones, J. Stanley, M. Zerbini, X. Zhou and C.M. Fauquet. 2008. Recommendations for the classification and nomenclature of the DNA-B satellites of *Begomoviruses*. *Arch. Virol.*, 153: 763–781. https://doi.org/10.1007/s00705-007-0013-6

Chahwala,F.D., B.S. Bhatt, S. Rathore and A.K. Singh. 2014. Chilli leaf curl Ahmedabad virus clone pChAhm-KpnI2, complete genome. Submitted (06-OCT-2014) School of Life Sciences, Central University of Gujarat, Sector-30, Gandhinagar, Gujarat 382030, India. https://www.ncbi.nlm.nih.gov/nuccore/KM880103.1/

Chenna, R., H. Sugawara, T. Koike, R. Lopez, T.J. Gibson, D.G. Higgins and J.D. Thompson. 2003. Multiple sequence alignment with the Clustal series of programs. Nucl. Acids Res., 31: 3497. https://doi.org/10.1093/nar/gkg500

Coelho, M.C., A.S. Rodrigues, J.A. Teixeira and M.E. Pintado. 2023. Integral valorisation of tomato by-products towards bioactive compounds recovery: Human health benefits. Food Chem., 410: 135319. https://doi.org/10.1016/j.foodchem.2022.135319

Crespi, S., E. Noris, A.M. Vaira and G.P. Accotto. 1995. Molecular characterization of cloned DNA from a tomato yellow leaf curl virus isolate from Sicily. Phytopathol. Medit., 34(2): 93-99.

Czosnek, H. and H. Laterrot. 1997. A worldwide survey of tomato yellow leaf curl viruses. Arch. Virol., 142: 1391-1406. https://doi.org/10.1007/s007050050168

Devendran, R., T. Namgial, K.K. Reddy, M. Kumar, F. Zarreen and S. Chakraborty. 2022. Insights into the multifunctional roles of geminivirus-encoded proteins in pathogenesis. Arch. Virol., 167(2): 307-326. https://doi.org/10.1007/s00705-021-05338-x

Dhaliwal, M.S., S.K. Jindal, A. Sharma and H.C. Prasanna. 2020. Tomato yellow leaf curl virus disease of tomato and its management through resistance breeding: A review. J. Hortic. Sci. Biotechnol., 95(4): 425-444. https://doi.org/10.1080/14620316.2019.1691060

FAOSTAT, 2023. http://www.fao.org/faostat/en/#data/QC

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M. Zerbini and X. Zhou. 2008. Geminivirus strain demarcation and nomenclature. Arch. Virol., 153: 783-821. https://doi.org/10.1007/s00705-008-0037-6

Fiallo-Olivé, E., L.L. Pan, S.S. Liu and J. Navas-Castillo. 2020. Transmission of begomoviruses and other whitefly-borne viruses: Dependence on the vector species. Phytopathology, 110(1): 10-17. https://doi.org/10.1094/PHYTO-07-19-0273-FI

Gerhardt, K.E., X.D. Huang, B.R. Glick and B.M. Greenberg. 2009. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Sci., 176: 20-30. https://doi.org/10.1016/j.plantsci.2008.09.014

German, T.L., D.E. Ullman and J.W. Moyer. 1992. Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. Ann. Rev. Phytopathol., 30: 315-348. https://doi.org/10.1146/annurev.py.30.090192.001531

Ghanim, M. and H. Czosnek. 2000. Tomato yellow leaf curl geminivirus (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. J. Virol., 74(10). https://

doi.org/10.1128/jvi.74.10.4738-4745.2000

Ghimirey, V., J. Chaurasia and S. Marahatta. 2024. Plant nutrition disorders: Insights from clinic analyses and their impact on plant health. Agric. Ext. Dev. Count., 2(1): 09-17. https://doi.org/10.26480/aedc.01.2024.09.17

Giovannucci, E., 1999. Tomatoes, tomato based product, lycopane and cancer. Review of the epidemiologic literature. J. Natl. Cancer Inst., 91: 317-331. https://doi.org/10.1093/jnci/91.4.317

