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Research Article

Detection of tomato leaf curl Palampur virus in new plant species from Iran

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Abstract: Tomato leaf curl Palampur virus (ToLCPalV; *Begomovirus solanumpalampurense*) is a bipartite begomovirus (*Geminiviridae*) that causes tomato leaf curl disease (TLCD). This study aimed to detect ToLCPalV in additional naturally infected plant species in Iran and analyze their phylogenetic relationships. Leaf samples were collected from symptomatic plants in Tehran Province. ToLCPalV was detected through PCR analysis followed by sequencing of the resulting amplicons in the following plant species: soybean *Glycine max* (Fabaceae), *Chenopodium album* (Amaranthaceae), *Acalypha australis* (Euphorbiaceae), cotton *Gossypium hirsutum*, (Malvaceae), *Malva sylvestris* (Malvaceae), and *Bougainvillea glabra* (Nyctaginaceae). Euphorbiaceae, Malvaceae, and Nyctaginaceae are reported here for the first time as plant families in which ToLCPalV was detected under natural conditions. In the Bayesian phylogenetic tree reconstructed using partial sequences of DNA-B from currently available sequences, two main clades were formed. One clade included sequences from isolates prevalent in Iran and sequences obtained in this study from Iraq, Oman, and Saudi Arabia; the second clade included sequences from India and Pakistan from various hosts. The spread of ToLCPalV from southeastern Iran to the central and northeastern regions poses a serious threat to cucurbit and tomato cultivation in these areas, underscoring the urgent need to implement monitoring and control programs, as these are primary production zones for these crops. Given the substantial damage observed in fields and greenhouses, ToLCPalV is emerging as a major threat to cucurbits, tomatoes, and other crops as its host range expands.

Keywords: Geminivirus, Begomovirus, ToLCPalV, Iran, Phylogenetic

Introduction

Plant viruses pose major threats to global agriculture, causing diseases that can severely affect crop yields. Tomato leaf curl Palampur virus (ToLCPalV; *Begomovirus solanumpalampurense*) is a notable *Begomovirus* in the family *Geminiviridae*, affecting a wide range of plant species across Asia. ToLCPalV is classified as a bipartite *begomovirus* belonging to the oldworld clade, comprising two single-stranded DNA genomic segments, each measuring approximately 2.6–2.7 kb in length, and encapsulated within a distinctive ‘geminate’ particle (Stanley *et al*., 2005).

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Researchers first identified ToLCPalV in 2008 in tomatoes in India, where it caused yellowing and leaf curling (Kumar *et al*., 2008), and later reported it in pepper, squash, cucumber, melon, and watermelon in Pakistan (Malik *et al*., 2011) and in squash and eggplant in India (Venkataravanappa *et al*., 2018). In Iran, it was initially detected in tomato fields in Kerman and Hormozgan provinces (Fazeli *et al*., 2009) and later in cucurbits, beans, and weeds across several provinces including Fars, Sistan-o-Baluchestan, Khorasan Razavi, and Yazd (Heydarnejad *et al*., 2009, 2013). Pepper and eggplant were also identified as new hosts in Sistan-o-Baluchestan (Abkhoo and Mehraban, 2020). By 2013, ToLCPalV became one of the most damaging viruses of tomatoes and cucurbits in central and southern Iran, with limited incidence in watermelon (Heydarnejad *et al*., 2009, 2013). The virus has also been reported in neighboring countries (India, Pakistan, Saudi Arabia, Iraq, Oman). It is spreading from eastern to western Iran, expanding its host range (Astaraki and Shams-Bakhsh, 2023).

In the present study, we observed iron deficiency-like symptoms, such as chlorosis of younger leaves with veins usually remaining green, and severe mosaic and leaf entanglement, the typical symptoms reported for ToLCPalV infection (Kazemi *et al*., 2026), in weeds and crop plants at the campus of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Molecular approaches were employed to examine the presence of ToLCPalV in these symptomatic plants. Accordingly, this study aimed to detect ToLCPalV in additional naturally infected plant species in Iran and investigate their phylogenetic relationships.

Materials and Methods

Field and greenhouse sampling

Leaf samples showing viral symptoms were collected from six plant species *Acalypha australis*, soybean (*Glycine max*), cotton (*Gossypium hirsutum*), *Malva sylvestris*, *Bougainvillea glabra*, and *Chenopodium album* from greenhouses and fields on the Faculty of Agriculture campus, Tarbiat Modares University, Tehran, Iran. Samples were labeled with collection details and stored at −80 °C.

