



Whole genome characterization, recombination, variation, satellites and phylogeny analyses of *Tomato leaf curl Palampur virus* infecting courgetti squash (*Cucurbita pepo* L.) in Iraq

Mohammed S. Mohammed* and Adnan A. Lahuf

Plant Protection Department, Agriculture College, University of Kerbala, Karbala, Iraq

*Corresponding author e-mail: mohammed.saadoun@s.uokerbala.edu.iq

<https://doi.org/10.59658/jkas.v10i3.1249>

Received:

July 17, 2023

Accepted:

Aug. 31, 2023

Published:

Sept. 10, 2023

Abstract

Durning season of 2021-2022, symptoms of leaf curl, yellowing, crumpling and stunting were observed commonly on courgetti crop in Al-Yusufiyah region, Baghdad Province, Iraq. Diseased courgetti leaves were collected randomly and sent for sequencing with Illumina MiSeq. Pairwise nucleotide analysis of the assembled contigs revealed the maximum identity of 98-99% and 95-99% with Tomato leaf curl Palampur virus (ToLCPalV) for DNA-A and DNA-B components, respectively. The DNA-A component (OQ693629.1) contains six main genes encoding six proteins, while DNA-B component (OQ693630.1) comprises only two genes encoding two proteins, with the presence of non-coding regions between genes in both the two segments. Furthermore, the recombination breakpoint analysis shows that ToLCPalV Baghdad-1/Iraq segment DNA-A (OQ693629.1) and ToLCPalV isolate Mosul segment A (OR052249.1) are the parent of the recombinant isolates: ToLCPalV isolate Kufa segment DNA-A (OP810506.1) and ToLCPalV segment A (ON254271.1) from Karbala. However, the ToLCPalV Baghdad-1/Iraq segment DNA-B (OQ693630.1) with ToLCPalV segment DNA-B (OP620406.1) from Kufa are the parent of ToLCPalV isolate Babylon1 segment DNA-B (ON229620.1). As well as, there was no evidence discovered for the association of alpha- and/or beta-satellites with ToLCPalV infecting courgetti crop. Single nucleotide polymorphisms (SNP) analysis indicates a common genetic variation within the current isolate under study and the other Iraqi isolates. Phylogenetic analysis confirms the genetic relationship between the genomics components of Iraqi ToLCPalV and various international isolates of the same virus, especially those distributed in Saudi Arabia and Iran that could be a possible the origin and introduction of ToLCPalV into Iraq.

Keywords: *Tomato leaf curl Palampur virus*, *Cucurbita pepo*, Molecular characterizations

Introduction

Courgetti squash (*Cucurbita pepo* L.) is important cucurbitaceous crop belonging to family, *Cucurbitaceae* and grown worldwide including in Iraq [1]. It was originated in North America and cultured since primeval times [2]. In Iraq, courgetti was grown in 2022 with the production of 39,000 tons [3]. Consumption of 100 g courgetti supplies 17 kcal of energy and is a good source of folate (24 µg), potassium (261 mg), provitamin A (200 IU) and vitamin C (12.9 mg) [4]. Squash and other cucurbitaceous are affected by many biotic and abiotic stresses causing significant economic yield losses [5]. The low productivity in this crop and others is mainly due to various diseases caused by fungi, bacteria, viruses, nematodes, and viruses [6, 7, 8]. Among these, the viral diseases caused by various RNA viruses and DNA viruses that are major constraint for courgetti cultivation worldwide [9, 1].

The family Geminiviridae is well-known for causing various diseases in economically important crop worldwide [10,11]. It consists of small single-stranded DNA viruses, namely Geminiviruses. These viruses have a circular single-stranded DNA genome that is encapsidated into twinned icosahedral particles. The family comprises of nine genera, differentiated based on genome structure, host range and insect vector [12]. Among these, Begomovirus is the largest group, with over 424 species reported so far and are transmitted by different cryptic species of whitefly [13,14]. The classification of Begomoviruses is based on their genome composition, with monopartite viruses possessing a single genome component and the bipartite viruses having two similar-sized components, DNA-A and DNA-B [15]. The begomoviruses can be divided into monopartite and bipartite viruses, which are also referred to as Old World (OW) and New World (NW), respectively [12]. Unlike bipartite viruses, monopartite begomoviruses are commonly associated with sub-genome segments known as alphasatellites and betasatellites. These satellites play a significant role in symptom modulation, pathogenesis, and in silencing suppressors [16]. However, recent reports indicate that bipartite begomoviruses can also be associated with DNA satellites [17, 18].

Production of vegetable crops including courgetti has drastically been reduced due to viral diseases. The common viruses involved in the infection of cucurbits are Cucumber mosaic virus, *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ring spot virus* (PRSV) and *Squash leaf curl China virus* (SLCCNV) [9]. *Tomato leaf curl Palampur virus* (ToLCPMV) has been also reported as an infectious agent of leaf curl disease in cucurbits, tomato, cucumber and muskmelon [19, 20]. This virus is relatively new introduction in agro-ecological zone of Iraq. Leaf samples of courgetti plants symptoms showing curling, yellowing, crumpling and stunting were observed commonly in the fields of Al-Yusufiyah region, Baghdad Province, Iraq. This study was conducted to identification of the whole genome characterization, recombination, variation, satellites and phylogeny analyses of the associated virus.

Materials and Methods

Leaf samples collection, RNA extraction and Illumina high-throughput sequencing

Different diseased leaf samples were collected from several courgetti fields of Al-Yusufiyah, Baghdad Province, Iraq in 2022. The leaf samples were immediately placed in DNA/RNA Shield™ (Zymo Research, USA), frozen at -20 °C and transported to the MacroGen, South Korea [21]. Based on the reports provided by MacroGen company, the total RNA was extracted from leaf samples utilizing Agilent RNA 6000 nano reagent's part 1 (Agilent Technologies, Germany) based on the manufacturer's instructions. The quantity and quality of RNA extracted were examined by Agilent 2100 and Fragment Analyzer. Preparation and high-throughput or next generation sequencing (NGS) of the RNA libraries to obtain data was accomplished on an Illumina HiSeq 3000 platform using the Metatranscriptomics method.

