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Genetic Diversity, Phylogeny and Recombination among Iraqi Isolates of *Tomato Leaf Curl Palampur virus*

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ABSTRACT

Background: The study was conducted to confirm the genetic diversity of several tomato leaf curl virus (TLCPV) isolates in the Molecular Biology Laboratory of the College of Agriculture, University of Kufa.

Methods: In this study, the full-length nucleotide sequences of the binary components of tomato palampur leaf curl virus (TLCPV) comprising 7 complete DNA-A sequences (OQ693629.1,ON229618.1, OP810506.1 PP230526.1 OR052249.1, OP620405.1 and OP479886.1) and 7 complete DNA-B sequences (PP230527.1, OP810507.1, OP620406.1, ON229620.1, OR052250.1, 1OQ693630.1, ON254270.1) were imported from the NCBI GenBank database dated November 10, 2025; Sequence alignment, genetic distance calculation and phylogenetic tree construction were performed using the Geneious Prime platform (version 2025.1.2), while recombination dynamics were evaluated using the software RDP (version 4.101).

Result: Genetic evolution analysis showed that Iraqi TLCPV isolates split into distinct strains; Isolates coming from Babylon and Baghdad showed high sequence similarity, while isolate OP810506.1 Kufa isolate (tomato) formed a distinct genetic strain. RDP4 analysis also revealed three recombination events within the DNA-A sequences and event 2 (which includes isolate OP810506.1 as a hybrid isolate generated by Minor parent ON229618.1) was a statistically confirmed event that met the strict criterion of support by 7 detection algorithms. In addition, two major recombination events were identified within the DNA-B region. The most affected strain was PP230527.1, which showed a different genomic pattern resulting from recombination with isolates such as ON229620.1 and OP810507.1. These results demonstrate the presence of genetic variation in Iraqi TLCPV isolates, highlighting potential challenges for virus management and biological control strategies in regional agroecosystems. These recombination events are statistically significant and acceptable because they are supported by ≥5 independent algorithms with a bonferron-corrected P<0.05 limit.

Key words: Multiple lineages, RDP4, *Tomato leaf curl Palampur virus*, Variation.

INTRODUCTION

The *Tomato leaf curl Palampur virus* (TLCPV) is a bipartite begomovirus belonged to the family of *Geminiviridae*, which causes important economic losses in many crops worldwide (ICTV, 2023). Substitution rate in *Begomovirus* have been estimated by analyzing DNA fragments amplified by polymerase chain reaction (PCR), which represent approximately 50% of the viral genome. The results indicate that substitution rate vary depending on the virus species, plant host and detection techniques used. The genetic diversity observed in *Begomovirus* arises from several molecular mechanisms. Point mutations, involving single-nucleotide changes, can lead to significant alterations in protein function, influencing viral fitness and host interactions. Recombination plays a crucial role by facilitating the exchange of genetic segments between different viral genomes, thereby generating novel variants with potentially enhanced adaptability. These evolutionary pressures are frequently driven by host genetic resistance pathways, for example those characterized against *Potato virus Y* (Torrance *et al.,* 2020), in addition to, complex selective forces shaping begomovirus genetic variation and control approaches in diverse local environments (Al-Abedy *et al.,* 2021). Recombination plays a crucial role in accelerating evolutionary adaptation by combining multiple advantageous traits into a single genome (Sheerali *et al.,* 2025). According to the International Committee on Taxonomy of Viruses (ICTV, 2023), the *Tomato leaf curl Palampur virus* (TLCPV) belongs to the family *Geminiviridae* and the genus *Begomovirus*. These are small, non-enveloped viruses with a single-stranded circular DNA (ssDNA) genome. The genome consists of two segments of DNA: the first is DNA-A, which contains 2,756 nucleotides and the second is DNA-B, comprising 2,719 nucleotides (Shafiq *et al.,* 2019; Materatski *et al.,* 2021; Alyasiry *et al.,* 2024).

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The DNA-A segment contains six genes are responsible for initiating infection by mediating viral protein-host protein interactions, as well as for viral replication, host gene silencing, virulence and systemic viral spread. The DNA-B segment contains only two genes: BC1, responsible for the production of the viral movement protein and BV1, which facilitates viral movement into and out of the host nucleus (Fiallo-Olivé *et al.,* 2021). This virus is transmitted by the vector whitefly (*Bemisia tabaci*), specifically of biotype B (known as MEAM1) (Hanley-Bowdoin *et al.,* 2013; Abkhoo and Mehraban, 2020; Fiallo-Olivé *et al.,* 2021). Symptoms generally include leaf curling, light mosaic patterns characterized by irregular green spots alternating with yellow spots, downward leaf curling, distortion of buds and fruits, stunted growth, small leaf size, severe yellowing and necrotic streaking. These symptoms vary depending on the plant species, cultivar and viral isolate (Dhkal *et al.,* 2020; Venkataravanappa *et al.,* 2021; Aljuaifari *et al.,* 2026). In a previous study, complete genomic sequencing and whole-genome and metatranscriptomic data were collected from infected cucumber leaves to determine the pathogen, which revealed the presence of Palampur tomato leaf curl virus (TLCPV) in its DNA-A (2,756 base pairs) and DNA-B (2,719 base pairs) forms and registered under the accession numbers ON229618 and ON229620 (Al-Yasiri, 2023; Alyasiry *et al.,* 2024). Based on these preliminary data, the histological relationships, genetic diversity and recombination dynamics of isolates of this virus in Iraq compared to regional isolates are still not precisely known. Accordingly, the present study aims to conduct a comprehensive comparative computational analysis (in silico comparative analysis) of the TLCPV genomic sequences available in the GenBank database to determine the genetic diversity and molecular evolution of the virus in the region.