DNA extraction, PCR and sequencing

The CTAB (Cetyl trimethylammonium bromide) method was employed to extract DNA from leaf samples (Doyle and Doyle 1987). The quality of DNA was examined using a nanodrop. Two primer pairs specific for ToLCPalV DNA-A and DNA-B were used for PCR amplification of the corresponding genomic regions (Sabouri and Heydarnejad 2013). The PCR program was followed as previously described (Heydarnejad *et al*., 2013). The PCR products were run on a 1% agarose gel, and the obtained amplicons using primer pair ToLCPalV-1079-F/ToLCPalV-2054-R were sequenced using the forward primer (Pishgam Corporation, Tehran, Iran). The newly generated sequences were deposited into the GenBank database under the accession numbers presented in the phylogenetic tree.

Phylogenetic analysis

The newly generated sequences were compared with other ToLCPalV isolates from GenBank using BLAST, and relevant sequences from different hosts and regions were retrieved. Outgroup sequences were selected according to previous studies (Heydarnejad *et al*., 2013). Sequences were aligned in Clustal X2 (Larkin *et al*., 2007), and poorly aligned positions were removed using Gblocks. The substitution model was determined with MrModelTest 2 (Nylander, 2004). Bayesian phylogenetic analysis was conducted in MrBayes v3.1.2 for five million generations, and posterior probabilities were calculated using MCMC with a 50% consensus majority rule (Ronquist and Huelsenbeck, 2003; Larget and Simon, 1999). Adequacy of sample size was checked in Tracer v1.6 (Rambaut and Drummond, 2009). Trees were visualized in Dendroscope v3.2.8 (Huson and Scornavacca, 2012) and drawn in CorelDRAW® 16.

Results

Plant species identification

The collected plant samples belonged to five families and six species, including Euphorbiaceae (*Acalypha australis*), Fabaceae (*Glycine max*, soybean), Malvaceae (*Gossypium hirsutum*, cotton; *Malva sylvestris*), Nyctaginaceae (*Bougainvillea glabra*), and Amaranthaceae (*Chenopodium album*). Except for *Malva sylvestris* and *Chenopodium album*, which were collected from the field, the remaining samples were collected from the greenhouse, all located on the campus of the Faculty of Agriculture at Tarbiat Modares University, Tehran, Iran. Among the reported families, Euphorbiaceae, Malvaceae, and Nyctaginaceae are reported here for the first time as plant families in which ToLCPalV was detected under natural conditions (Table 1).

Table 1 Accession Numbers of Six Plant Samples in NCBI.

Plants	Accession numbers
Acalypha australis	PV776867
cotton	PV776869
Chenopodium album	PV776872
Malva sylvestris	PV776870
Bougainvillea glabra	PV776868
soybean	PV776871

Disease symptoms

The observed symptoms on collected plants were severe twisting of leaves and reduction of leaf size in *Malva sylvestris*; pale leaves, severe mosaic in cotton, severe leaf curl, interveinal chlorosis, reduction in leaf size, and slight yellowing in *Acalypha australis*; severe mosaic in *Bougainvillea glabra*; severe mosaic, chlorosis, very slight yellowing of the leaf margins of plants in soybean, and interveinal chlorosis and yellowing in *Chenopodium album* (Figures 1 and 2).