Sequence analysis of the NGS data, virus genome assembly and phylogenetic relationships identification

The FastQC software was operated to approve the quality of raw reads received (Babraham Bioinformatics, Cambridge, UK). These raw reads were then assembled utilizing SPAdes or/and Trinity programs to generate numerous contigs [22] that were scanned against a local virus sequence database, retrieved from NCBI viral sequences database (<https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/virus?> (Accessed in 25/9/2022) using BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (E-value < 0.01) in order to identify potential similar sequences to viral RNA sequences [11]. The RefSeq of the candidate viruses detected were imported from NCBI Reference Sequence Database. Bowtie 2 program was functioned to RefSeq-based assembly search and matched contigs that are similar to viral sequences were selected and additional annotated and reported using the Geneious Prime® 2021.1.1. [23]. The current study's sequences were also compared to those retrieved from GenBank using the Sequence Demarcation Tool (SDT version 1.2) (Muhire et al. 2014) to determine their pairwise nucleotide identity. The resulting percent pairwise identity was then calculated. The viral sequences of the predicted conserved and functional domains were recognized via InterProScan program (<https://www.ebi.ac.uk/interpro/search/sequence/>). The fundamental motifs sequences of genes and other genetic regions identified were downloaded and aligned with several similar sequences using the ClustalW method embedded in MEGA v.10.1.5 that was also operated for building of the phylogenetic trees applying the neighbor joining approach with 1000 bootstrap replicates [24, 25, 26, 27].

Investigation of presence of alpha- and/or beta-satellites

The raw reads data were also checked for the potential presence of alpha- and/or beta-satellites component applying Bowtie 2 program to RefSeq-based assembly search using the reference sequences of the main satellites: Cotton leaf curl Multan betasatellite (CLCuMuB), Cotton leaf curl Gezira virus betasatellite (CLCuGeV), Okra yellow crinkle Cameroon alphasatellite (OYCrCMA), Pepper leaf curl betasatellite

(PepLCB), and Okra leaf curl Oman betasatellite (OLCuOMB). Additional annotation and reporting using the Geneious Prime® 2021.1.1. was applied to confirm results of the analysis [18].

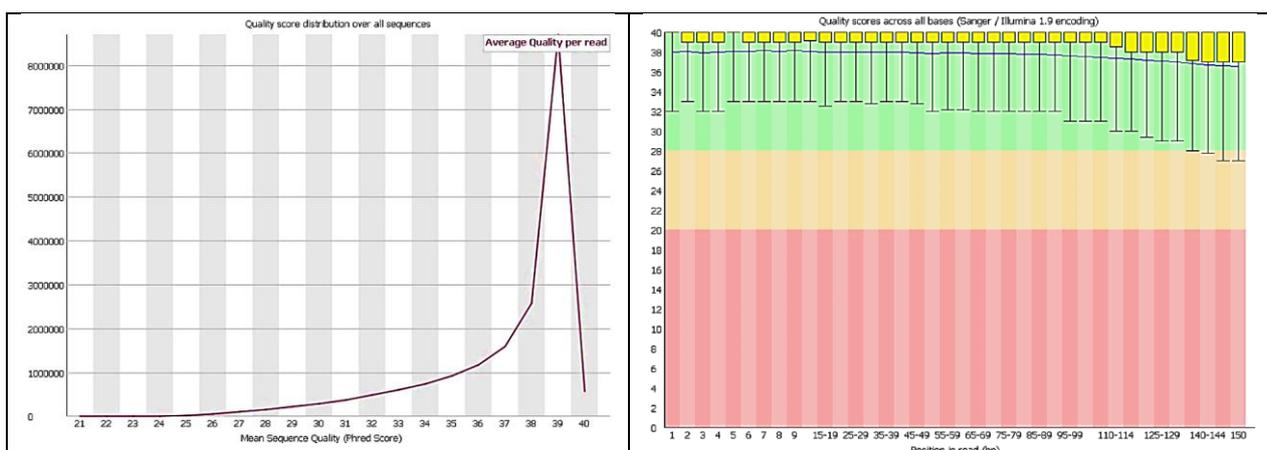
Recombination analysis and detection of SNP

To detect potential recombination events in the genomes of ToLCPaIV under study and other Iraqi isolates of the same virus. The recombination detection program (RDP v.4.101) [28]. The criteria demonstrated previously [29] was followed to accept any potential recombination event. Estimation of SNP rate was estimated in the ORFs encoded by ToLCPaIV via employing tool of find variations SNPs/INDELS in the Geneious Prime platform.

Results and Discussion

Illumina NGS Data Analysis

The results of the analysis of the quality of the raw reads of the Illumina NGS data using FastQC program (Figure 1) showed that it was suitable for analysis with bioinformatics tools and programs. The NGS data received was 41,802,108 paired ends reads, with a length of 150 base pairs for each read. As it was found that the data was of high quality, an average score of 39 degrees. This means, according to the Phred quality score, the accuracy of the sequence is more than 99.9%, meaning that the probability of an incorrect nucleotide within the sequence of the reads was 1 in 10,000. The recommended rates of Phred quality score start from 30 degrees and above to conduct the process of analysis and study of the raw reads. The analysis also proved the equality of the lengths of the raw reads, the absence of undefined nitrogen bases (N), the absence of the added adapters sequences, and the percentage of guanine and cytosine bases (GC%) for the sample was 48%.