MATERIALS AND METHODS

Phylogenetic tree

All Iraqi viral isolates of *Tomato leaf curl Palampur virus* (TLCPV) registered in the NCBI database were retrieved on November 10, 2025, TLCPV segment DNA-A isolate Baghdad-1/Iraq OQ693629.1 (zucchini) , isolate Babylon-1ON229618.1 (cucumber), isolate Kufa OP810506.1 (tomatoes) ,isolate, Baghdad MS89 PP230526.1 (pumpkin), isolate Mosul OR052249.1 (squash), isolate Baghdad OP620405.1 (zucchini) and isolate Baghdad Al-Yusufiyah OP479886.1), along with an additional Tomato leaf curl New Delhi virus isolate from a different lineage (OM102562), serving as an outgroup for comparison and tree rooting. while TLCPV segment DNA-B (isolate Baghdad MS89 PP230527.1 (squash) ,isolate Kufa OP810507.1 (tomato) , isolates Baghdad OP620406.1 (zucchini), isolate Babylon-1 ON229620.1 (cucumber), isolate Mosul OR052250.1 (squash), isolate Baghdad-1OQ693630.1 (zucchini) ,isolate Karbala-1 ON254270.1 (tomato), along with an additional (ToLNDV, OP356208) serving as an outgroup for comparison and tree rooting. These genetic sequences were subsequently imported into the Geneious Prime platform (version 2025.1.2), where a multiple alignment was performed using the clustal omega alignment tools integrated within the software. Following the alignment process, sequence ends were trimmed in order to standardize sequence lengths and enhance the accuracy of downstream phylogenetic analyses. A phylogenetic tree was then constructed in Geneious Prime using the Neighbor-Joining (NJ) method based on the Hasegawa-Kishino-Yano (HKY) nucleotide substitution model available algorithms, incorporating the designated outgroup to properly root the tree and facilitate interpretation of evolutionary relationships among the analyzed isolates Statistical support for internal nodes was evaluated using bootstrap analysis with a 1000-repeat procedure.

Recombination and genetic variation analysis using RDP4

To investigate recombination events and assess genetic variation among isolates of TLCPV, full-length genomic sequences of eight DNA-A isolates and eight DNA-B isolates were analyzed using the Recombination Detection Program (RDP) version 4.101. The analysis was conducted under the 'program's default settings, with the activation of seven recombination detection algorithms: RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq. Multiple-testing corrections were applied using the Bonferroni method and nested or duplicated recombination signals were automatically merged according to Standard algorithm thresholds. Recombination events were considered statistically significant only if detected by at least 5 methods and supported by a Bonferroni-corrected p-value <0.05, thereby ensuring robustness of the results. The software automatically identified recombination breakpoints and where possible, predicted the major and minor parental sequences for each recombinant isolate. Additionally, a UPGMA phylogenetic tree was constructed in RDP4 using the genomic region spanning the identified recombination breakpoints to illustrate evolutionary relationships local clustering patterns and place recombinant isolates within their broader phylogenetic context. UPGMA was utilized strictly as an exploratory visual screening tool for recombinant tracts rather than as independent statistical proof of parental ancestry. Comprehensive recombination event summaries were also extracted and tabulated, as documented in the results, including each event, its associated recombinant sequence, putative parents, exact breakpoint coordinates and the supporting detection methods.

RESULTS AND DISCUSSION

Phylogenetic analysis of tomato leaf curl palampur virus (TLCPV) - DNA-A and DNA-B Segment

The phylogenetic tree illustrates the evolutionary relationships among DNA- Segment sequences of tomato leaf curl Palampur virus (TLCPV), collected from various regions of Iraq and different host plant species. Tomato

leaf curl New Delhi virus (ToLNDV, OM102562) was used as an outgroup to root the tree (100% bootstrap). tomato leaf curl New Delhi virus (OM102562) has been used as an exogenous group for evolutionary tree rooting. Cluster analysis showed that isolate Baghdad-1/Iraq OQ693629.1 (zucchini) and isolate Babylon-1ON229618.1 (cucumber) form distinct early branching lineages, while the isolate Kufa OP810506.1 (tomatoes) was collected directly with the external group ToLNDV with 100% statistical support (100% bootstrap support). In contrast, the other main branch, supported by a bootstrap value of 85.6%, was characterized by the presence of an isolate, Baghdad MS89 PP230526.1 (pumpkin), which branched out separately from a sub-branch with very strong statistical support (100% bootstrap support). Under this sub-branch, isolate Mosul OR052249.1 (squash) was merged with isolate Baghdad OP620405.1 (zucchini) and isolate Baghdad Al-Yusufiyah OP479886.1 with statistical support of 96.4% (Fig 1).