Glick, E., Y. Levy and Y. Gafni. 2009. The viral etiology of tomato yellow leaf curl disease. A review. Plant Prot. Sci., 45: 81–97. https://doi.org/10.17221/26/2009-PPS

Gronenbor, B., 2007. The tomato yellow leaf curl virus genome. In: Tomato yellow leaf curl virus disease: Management, molecular biology, breeding for resistance, (ed. H. Czosnek). 8(1): 67–84. Springer, Netherlands, 2007. https://doi.org/10.1007/978-1-4020-4769-5_5

Harrison, B.D. and D.J. Robinson. 1999. Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). Ann. Rev. Phytopathol., 37: 369-398.

Hong, Y.G. and B.D. Harrison. 1995. Nucleotide sequences from tomato leaf curl viruses from different countries: Evidence for three geographically separate branches in the evolution of the coat protein of whitefly-transmitted geminiviruses. J. Gen. Virol., 76(2): 43-49. https://doi.org/10.1099/0022-1317-76-8-2043

Iftikhar, Y., M. Mubeen, A. Sajid, M. Zeshan, Q. Shakeel, A. Abbas and H. Anwaar. 2021. Effects of tomato leaf curl virus on growth and yield parameters of tomato crop. Arab J. Plant Prot., 39(1).

Iquebal, M., A.V. Sarika, V.N. Arora, N. Verma, A. Rai and D. Kumar. 2013. First whole genome based microsatellite DNA marker database of tomato for mapping and variety identification. BMC Plant Biol., 13: 197-203. https://doi.org/10.1186/1471-2229-13-197

Junaid, M., M. Ahmad and Saifullah. 2021. Evaluating soil amendments for the management of bacterial wilt disease of tomato. Sarhad J. Agric., 37(2): 690-696. https://doi.org/10.17582/journal.sja/37.2.690.696

Khan, A.A., G. Qadar, A.A. Abro and M. Awais. 2023. Tomato yield losses due to attack of insects/pests in Pakkhal Valley of District Mansehra Khyber Pakhtunkhwa, Pakistan. Sarhad J. Agric., 39(1): 21-28. https://doi.org/10.17582/journal.sja/2023/39.1.21.28

Khan, S., S. Ullah and M. Sajid. 2021. Effect of selenium on growth and chemical properties of tomato hybrid salar F1. Sarhad J. Agric., 37(2): 444-455. https://doi.org/10.17582/journal.sja/2021/37.2.444.455

Kheyr-Pour, A., M. Bendahmane, M. Matzeit, G.P. Accotto, S. Crespi and B. Gronenborn. 1991. Tomato yellow leaf curl virus from Sardinia is a white¯y-transmitted monopartite geminivirus. Nucl. Acids Res., 19(24): 6763-6769. https://doi.org/10.1093/nar/19.24.6763

Kumar, J., P.S. Sudhir, A. Kumar, J.A. Khan and R. Tuli. 2013. Detection and characterization of a new betasatellite: variation in disease symptoms of tomato leaf curl Pakistan virus-India due to associated betasatellite. Arch. Virol., 158: 257–261. https://doi.org/10.1007/s00705-012-1486-5

Kumar, P. and M. Kumar. 2018. Leaf curl disease: A significant constraint in the production of tomato in India. https://doi.org/10.5772/intechopen.76049

Lata, S., Z. Hussain, R.K. Yadav, G.S. Jat, P. Kumar and B.S. Tomar. 2024. Insights into the genetic improvement of tomato. In: Genetic engineering of crop plants for food and health security: 2: 165-184. https://doi.org/10.1007/978-981-97-3119-0_7

Li, F., R. Qiao, X. Yang, P. Gong and X. Zhou. 2022. Occurrence, distribution, and management of tomato yellow leaf curl virus in China. Phytopathol. Res., 4(1): 28. https://doi.org/10.1186/s42483-022-00133-1

Lucier, G., B.H. Lin, J. Alsshouse and L.S. Kantor. 2000. Factors affecting tomato consumption in the United States. ERS/USDA. Vegetables and Specialties/VGS-282/November.