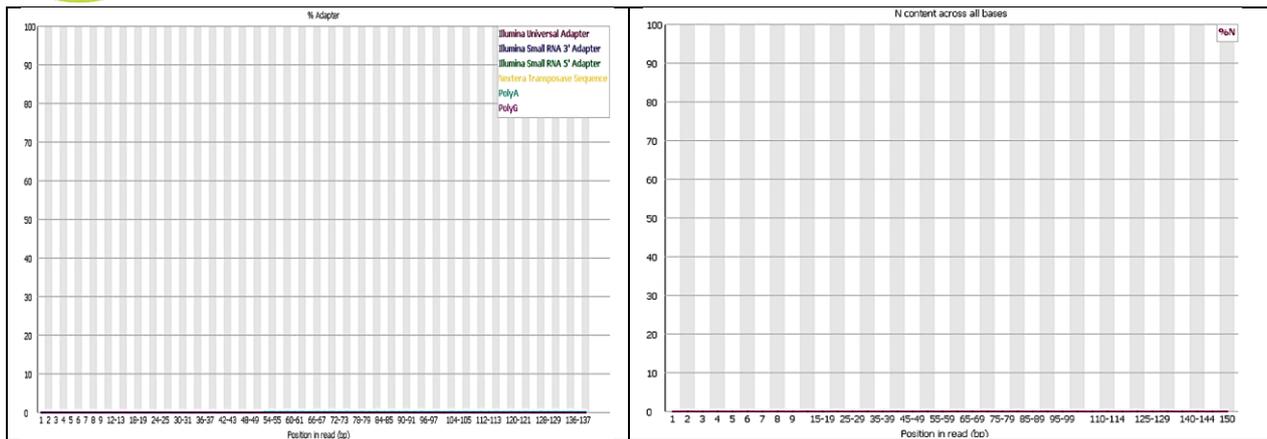


Figure (1): Quality of raw data reads using FastQC software

Sequence Comparisons and Identification of ToLCPaLV and satellites

After deletion of sequence data similar to the genome sequence of the courgetti plant (*Cucurbita pepo*; PRJNA421955), the number of raw reads remaining was 4,519,992 reads. The remaining data were assembled first using the DeNovo assembly method that produced 65,578 overlapping contigs. The comparison results of these contigs with the data of plant virus's genome sequences [14] (showed a high similarity with many plant viruses belonging to different families. As well as, the overlapping contigs of size 200 bp and above that are similar to plant virus sequences were approved, and less than that, even if they were similar to one of the virus sequences, were ignored. To confirm the accurate identification of the plant viruses that were identified from the first comparison process, the reference assembly method was applied using the reference genome of viruses [22].

The results revealed that there were many overlapping contigs similar to the first and second segments (DNA-A and DNA-B) of the genome of the bipartite ToLCPaLV virus belonging to the genus Begomovirus. It was also noted that the sequence of raw reads similar to the virus genome possesses a genetic organization identical to the genome of this virus, in that the first segment contains six genes encoding six proteins, while the second segment comprises only two genes encoding two proteins, in addition to the presence of non-coding regions between genes called intergenic region in both the two segments (Figure 2).

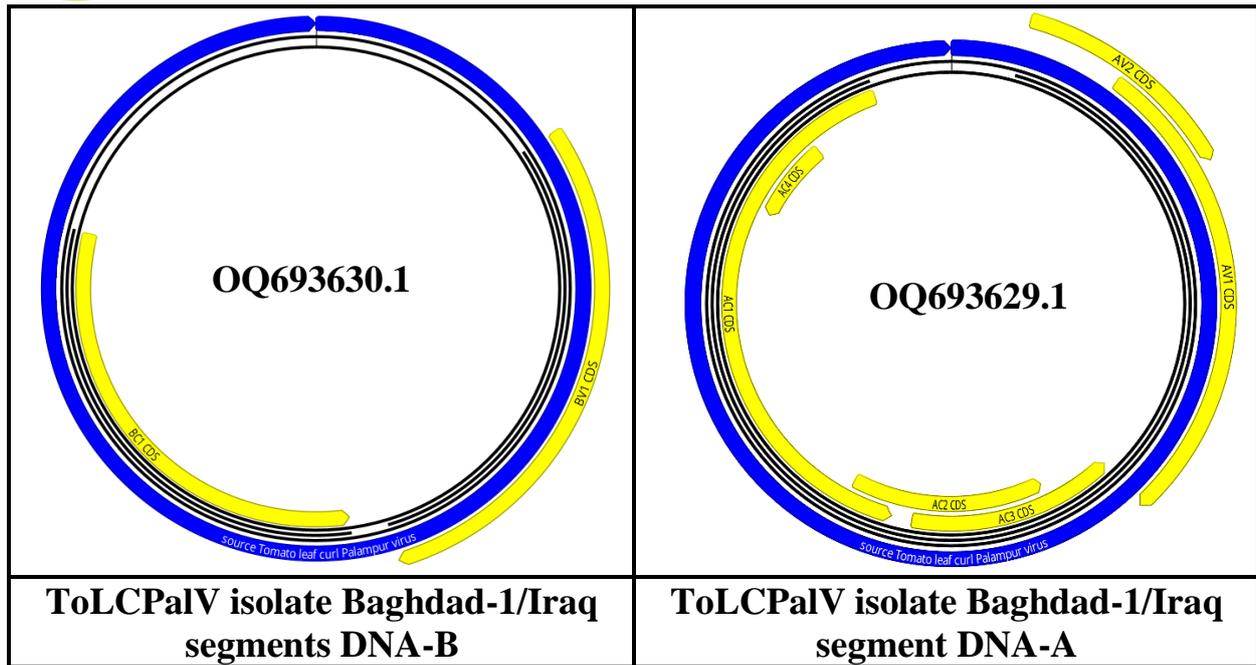
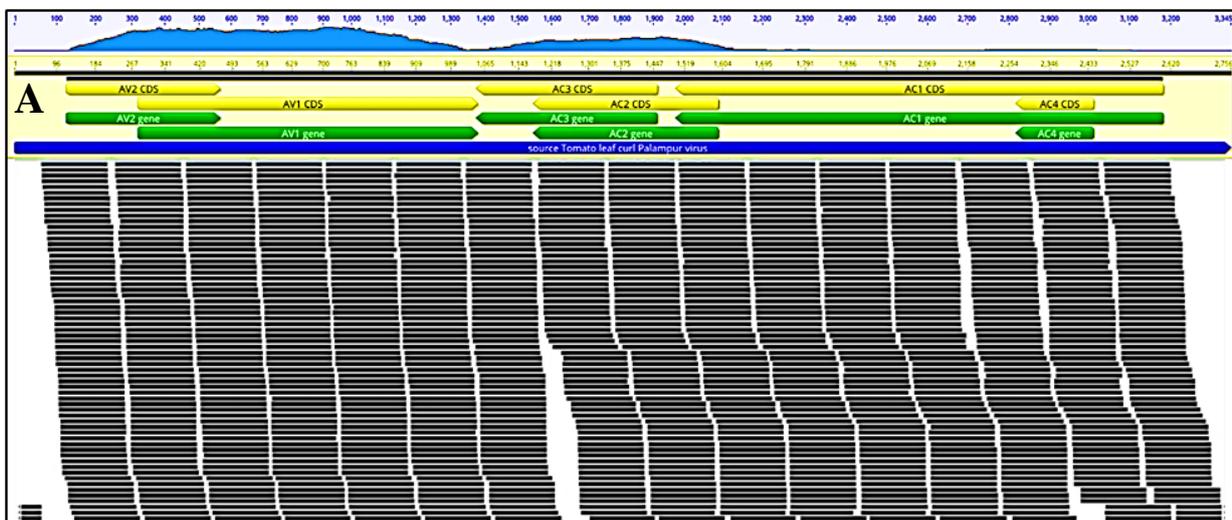