Sequences of *Tomato leaf curl Palampur virus* (TLCPV), isolated from different locations across Iraq and various host plants. Tomato leaf curl New Delhi virus (ToLNDV, OP356208) was used as an outgroup to root the tree. The tree resolved into two main clades branching from the node supported by a 100% bootstrap value. The first main clade (79.9% bootstrap support) included isolate Baghdad MS89 PP230527.1 (squash) branching separately from a strongly supported sister pair (100% bootstrap support) comprising isolate Kufa OP810507.1 (tomato) and isolates Baghdad OP620406.1 (zucchini). The second main clade, supported by a 76.8% bootstrap value, consisted of isolate Babylon-1 ON229620.1 (cucumber) alongside a highly supported sub clade (100% bootstrap support). Within this sub-clade, isolate Mosul OR052250.1 (squash) grouped separately from isolate Baghdad-1OQ693630.1 (zucchini) and isolate Karbala-1ON254270.1 (tomato), which formed a closely related pair with an 82.6% bootstrap value. Comprehensive Molecular Analysis of Genetic Diversity and Recombination in *Tomato Leaf Curl Palampur Virus* DNA-A Isolates Using RDP4 Software (Fig 2).

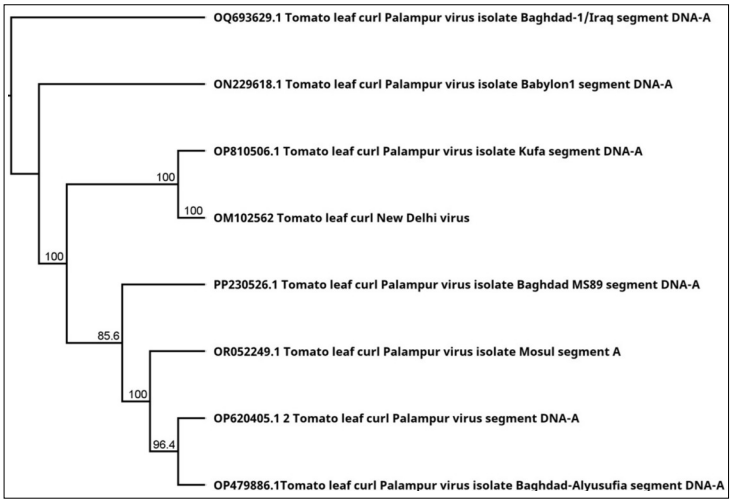


Fig 1: The phylogenetic tree of iraqi isolates of tomato leaf curl palampur virus (TLCPV) DNA-A.

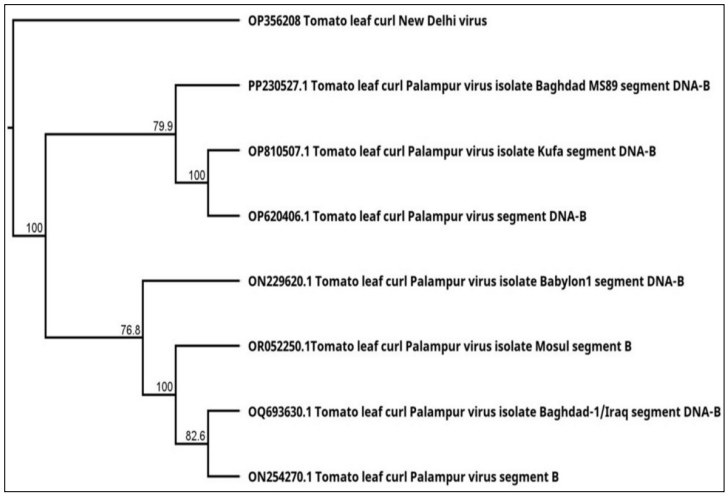


Fig 2: The phylogenetic tree of iraqi isolates of tomato leaf curl palampur virus (TLCPV) DNA-B.

A comprehensive molecular analysis was performed to examine genetic variation and recombination between eight Iraqi DNA- A isolate of tomato leaf curl virus Palampur (TLCPV), along with sequencing of one exogenous group tomato leaf curl virus New Delhi-ToLNDV, (OM102562). In this study, RDP4 v4.101 was used, which integrates algorithms including (RDP, SiScan, BootScan, MaxChi, Chimaera, GENECONV and 3Seq) to identify recombination sites and potential parental lineages.

In Fig 3, which shows the analysis of SiScan, graph transitions are evaluated based on the degree of SiScan Z. A significant shift in the Z-score curve was observed around alignment positions ~2150 to ~2480, exceeding the corrected Bonferroni threshold (P<0.05). This graph transition provides evidence consistent with a recombination event in this genomic region.

Furthermore, Fig 4 depicting the pairwise identity diagram from the RDP analysis supports these results, demonstrating a transition in sequence identity consistent with recombinant origin within the identified region (positions ~2150-~2480; 95% breakpoint confidence interval). The recombination event detected in isolate OP810506.1 was statistically confirmed across all applied algorithms, supported by highly significant Bonferrni-corrected P values ranging from 1.315×10^{-10} (MaxChi) to 7.527×10^{-24} (SiScan)(Table 2).