Majid, A., M.T. Shakeel, M.N. Aslam, W. Ashraf, M. Raheel, A. Maqsood and M.U. Shafiq. 2023. Unraveling the devastating impact of tomato leaf curl New Delhi Virus (ToLCNDV): Understanding its characteristics, host range, and management strategies. Adv. Virol., 1(2): 10-19. https://doi.org/10.58801/2k23rv2utd

Minfal, 2007. Agriculture Statistics of Pakistan. Govt. of Pakistan, Ministry of Food, Agriculture and Livestock Division, (MINFAL,) Economic Wing: 82.

Miozzi, L., C. Napoli, L. Sardo and G.P. Accotto. 2014. Transcriptomics of the Interaction between the monopartite phloem-limited geminivirus tomato yellow leaf curl sardinia virus and solanum lycopersicum highlights a role for plant hormones, autophagy and plant immune system fine tuning during infection. PLoS One, 9(2): e89951. https://doi.org/10.1371/journal.pone.0089951

Moriones, E. and J. Navas-Castillo. 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res., 71: 123–134. https://doi.org/10.1016/S0168-1702(00)00193-3

Noris, E., E. Hidalgo, G.P. Accotto and E. Moriones. 1994. High similarity among the tomato yellow leaf curl virus isolates from the western Mediterranean basin: The nucleotide sequence of an infectious clone from Spain. Arch. Virol., 135: 165-170. https://doi.org/10.1007/BF01309774

Pandey, P., S. Mukhopadhya, A.R. Naqvi, S.K. Mukherjee, G.S. Shekhawat and N.R. Choudhury. 2010. Molecular characterization of two distinct monopartite begomoviruses infecting tomato in India. Virol. J., 7: 337-346. https://doi.org/10.1186/1743-422X-7-337

Petrov, N.M., M.I. Stoyanova and R. Gaur. 2024. Viruses as stress factors and their management in vegetable crops. In: Molecular dynamics of plant stress and its management. pp. 331-350. https://doi.org/10.1007/978-981-97-1699-9_14

Porebski, S., L. Bailey and B. Baum. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Rep., 15: 8–15. https://doi.org/10.1007/BF02772108

Prajapat, R., A. Marwal, A. Sahu and R.K. Gaur. 2011. Phylogenetic and *in silico* docking studies between coat protein of mimosa yellow vein virus and whey α-lactalbumin. Am. J. Biochem. Mol. Biol., 1(3): 265-274. https://doi.org/10.3923/ajbmb.2011.265.274

Prasad, C.V.S.S., S. Gupta, A. Gaponenko and M. Dhar. 2012. *In-silico* comparative study of inhibitory mechanism of plant serine proteinase inhibitors. Bioinformation, 8(14): 673. https://doi.org/10.6026/97320630008673

Rochester, D.E., J.J. DePaulo, C.M. Fauquet and R.N. Beachy. 1994. Complete nucleotide sequence of the geminivirus tomato yellow leaf curl virus, Thailand isolate. J. Gen. Virol., 75: 477-485. https://doi.org/10.1099/0022-1317-75-3-477

Sato, S., S. Tabata, H. Hirakawa, A. Erika, S. Kenta, I. Sachiko, K. Takakazu, N. Yasukazu, S. Daisuke, A. Koh, E. Michael, K. James. B. Robert, L. Changbao, S. Yang, X. Xun, P. Shengkai, C. Shifeng, L. Xin, R. Yuanyuan, W. Jun, A. Alessandro, D. P. Francesca, T. Sara and Y.S.G. Giulio. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature, 485: 635-641. https://doi.org/10.1038/nature11119

Shahidi, F., A. Chandrasekara and Y. Zhong. 2011. Bioactive phytochemicals in vegetables. In: (eds. N.K. Sinha, Y.H. Hui, E.O. Evranuz, M. Siddiq, J. Ahmed). Handbook of vegetables and vegetable processing. Wiley-Blackwell; Ames, IA, USA: 2011. pp. 125–158. https://doi.org/10.1002/9780470958346.ch6