Figure (2): Genomic organization of the Iraqi isolate ToLCPaIV isolate Baghdad-1/Iraq

The analysis also displayed that the percentage of coverage of the virus genome by the raw reads sequences was 100%, while the percentage of similarity between these raw read's sequences and the virus genome sequence was 98.2% (Figure 3). As for the average coverage or depth of sequencing the whole virus genome, it reached 3198.48 X. The analysis also indicated that the presence of the virus in the infected plant was in an active state through the high rates of gene expression for genes important in the life cycle of the virus, such as the gene expressed in the DNA-A segment was AV1 and AV2, which is related to the capsid protein, and AC2 and AC3, which are related to the production and enhancing proteins related to virus replication process. Also, the gene expression ratio of BC1 and BV1 genes in the DNA-B segment, which are also related to the movement protein and pathogenicity factor protein respectively.



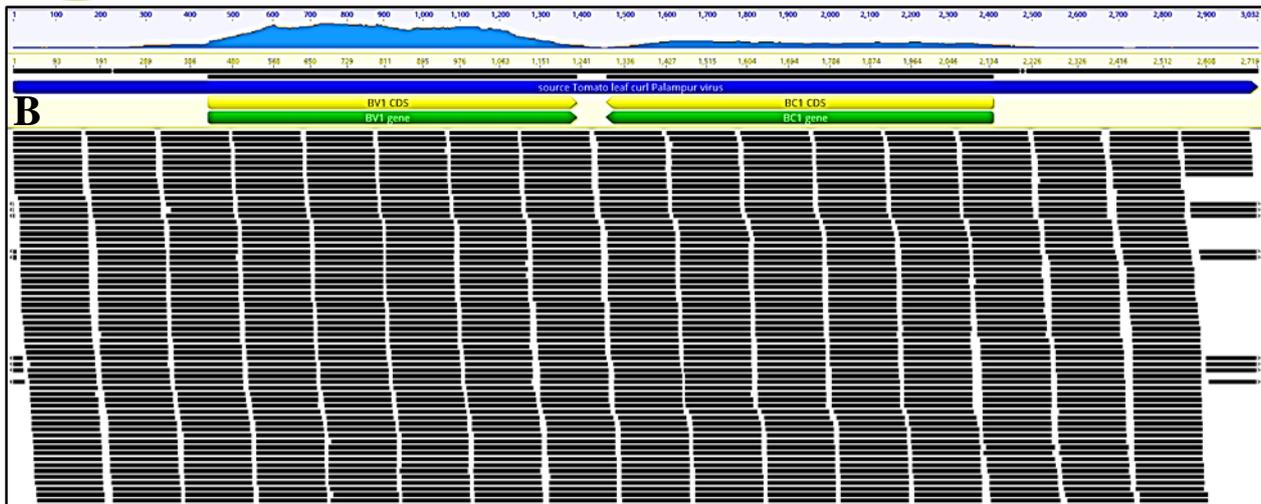


Figure (3): The raw reads similar to the DNA-A genome segment (A) and the DNA-B genome segment (B) of the Iraqi isolate of ToLCPaLV isolate Baghdad-1/Iraq

The complete genome of ToLCPaLV virus containing DNA-A and DNA-B segments of length 2,756 and 2,720 base pairs respectively was recorded in the NCBI-GenBank database under the names *Tomato leaf curl Palampur virus* isolate Baghdad-1/Iraq segment DNA-A. and *Tomato leaf curl Palampur virus* isolate Baghdad-1/Iraq segment DNA-B and under OQ693629.1 and OQ693630.1, respectively.

The results of the analysis of the pairwise nucleotide identity (Figure 4) and the phylogenetic analysis (Figure 5) of these two segments of the ToLCPaLV virus genome confirmed the extent of the genetic relationship between them and many of the global isolates and strains corresponding to them, especially those spread in Iraq, Saudi Arabia and Iran. The similarity rate was more than 98% for the isolate ToLCPaLV isolate Baghdad-1/Iraq segment DNA-A (OQ693629.1) that was with the isolate ToLCPaLV isolate Babylon1 segment DNA-A (ON229618.1) in the same branch. This indicates the great similarity between them, given that they are from the same country and infect plants belonging to the same family. This clearly indicates that they have one common ancestor. The Saudi isolates (OL416211.1 and OL416213.1) were as the other isolates close to it (Figure 28 and 29).