In Fig 5 based on the MaxChi algorithm, a similar pattern of genetic variation was identified, with a sudden

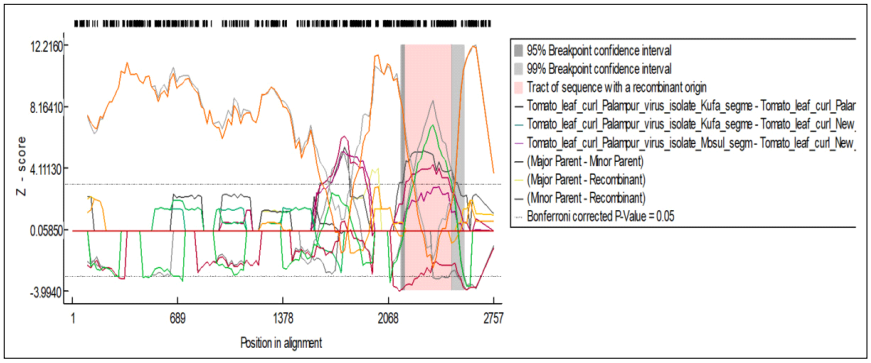


Fig 3: SiScan-based detection of recombination breakpoints in tomato leaf curl palampur virus DNA-A isolates revealing significant genetic divergence at genomic.

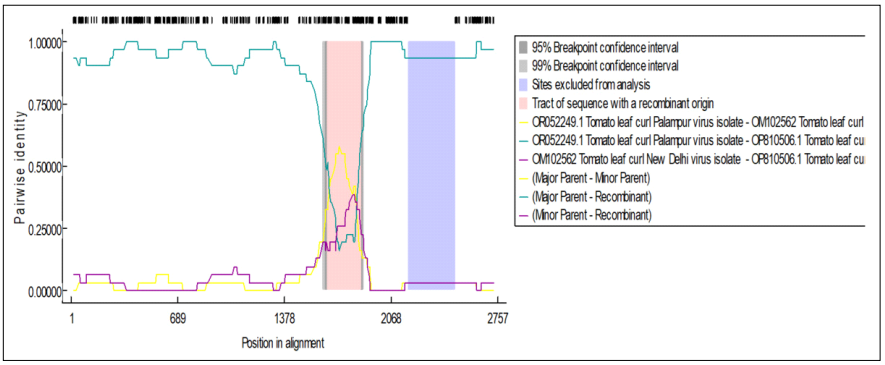


Fig 4: Pairwise identity analysis generated by the RDP algorithm highlighting genetic variability and recombination regions among tomato leaf curl palampur virus DNA-A isolates along the genome alignment.

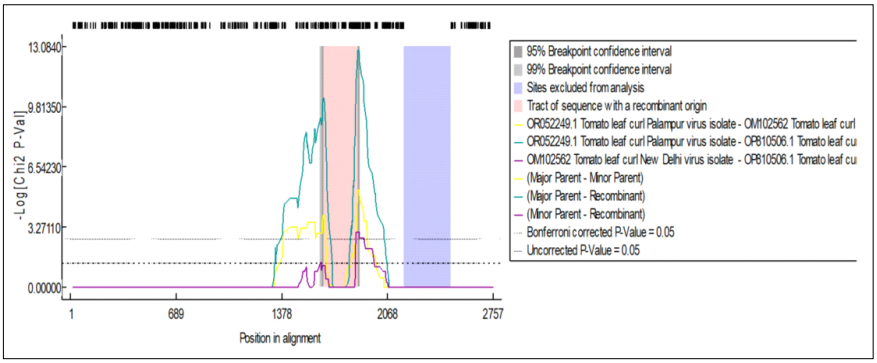


Fig 5: Identification of recombination breakpoints in tomato leaf curl palampur virus DNA-A genome using MaxChi statistical analysis.

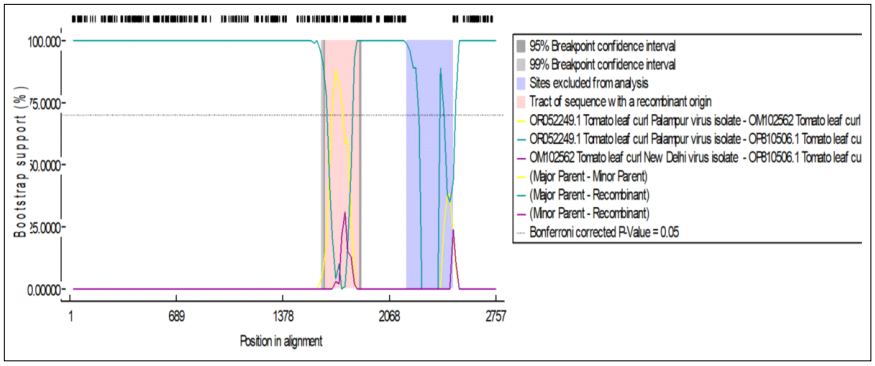


Fig 6: Bootscan analysis revealing dynamic recombination patterns and bootstrap support across the genome of tomato leaf curl palampur virus DNA-A isolates.

change in sequence similarity between isolates, particularly within the previously defined region (760-2279 base pairs). The curves in this analysis show a clear genotype crossover, strongly supporting the hypothesis that an actual recombination event occurred in that genomic region.

These combined results were further strengthened by the analysis shown in Fig (6), based on the BootScan tool, which relies on the bootstrap method. The results show clear fluctuations in bootstrap support values across the genome between different isolates, with the early parts of the genome showing genetic similarity between the reference isolate and one of the potential parents. In contrast, the later parts show greater similarity to a different isolate. This change in the similarity pattern indicates the presence of a genomic segment introduced by recombination, providing strong evidence of a true recombination event that contributed to the current genetic makeup of the reference isolate.