Shahwar, D., N. Ahn, D. Kim, W. Ahn and Y. Park. 2023. Mutagenesis-based plant breeding approaches and genome engineering: A review focused on tomato. Mutat. Res. Rev. Mutat. Res., pp. 108473. https://doi.org/10.1016/j.mrrev.2023.108473

Shakoor, A., M.A. Sabri, M. Afzal and M.H. Bashir. 2010. Role of plant morphological characters towards resistance of some cultivars of tomato against phytophagous mites (Acari) under green house conditions. Pak. J. Life Soc. Sci., 8(2): 131-136.

Shikhi, M., A.R.K. Geraldine, D.V. Scindia and L. Roopa. 2013. *In silico* target deconvolution of curcumin (Diferuloylmethane) against respiratory syncytial virus. Int. J. Agric. Environ. Biotechnol., 6: 753-758.

Shirasawa, K. and T. Ariizumi. 2024. Near-complete genome assembly of tomato (*Solanum lycopersicum*) cultivar micro-tom. Plant Biotechnol., 41(4): 367-374. https://doi.org/10.5511/plantbiotechnology.24.0522a

Simone, G.W., J.K. Brown, E. Hiebert and R.E. Cullen. 1990. New geminivirus epidemic in Florida tomatoes and peppers. Phytopathology, 80: 1063. https://doi.org/10.1094/PDIS.2000.84.1.103C

Vander-Vlugt, R., C. Stijger, J. Verhoeven, J. Verhoeven and D. Lesemann. 2000. First report

of *Pepino mosaic* virus on tomato. Plant Dis., 84: 103.

Varma, A. and V.G. Malathi. 2003. Emerging geminivirus problems: A serious threat to crop production. Ann. App. Biol., 142: 145-164. https://doi.org/10.1111/j.1744-7348.2003.tb00240.x

Verma, Y.S., S.Y. Bhardwaj and R. Rathore. 2016. Molecular diagnosis and characterization of a geminivirus causing leaf curl disease of tomato in mid hills of northern India. Int. J. Ari. Env. Biotech., 9(6): 951-965 https://doi.org/10.5958/2230-732X.2016.00122.4

Wang, Y., C. Sun, Z. Ye, C. Li, S. Huang and T. Lin. 2024. The genomic route to tomato breeding: Past, present, and future. Plant Physiol., kiae248. https://doi.org/10.1093/plphys/kiae248

Wani, A.H., 2011. An overview of the fungal rot of tomato. Mycopathology, 9(1): 1-5.

Wyatt, S.D. and J.K. Brown. 1996. Detection of subgroup III Gemini-virus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathoogy, 86: 1288-1293. https://doi.org/10.1094/Phyto-86-1288

Xie, Y., X. Jiao, X. Zhou, H. Liu, Y. Ni and J. Wu. 2013. Highly sensitive serological methods for detecting tomato yellow leaf curl virus in tomato plants and whiteflies. Virol. J., 10: 142. https://doi.org/10.1186/1743-422X-10-142

Yan, Z., A.M.A. Wolters, J. Navas-Castillo and Y. Bai. 2021. The global dimension of tomato yellow leaf curl disease: Current status and breeding perspectives. Microorganisms, 9(4): 740. https://doi.org/10.3390/microorganisms9040740

Zambrano, K., O. Carballo, F. Geraud, D. Chirinos, C. Fernandez and E. Marys. 2007. First report of Tomato yellow leaf curl virus in Venezuela. Plant Dis., 91(6): 768. https://doi.org/10.1094/PDIS-91-6-0768A

Zhu, Z., Y. Zhang, J. Liu, Y. Chen and X. Zhang. 2018. Exploring the effects of selenium treatment on the nutritional quality of tomato fruit. Food Chem., 252: 9-15. https://doi.org/10.1016/j.foodchem.2018.01.064