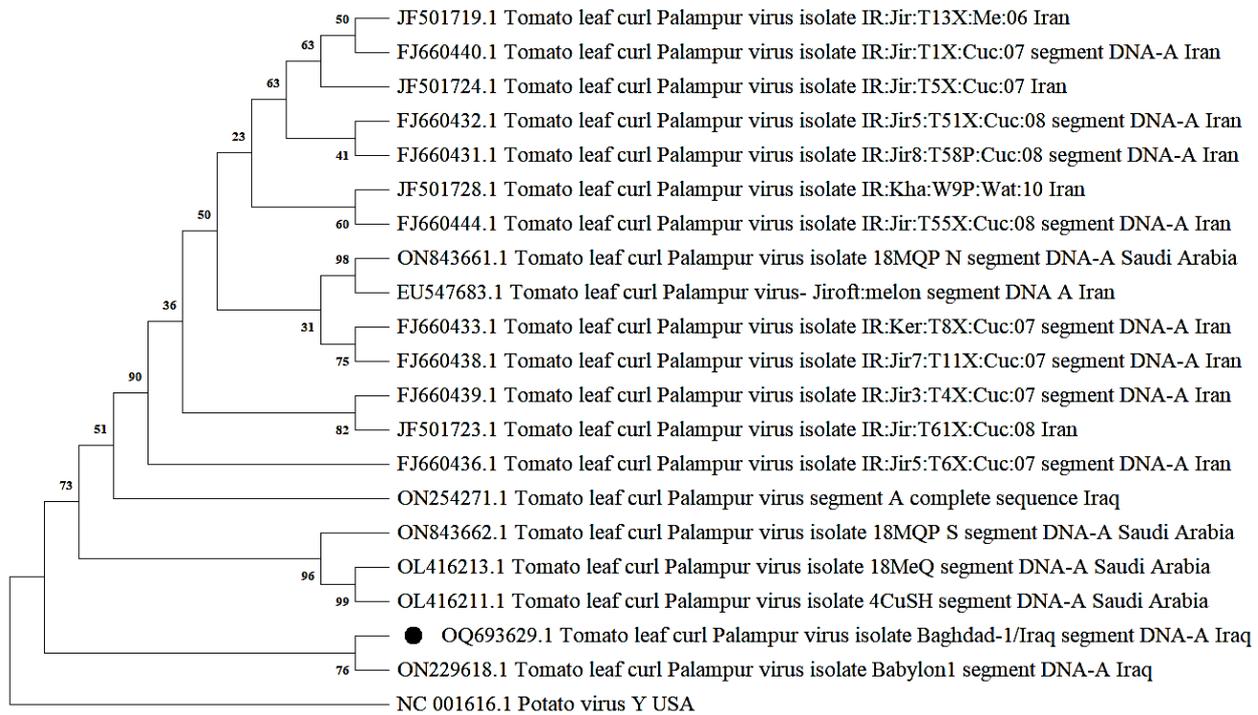


Figure (5): The genetic relationship between ToLCPaV isolate Baghdad-1/Iraq segments DNA-A (marked with a black dot) with other global strains and isolates of the same virus. Potato virus Y (NC001616.1) was out of the group to confirm the analysis.

Additionally, there was a high similarity between (95-99%; Figure 6) between the sequence of ToLCPaV isolate Baghdad-1/Iraq segment DNA-B (OQ693630.1) with a number of strains and isolates corresponding to the same genomic segment disperse in Iraq, Saudi Arabia and Iran. However, the Iraqi isolates were the closest as they were in one group within one branch in the genetic tree. This means that they have the same history of evolution and the common ancestor (Figure 7). As well as, there was no evidence discovered for the association of alpha- and/or beta-satellites with ToLCPaV infecting Courgetti crop .

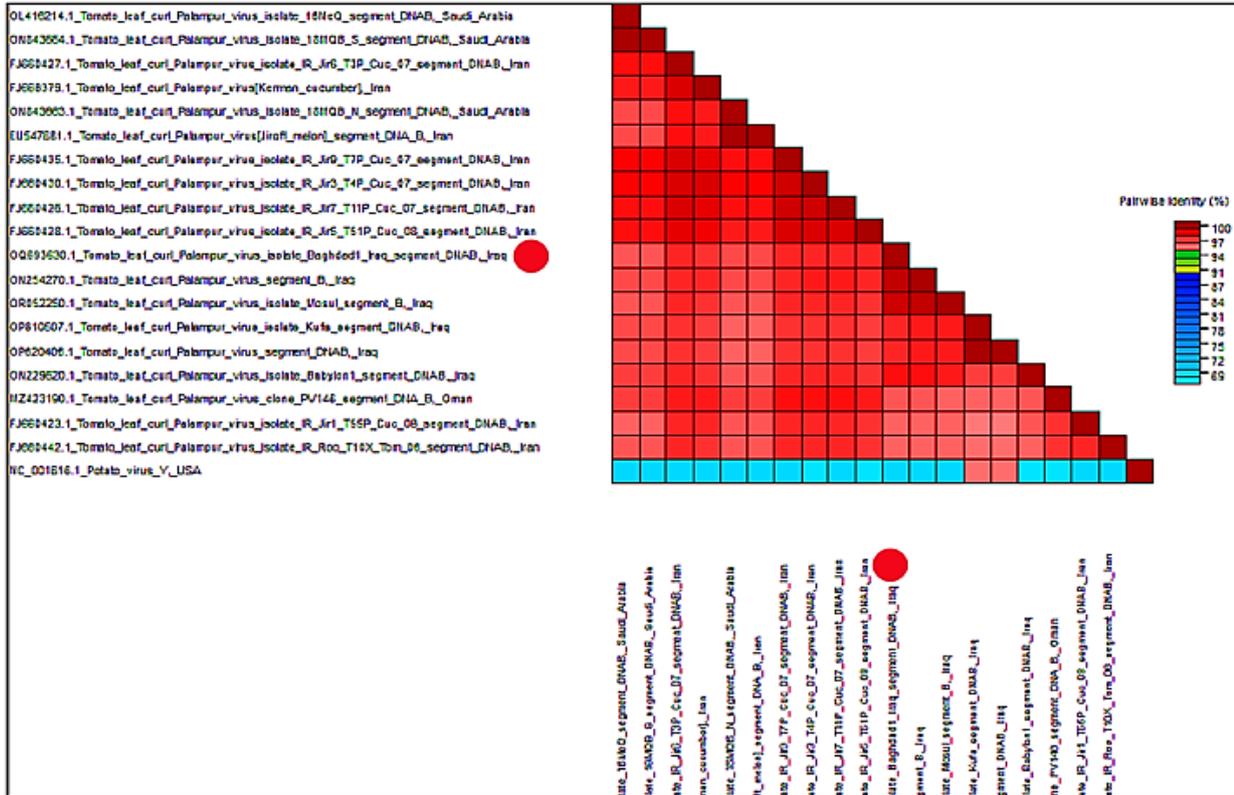


Figure (6): The similarity ratio of the nucleotide of the DNA-B segment of the Iraqi isolate of the ToLCPaLV isolate Baghdad-1/Iraq virus (marked with a red dot) with the international strains and isolates of the same virus.