Turning to Fig (7), which represents the MaxChi/χ^2 statistical regrouping matrix used to assess the potential locations of genomic breakpoints (breakpoints), a distinct statistical pattern is observed across the examined alignment. The color gradient in this matrix reflects the magnitude of the MaxChi/χ^2 test statistic. The red and orange regions in the matrix indicate higher values for the χ^2 statistic, representing statistically significant support for the regrouping breakpoints ($P<0.05$). In contrast, the blue-green regions represent lower values for the χ^2 test. This statistical stereopsis provides evidence consistent with potential regrouping events and areas of mutual fracture points between isolates, reinforcing and confirming the results of previous analyses.

In Table 1, which summarizes the genetic variation and recombination events of TLCPV-DNA-A, three distinct recombination events that met the statistical significance requirement (support from 5 detection algorithms) were documented. Only eligible events out of four (events 1, 2 and 3) were shown as statistically significant. Table 2 contains eligible event data where Event 1 included the hybrid sequence OM102562, with breakpoints starting at

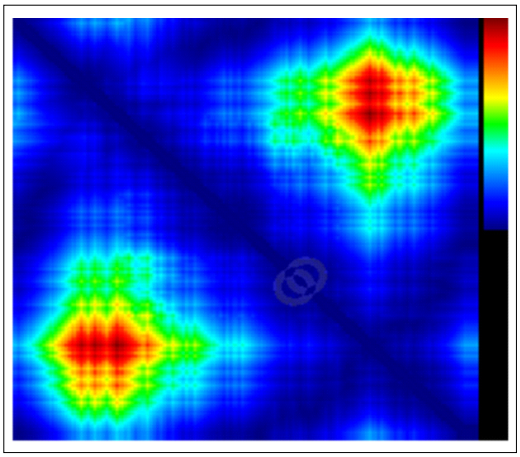


Fig 7: MaxChi/χ^2 statistical matrix for assessing genomic breakpoint locations and the statistical significance of regrouping among DNA-A isolates of tomato leaf curl Palampur virus.

2176 and ending at 2480, where isolation OR052249 was identified as a secondary father, while the primary father remains unknown, which is supported by seven complete algorithms (7/7) and with a corrected RDP statistical value of 6.905×10^{-18}.

Fig 8, which presents the color-coded phylogenetic tree, provides additional support for interpreting the inferred genetic relationships among the isolates. According to RDP the color scheme, isolate OP810506.1 is marked in red, identifying it as a putative recombinant isolate. Meanwhile, isolate ON229618.1 is highlighted in blue, representing the inferred minor parental sequence and other sequences are shown in green as reference parental candidates used to infer the unknown major parent.

Comprehensive molecular analysis of genetic diversity and recombination in tomato leaf curl palampur virus DNA-B isolates using RDP4

A comprehensive molecular analysis was performed to examine genetic variation and recombination between eight Iraqi samples of tomato leaf curl virus Palampur (TLCPV) DNA, along with the sequencing of one exogenous

Table 1: Demonstrates statistically significant recombination events (P≤0.05) in the genome ways tomato leaf curl palampur virus DNA-A isolates using RDP4 software algorithms, stop point start/end coordinates and confidence intervals (CI) 99%, in more than 5.

Event no.	Recombinant	Major parent	Minor parent	Detection methods							Ending break point	Beginning break point
				RDP	GENECONV	Boot Scan	MaxChi	Chimaera	SiScan	3Seq		
1	OM102562	Unknown	OR052249	6.905×10^{-18}	9.684×10^{-12}	6.787×10^{-14}	1.315×10^{-10}	7.483×10^{-12}	7.527×10^{-24}	2.220×10^{-15}	2176	2480
2	OP810506	Unknown	OR052249	1.088×10^{-10}	9.029×10^{-03}	1.948×10^{-07}	1.881×10^{-12}	3.814×10^{-06}	8.761×10^{-07}	6.555×10^{-08}	1654	1876
3	OP810506	Unknown	PP230526	9.508×10^{-08}	1.195×10^{-05}	9.609×10^{-07}	1.345×10^{-10}	-	4.424×10^{-16}	6.539×10^{-06}	2126	2729

Table 2: Demonstrates statistically significant recombination events (P≤0.05) in the genome ways tomato leaf curl palampur virus DNA-B isolates using RDP4 software algorithms, stop point start/end coordinates and confidence intervals (CI) 99%, in more than 5.

Event no.	Recombinant	Major parent	Minor parent	Detection methods							Beginning break point	Ending break point
				RDP	GENECONV	Boot Scan	MaxChi	Chimaera	SiScan	3Seq		
1	PP230527	ON229620	OP620406	8.325×10^{-6}	1.963×10^{-2}	1.380×10^{-5}	8.500×10^{-9}	3.975×10^{-3}	2.455×10^{-8}	2.818×10^{-7}	2401	278
2	ON229620	OQ693630	Unknown	1.195×10^{-2}	2.858×10^{-2}	-	9.040×10^{-6}	3.976×10^{-4}	-	1.996×10^{-3}	1941	244

group of tomato leaf curl virus New Delhi-ToLNDV (OP356208). In this study, RDP4 v4.101, which integrates algorithms including RDP, SiScan, BootScan, MaxChi, Chimaera, GENECONV and 3Seq, was used to identify recombination sites and potential parental lineages. The results showed significant genetic variation between the isolates studied. Recombination analysis identified PP230527 and ON229620 as potential recombinant isolates (Table 2).