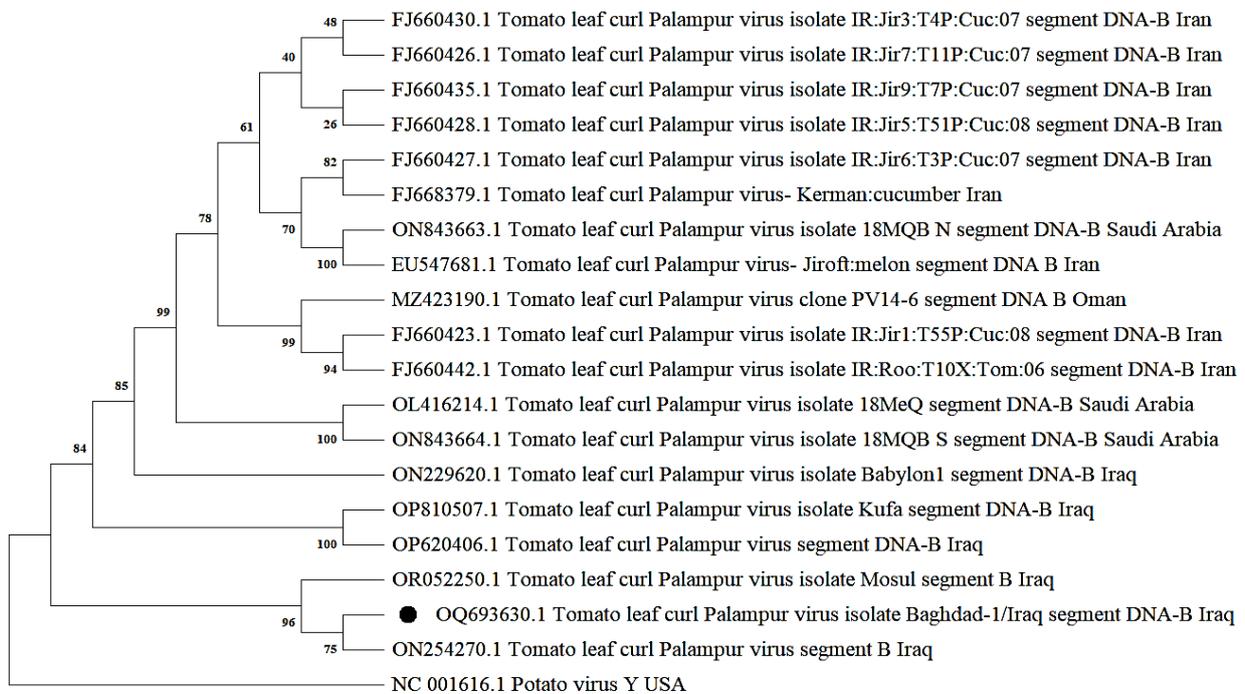


Figure (7): The genetic relationship between ToLCPaLV isolate Baghdad-1/Iraq segments DNA-B (marked with a black dot) with other global strains and isolates of the same virus. Potato virus Y (NC001616.1) was out of the group to confirm the analysis.

Identification of putative recombination and SNP events

The data obtained from RDP analysis discovered that potential recombination breakpoints were detected in both segments of the Iraqi ToLCPaIV isolates. Potentially, the ToLCPaIV isolate Mosul segment-A (OR052249.1) and the ToLCPaIV isolate Baghdad-1/Iraq segment DNA-A (OQ693629.1; identified in this study) were identified as major and minor parents respectively (Figure 5; Table 1) for the recombinants ToLCPaIV isolate Kufa segment DNA-A (OP810506.1) and ToLCPaIV segment DNA-A (ON254271.1) isolated from Karbala/Iraq. On the other hand, the ToLCPaIV isolate segment-B (OP620406.1) isolated from Kufa/Najaf and the ToLCPaIV isolate Baghdad-1/Iraq segment DNA-B (OQ693630.1; identified in this study) were found as major and minor parents respectively (Figure 5; Table 1) for the recombinant ToLCPaIV isolate Babylon1 segment DNA-B (ON229620.1).

Table (1): Recombination detection via RDP analysis

Segment	Recombinant	Breakpoints		Parents	
		Begin	End	Major	Minor
DNA-A	ToLCPaIV (OP810506.1)	1646	2538	ToLCPaIV (OR052249.1)	ToLCPaIV (OQ693629.1)
	ToLCPaIV (ON254271.1)	2428	2734	ToLCPaIV (OR052249.1)	ToLCPaIV (OQ693629.1)
DNA-B	ToLCPaIV (ON229620.1)	14	167	ToLCPaIV (OP620406.1)	ToLCPaIV (OQ693630.1)

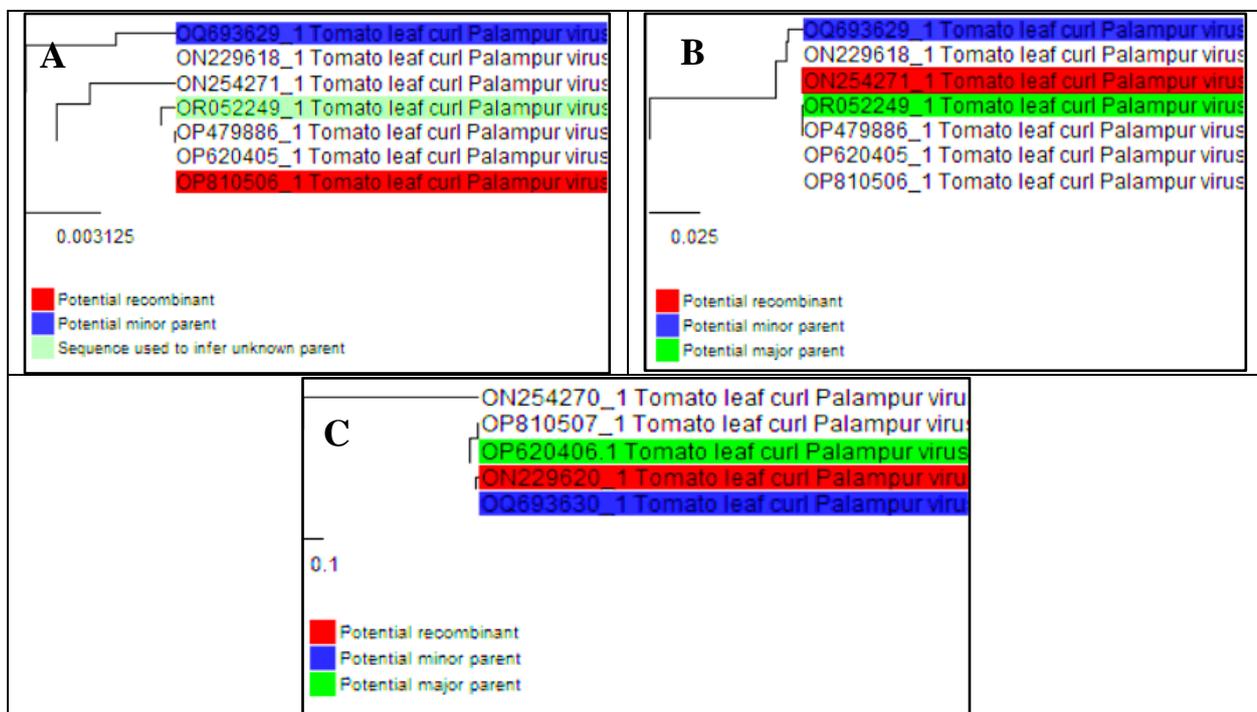


Figure (8): Discovery of recombination events operating RDP5.101. (A and B) the potential recombinants in segment DNA-A of ToLCPaIV (C) the possible recombinant in segment DNA-B of ToLCPaIV

Single nucleotide polymorphisms (SNP) analysis indicates a common genetic variation within the current isolate under study and the other Iraqi isolates. It was detected 74 SNPs in segment DNA-A, while in segment DNA-B there were 138 SNPs. However, the most interesting SNP is located in the position 2706 bp that was identified in the four Iraqi isolates (OQ693629.1, OR052249.1, OP479886.1, and OP620405.1) infecting courgetti crop only in Iraq (Figure 9). This SNP could be related to host specificity. Hence, further studies are needed to investigate this point.

Identity	2,680	2,690	2,700
1. OQ693629.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	T G
2. ON254271.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	G
3. OP810506.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	G
4. OR052249.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	T
5. OP479886.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	T
6. OP620405.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	T
7. ON229618.1	T A A G A C C C T	A A T G G C A A T A T T G T A A T T T T A G	G

Figure (9): Detection SNPs in Iraqi ToLCPaIV segment DNA-A. The indicated SNP is found in the Iraqi isolates of ToLCPaIV segment DNA-A infecting courgetti crop

ToLCPaIV virus was first recorded in the Indian city of Palampur in 2008 [30], then it was recorded in Pakistan [31]. In Iraq, it was reported on squash and Datura [32] cucumber and tomato [18, 20] in many Iraqi governorates, which indicates the wide range of hosts and spread. So far, the full genome and phylogenetic analysis of the present Iraqi ToLCPaIV isolates confirmed the evidence for existing only the bipartite genome of this virus in Iraq. The alpha and betasatellites association were not identified in all the symptomatic leaf samples. However, further studies are required to confirm this conclusion. The occurrence of recombination in the past has been proven by analyzing recombination breakpoints in begomoviruses. The process of recombination, both inter and intra species, plays a crucial role in the development of new begomoviruses and their ability to adapt to new hosts in agricultural ecosystems. This evidence highlights the significance of recombination in the evolution of these viruses [33]. Considering the rate at which the host range of ToLCPaIV is expanding, it is anticipated that diseases caused by this virus will significantly impede production of vegetable crops belonging to families such as Solanaceae, Cucurbitaceae, Leguminaceae, Basellaceae, and Malvaceae. This will serve as a major challenge for crop production in these families [34]. Furthermore, the molecular identification techniques should be applied as a vital step in any disease management to achieve efficient consequences in control them and reduce their harmful impact [35].

References

- 1) Mohammed, M.S. and Lahuf, A.A. (2023). First report of *Cucumber vein yellowing virus* in Iraq. *New Disease Report*, 47 (2):1-2.
- 2) Paris, H.S. and Lust, T.A. (2020). Origin of the zucchini squash, *Cucurbita pepo* subsp. *pepo* Zucchini group. *Acta Horticulture*, 1294: 1-8.