In Fig 9, which shows the SiScan analysis, the graph transitions are evaluated based on the SiScan Z score. A significant shift and sharp rise in the Z-score curve were observed to reach a peak of about 7.08 at the two genomic fracture point regions (around alignment positions 2401 to 278), clearly exceeding the corrected Bonferroni threshold ($P<0.05$). This sharp transition in grade Z provides conclusive statistical evidence consistent with the occurrence of a genetic recombination event in this genomic region of the hybrid isolate PP230527.1, acquired from the primary parent ON229620.1 and the secondary parent OP620406.1.

Furthermore, Fig 10 depicting the Pairwise Identity Plot induced by RDP analysis supports these results, demonstrating a clear transition in sequence identity consistent with recombinant origin within the identified genomic region (positions 2401-278, with confidence

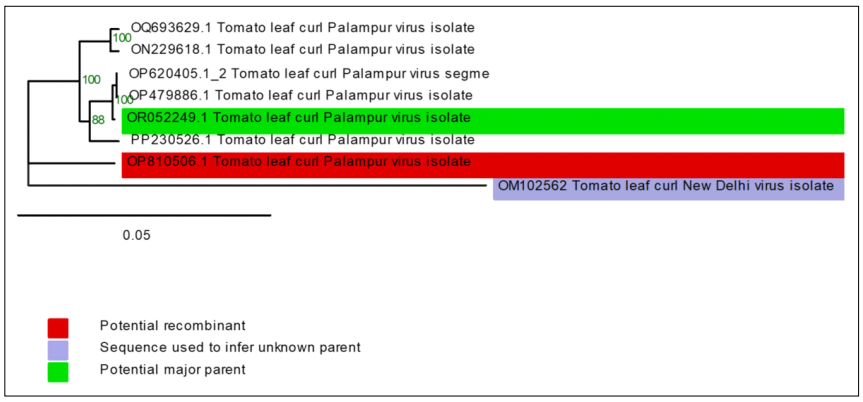


Fig 8: Color-coded phylogenetic tree illustrating the recombinant isolate (Red), minor parental isolate (Blue) and reference sequences (Green) of tomato leaf curl palampur virus DNA-A.

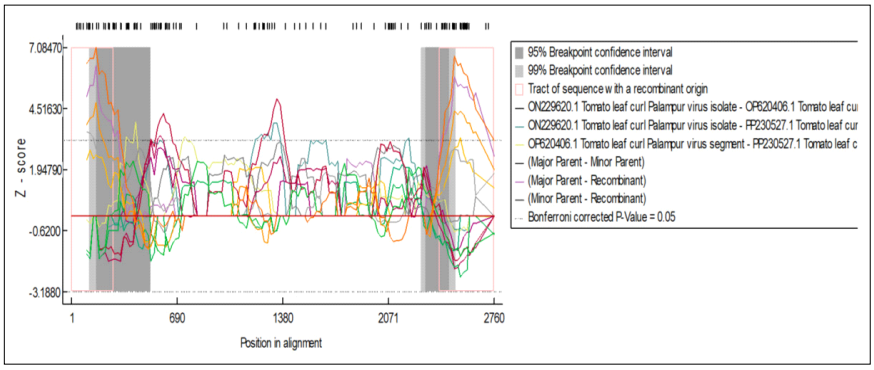


Fig 9: SiScan analysis revealing wave-like patterns of genetic similarity among TLCPV DNA-B Isolates.

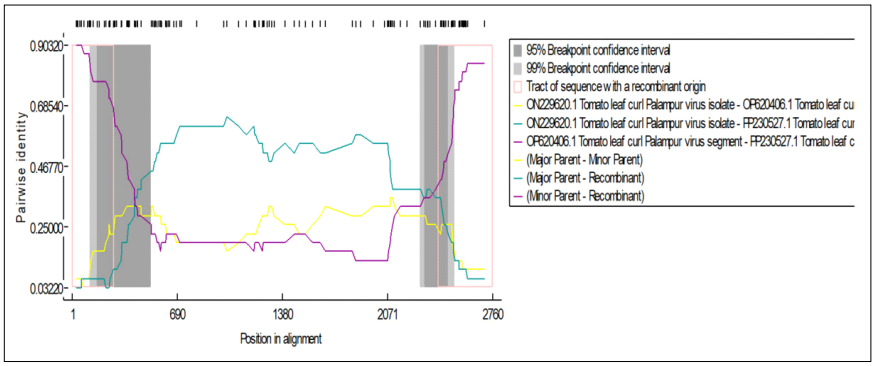


Fig 10: Detection of recombination breakpoints and parental contributions in TLCPVDNA-B isolates using RDP analysis.

intervals for breakpoints of 95% and 99%). The results show the genotype of the PP230527.1 hybrid isolate, transitioning from high similarity to minor parent isolate OP620406.1 in the periphery to high similarity to major parent isolate ON229620.1 in the central region. This recombination event was statistically confirmed across all seven applied algorithms (7/7), supported by highly significant corrected P values ranging from 1.963×10^{-2} (GENECONV) to 2.455×10^{-8} (SiScan) and 8.325×10^{-6} for the RDP algorithm (Table 2).

In Fig 11, based on the MaxChi test, a sharp peak is shown in the negative logarithm of the probability value (P-value) at the intersection points, reflecting a significant genetic differentiation within the PP230527.1 hybrid isolation segments compared to the potential parental sequences (major parent ON229620.1 and minor parent OP620406.1). This result strongly supports the precise identification of recombination fracture points at positions 2401 and 278.

Fig 12, based on the BootScan algorithm that evaluates bootstrap support values across alignment length, shows: The results show clear transitions in sequence identity, with the first and last genomic regions showing high support linking the isolate PP230527.1 to minor parent (OP620406.1), while the middle genomic region shifts to high similarity to major parent (ON229620.1). This transition provides conclusive evidence of a real recombination event that contributed to the current genetic structures of the isolation.

Moving on to Fig 13, the MaxChi/χ^2 statistical regrouping matrix i, a distinct statistical pattern is observed that identifies recombination sites and fracture points. The color gamut in this map expresses the calculated χ^2 statistic test value for possible pairs of fracture points; warm colors (red and orange) represent the maximum χ^2 statistic values (indicating the highest statistical support for fracture points at ($P<0.05$), while cool colors (blue) express the low χ^2 statistic values (absence of recombination signals).

In Table 2, eligible events that met strict statistical criteria (≥5 algorithms) were documented. Event 1 includes hybrid isolation PP230527 with breakpoints starting from 2401 and ending at 278, where isolation ON229620 AS major parent and isolation OP620406 were identified as minor parent, supported by all seven applied algorithms (7/7) and with an RDP-corrected Pvalue of 8.325×10^{-6}. While Event 2 includes the hybrid isolate ON229620 with break points from 244 to 1941and is a statistically proven event across 5 algorithms out of 7 (5/7): with an RDP-corrected P-value of 1.195×10^{-2}.

Fig 14 presents the UPGMA genetic evolution tree based on the continuous non-recombinant genomic structure extending between loci 278-2114. This genomic separator was specifically chosen because it represents the non-recombinant major sector located outside the

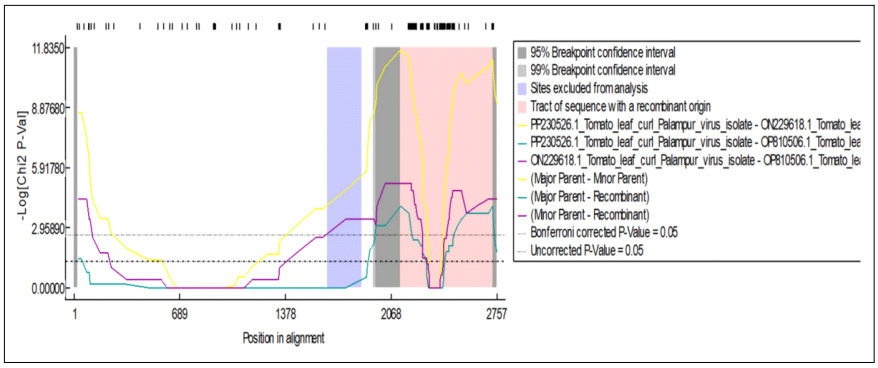


Fig 11: MaxChi analysis identifying significant recombination breakpoints in TLCPV DNA-B genome.

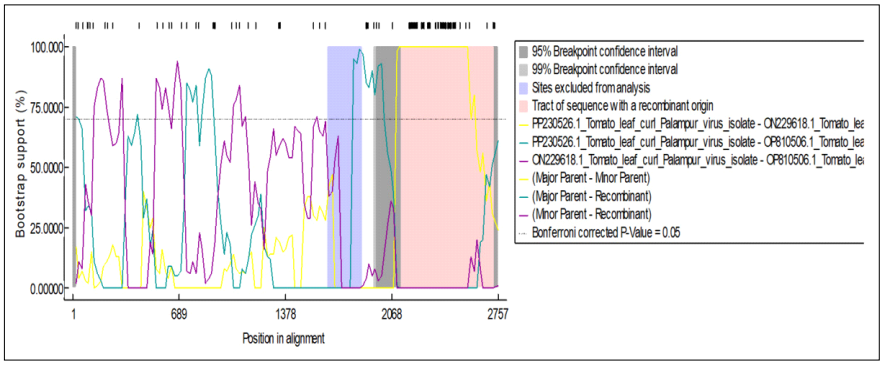


Fig 12: bootscan analysis revealing dynamic recombination patterns and bootstrap support across the genome of tomato leaf curl palampur virus DNA-A isolates.

range of hybrid fracture points (2401-278), ensuring that the tree shape is not distorted by recombination signals. In this tree, the hybrid isolate PP230527.1 clusters closely with the minor parent (OP620406.1 with 100% potstrap support) in this region, while the major parental isolate ON229620.1 branches into an independent branch, suggesting a complex evolutionary history for these isolates.

The results indicate that TLCPV has evolved through multiple recombination events among genetically diverse isolates, leading to the emergence of complex mosaic genomic patterns. The genetic variation arising from recombination serves as a key driver of the virus's genetic diversity and environmental adaptability, potentially contributing to expanded host range and increased disease severity. Isolates ON229620.1 and FP230527PP230527.1 exhibit the highest levels of genetic variation, whereas OP810507.1 remains the most conserved.