- 3) CSO (2022). CSO (The Central Statistical Organization at the Iraqi Ministry of Planning). Retrieved on August 25, 2023, from <https://mop.gov.iq/archives/11935>.
- 4) Brown, K. (2003). Edible Flowers. Anness Publishing Ltd, London, UK.
- 5) Abdalmoohsin, R.G.; Alhumairy, Y.N.; Abood, N.T. and Lahuf, A.A. (2019) Bioefficacy of *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* against *Pythium aphanidermatum* under laboratory and greenhouse aquaculture conditions in cucumber plants, *Cucumis sativus*. *Biopesticides International*, 15: 15–22.
- 6) Keinath, A.P.; Wintermantel, W.M. and Zitter, T.A. (2017). Compendium of Cucurbit Diseases and Pests. APS Press, USA.
- 7) Lahuf, A.A.; Jaafar, O.H.; Al-mosoy, M. and Hameed, Z.L. (2018). First record of the crown rot fungus *Fusarium equiseti* affecting *Triticum aestivum* L. and *Aptenia cordifolia* in Iraq. *Asian Journal of Agriculture and Biology*, 6 (4): 543-548.
- 8) Lahuf, A.A. (2019). *Alternaria alternata* causes leaf blight of rosy periwinkle (*Catharanthus roseus*) in Iraq. *Australasian Plant Disease Notes*, 14(4): 1-4.
- 9) Makouk, K. M.; Fajla, J. I. and Qamari, S. G. (2008). Viral diseases of important agricultural crops in the Arab region. Arab renaissance house, Beirut, Lebanon.
- 10) Navas-Castillo, J.; Fiallo-Olive, E. and Sanchez-Campos, S. (2011) Emerging virus diseases transmitted by whiteflies. *Annual Review Phytopathology*, 49:219–248.
- 11) Abass, M. and Lahuf, A. (2023). First report of *Tomato leaf curl Palampur virus* infecting tomato in Iraq. *Journal of Plant Pathology*, 105: 385.
- 12) Zerbini, F.M.; Briddon, R.W.; Idris, A. et al. (2017). ICTV virus taxonomy profile: geminiviridae. *Journal of General Virology*, 98(2):131–133.
- 13) Abd, D.A.; Kareem, A.A. and Lahuf, A.A. (2021). Molecular identification of sweet potato whitefly *Bemisia tabaci* putative species in Karbala province, Iraq and possibility control it using the nanoparticles of MgO and ZnO. *Plant Cell Biotechnology and Molecular Biology*, 22:175-184.
- 14) Salman, M. D. and Lahuf, A. A. (2022). A New Procedure to Identify Plant RNA Viruses Associated with the Whitefly (*Bemisia tabaci*) Using Next-Generation Sequencing. *Arab Journal of Plant Protection*, 40(2):169-174.
- 15) Gilbertson, R.L.; Batuman, O.; Webster, C.G. and Adkins, S. (2015) Role of the insect super vectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. *Annual Review Virology*, 2:67–93.
- 16) Zhou, X. (2013). Advances in understanding begomovirus satellites. *Annual Review of Phytopathology*, 51:357–381.
- 17) Venkataravanappa, V.; Lakshminarayanareddy, C.N.; Shankarappa, K.S.; Jayappa, J., Pandey S, Reddy MK (2019) Characterization of Tomato leaf curl New Delhi virus and DNA-satellites association with mosaic disease of cucumber. *International Journal of Biotechnology & Bioengineering* 5(6):93–109.



- 18) Abass, M.O. and Lahuf, A.A. (2022). First Report of the Satellite DNA Beta Associated with *Tomato Yellow Leaf Curl Virus*–Mild on Tomato in Iraq. *Plant Health Progress* 23 (4): 480-481.
- 19) Abass, M.O. and Lahuf, A.A. (2023). High-throughput sequencing and bioinformatic analysis reveal presence of the Endogenous Pararetrovirus Tobacco vein clearing virus Genome in the tomato (*Solanum lycopersicum*) host genome. *Arab journal of plant protection*, 41(1): 77-84.
- 20) Al-Yasiri, H. I. A. (2023). Identification and complete characterization of some pathogenic and endogenous viruses of cucumber plants and their resistance using some biotic and abiotic agents. PhD thesis, University of Kufa, Iraq.
- 21) Lahuf, A. A.; Kadhim, A. Q.; Hameed, Z.L. and Almulla, A.M.N. (2022). First report of *Paecilomyces* rot on rose (*Rosa* sp.) in Iraq. *Journal of Plant Pathology*, 104: 1195-1195.
- 22) Ye, Z-X; Lahuf, A. A.; Salman, M. D.; Zhang, Y. and Li, J-M. (2022). Draft genome sequence of a novel Iflavirus from Leafhoppers (*Exitianus capicola*) in Iraq. *Microbiology Resource Announcement* 11 (5):1-2.
- 23) Lahuf, A. (2021). RNA-Seq data analysis reveals various viral sequences associated with genome of date palm (*Phoenix dactylifera*). *IOP Conference Series: Earth and Environmental Science*, 735: 012027.
- 24) Kumar, S.; Strcher, G. and Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular and Biological Evolution*, 33: 1870-1874.
- 25) Abdulmoohsin, R. G.; Lahuf, A. A.; Husain, Y. N. and Hameed, Z. L., (2019). Bio-efficiency of some indigenous biocontrol agents against *Rhizoctonia solani* causing cowpea seed rot and preemergence damping-off. *IOP Conference Series: Earth and Environmental Science*, 388: 012011.
- 26) Lahuf, A. A. (2019). First report of *Fusarium proliferatum* causing stem and root rot on lucky bamboo (*Dracaena braunii*) in Iraq. *Hellenic Plant Protection Journal*, 12: 1–5.
- 27) Shehan, S.J.; Abdalmoohsin, R.G. and Lahuf, A.A. (2022). First report of *Ectophoma multirostrata* causing root rot of *Celosia argentea* in Iraq. *New Disease Report*, 46: e12126.
- 28) Martin, D.P.; Varsani, A.; Roumagnac, P. et al. (2021).RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. *Virus Evolution*, 7(1): veaa087.
- 29) Sattar, M.N.; Iqbal, Z.; Ali, S.N. et al. (2021). Natural occurrence of mesta yellow vein mosaic virus and DNA-satellites in ornamental sunflower (*helianthus* spp.) in Pakistan. *Saudi Journal of Biological Sciences*, 28(11):6621–6630.



- 30) Kumar, Y., Hallan, V., & Zaidi, A. A. (2008). Molecular characterization of a distinct bipartite begomovirus species infecting tomato in India. *Virus genes*, 37(3), 425–431.
- 31) Ali, I.; Malik, A.H. and Mansoor, S. (2010). First report of *Tomato leaf curl Palampur virus* on bitter melon in Pakistan. *Plant Disease*, 94(2):276.
- 32) Al-Kuwaiti, N. (2017). Molecular characterization of a leaf curl disease infecting zucchini squash in Iraq. *International journal of agriculture & biology*, 19: 649–653.
- 33) Lefeuvre, P.; Lett, J.M.; Reynaud, B. and Martin, D. P. (2007). Avoidance of protein fold disruption in natural virus recombinants. *PLoS Pathogen* 3:e181.
- 34) Hanamasagar, Y.; Naganur, P.; Shankarappa, K.S. et al. (2021). Characterization of Tomato leaf curl Palampur virus associated with leaf curl and yellowing disease of watermelon from India. *Indian Phytopathology*, 74: 1075–1088.
- 35) Jaber, M.H.; Lahuf, A.A. (2020). Survey, pathogenicity and molecular identification of novel *Fusarium* species causing seed decay and damping-off disease of wheat crop in Kerbala Province, Iraq. *Plant Cell Biotechnology and Molecular Biology*, 21, 1–14.