These findings underscore the importance of integrating diverse analytical tools to achieve a comprehensive understanding of the molecular evolution of plant viruses, which is critical for developing effective strategies to mitigate their spread in agricultural ecosystems. *Begomoviruses*, members of the *Geminiviridae* family, are among the most genetically diverse plant viruses. They possess a small, circular, single-stranded DNA (ssDNA) genome encapsulated in twin icosahedral protein particles (Navas-Castillo *et al.,* 2011). These viruses, particularly the tomato leaf curl Palampur virus (TLCPV), exhibit an evolutionary substitution rate for tomato yellow leaf curl disease-associated begomoviruses in substitutions per site per year (approximately 2.88 × 10^{-4}) per nucleotide per replication cycle-rates comparable to those of RNA viruses (Duffy and Holmes, 2008). Studies have shown that mutations are not entirely randomly distributed; rather, there are specific regions in the genome known as "mutation hotspots", indicating varying nucleotide stability across different parts of the genome (García-Arenal *et al.,* 2001). While mutations contribute to genetic diversity, recombination plays a crucial role in generating new viral strains. Recombination occurs during co-infection in plants, where genome segments are exchanged between different viruses, leading to the emergence of hybrid strains with greater adaptability (García-Arenal *et al.,* 2003; Muteab and Al-Abedy, 2025). In addition, factors such as geographical variability, climatic conditions and host plant diversity significantly enhance the likelihood of mutations and recombination events. For example, Iraqi isolates of TLCPV, collected from various provinces, have shown notable genetic variability, likely driven by differences in environmental conditions, cultivated plant varieties and selective pressures across regions (García-Arenal *et al.,* 2003; Hanley-Bowdoin *et al.,* 2013). Whole-genome sequencing of infected cucumber samples in Iraq revealed variations in the DNA-A and DNA-B sequences, documented under accession numbers ON229618 and ON229620, indicating genetic diversity among the isolates (Al-Yasiri, 2023; Alyasiry *et al.,* 2024). In bipartite viruses such as TLCPV, genetic reassortment also contributes to diversity by exchanging DNA-A and DNA-B segments between different strains. The cumulative effect of these mechanisms-including mutation, recombination, complementation and reassortment-leads to extensive

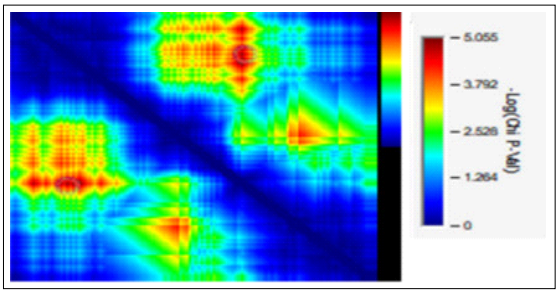


Fig 13: MaxChi/χ^2 statistical matrix for assessing genomic breakpoint locations and the statistical significance of regrouping among DNA-B isolates of tomato leaf curl Palampur virus.

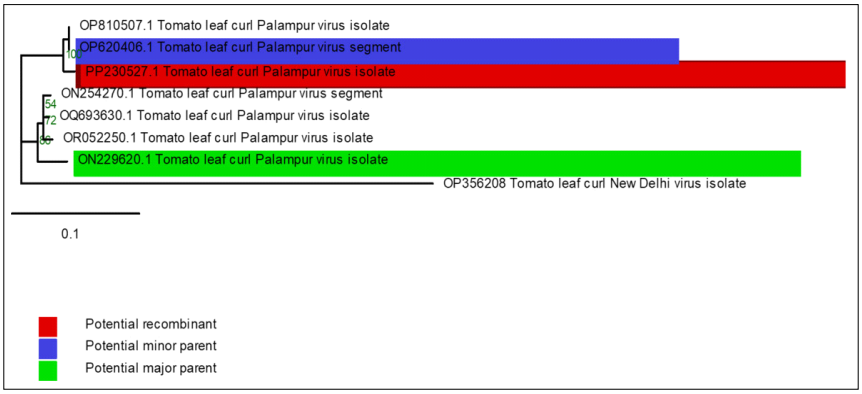


Fig 14: Color-coded phylogenetic tree illustrating the recombinant isolate (Red), minor parental isolate (Blue) and reference sequences (Green) of tomato leaf curl palampur virus DNA-A.

genetic variation among viral isolates, even within the same country or geographical environment, as seen in the Iraqi isolates. This genetic dynamism is a primary reason for 'TLCPV's ability to adapt to a wide range of host plants, such as tomato, cucumber, watermelon, squash, pepper and eggplant, as well as weed species like *Chenopodium* sp. and *Heliotropium europaeum* (Hanley-Bowdoin *et al.,* 2013; Fiallo-Olivé *et al.,* 2021; Al-Abedy *et al.,* 2021). In conclusion, the frequent occurrence of mutations, along with recombination mechanisms and host and geographic diversity, directly explains the genetic variability and recombination observed among different Iraqi TLCPV isolates.

CONCLUSION

This study was mapped the phylogenetic relationships, besides recombination dynamics of Iraqi TLCPV DNA-A and DNA-B segments, revealing important genetic diversity, recombination events, as well as geographic associations.

Conflict of interest

The authors declare that they do not have any conflicts of interest related with the publication of this research.

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