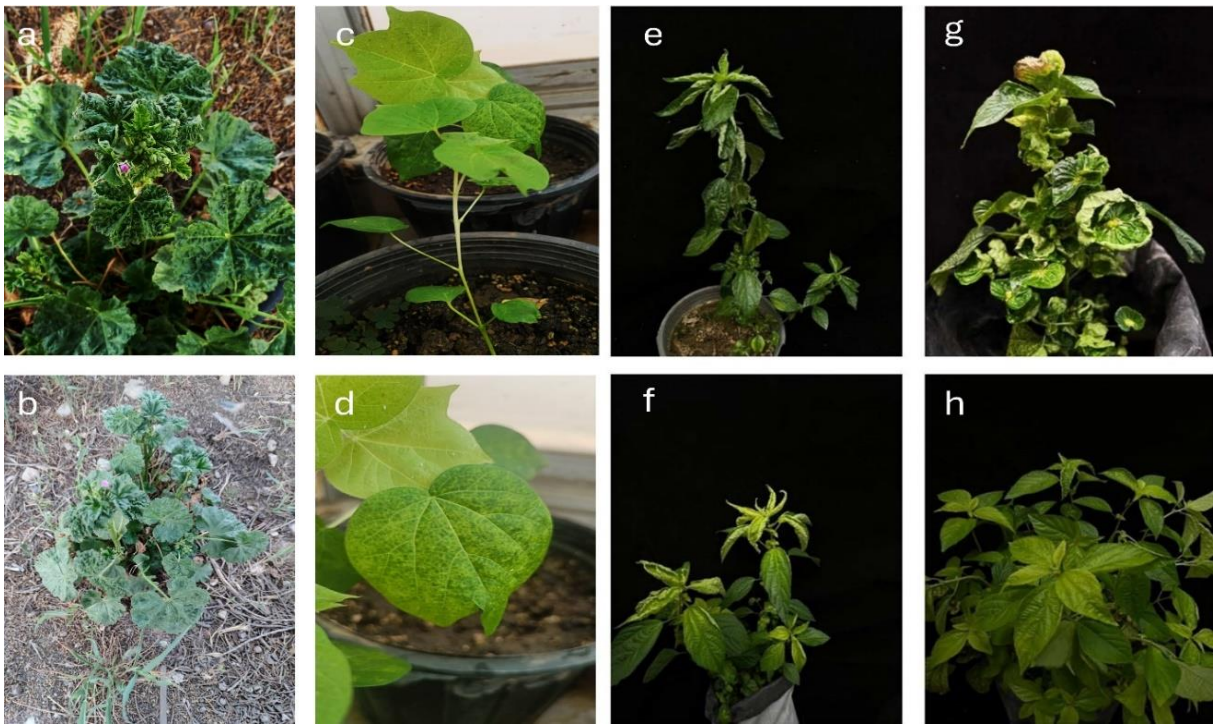


Figure 1 Symptoms of tomato leaf curl Palampur virus (ToLCPalV) on (a, b): *Malva sylvestris* (extreme twisting of leaves, reduction in leaf size); (c, d): cotton (Pale leaves, severe mosaic); (e, f, g, h): *Acalypha australis* (very severe leaf curl, interveinal chlorosis, reduction in leaf size, slight yellowing).

PCR, sequencing and phylogenetic profile

PCR using the primer pair ToLCPalV-1079-F/ToLCPalV-2054-R yielded successful amplification of the expected ~1000 nt band for six samples (Figure S3). The sequencing trials yielded six new sequences 500-700 nt long (the accession number and related host plant are presented on the phylogenetic tree). PCR with the primer pair ToLCPalV-F1217-A/ToLCPalV-R1709-A amplified the expected ~500 nt band in all six samples (Figure 3). No sequencing was performed for these amplicons. The detection of ToLCPalV in these samples was based on PCR amplification using two independent primer pairs targeting different genomic regions. The virus was detected in *Malva sylvestris* (21/25), *Chenopodium album* (49/52), *Bougainvillea glabra* (8/12), *Acalypha australis* (83/84), soybean (41/44), and cotton (30/38). A total of 65 ingroup and two outgroup sequences were included in the phylogenetic tree. Figure 4 shows the Bayesian phylogenetic tree including the sequences obtained in this study. In this tree, sequences from isolates originally isolated/sequenced from India, Pakistan, Saudi Arabia, Iran, Iraq, and Oman formed two major clades, with a maximally supported relationship between them. Clade A harbors sequences from Iran, Oman, Saudi Arabia, and Iraq. Clade B harbors sequences from Pakistan and India. The newly generated sequences in this study, including *Acalypha australis*, soybean, cotton, Bougainvillea glabra, *Malva sylvestris*, and *Chenopodium album*, formed a clade with three other sequences (*Cucumis sativus*) from Iran within clade A.



Figure 2 Symptoms of tomato leaf curl Palampur virus (ToLCPalV) on (a, b): *Bougainvillea glabra* (severe mosaic), soybean (severe mosaic, chlorosis, very slight yellowing of the leaf margins of plants); (e, f): *Chenopodium album* (interveinal chlorosis, yellowing).

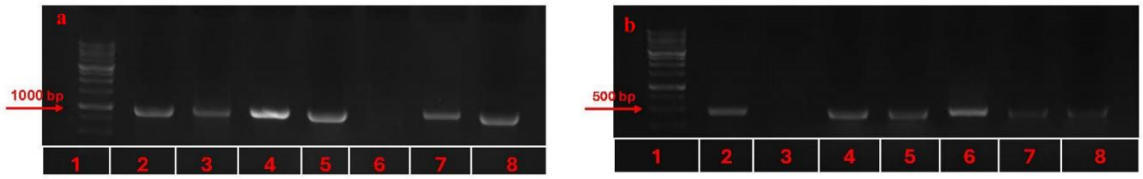


Figure 3 Electrophoretic patterns of PCR products showing 1000 bp (a) and 500 bp (b) fragments replicated by specific primer pairs ToLCPMV-F1217A/ToLCPMV-R1709A and ToLCPMV-F1079B/ToLCPMV-R2054B, respectively, in a 1% agarose gel. a: (1: Marker, 2: *Malva sylvestris*, 3: *Chenopodium album*, 4: *Acalypha australis*, 5: *Bougainvillea glabra*, 6: negative control of PCR, 7: soybean, 8: cotton)" b: (1: Marker, 2: *Malva sylvestris*, 3: negative control of PCR, 4: *Chenopodium album*, 5: *Bougainvillea glabra*, 6) *Acalypha australis*, 7: soybean, 8: cotton).

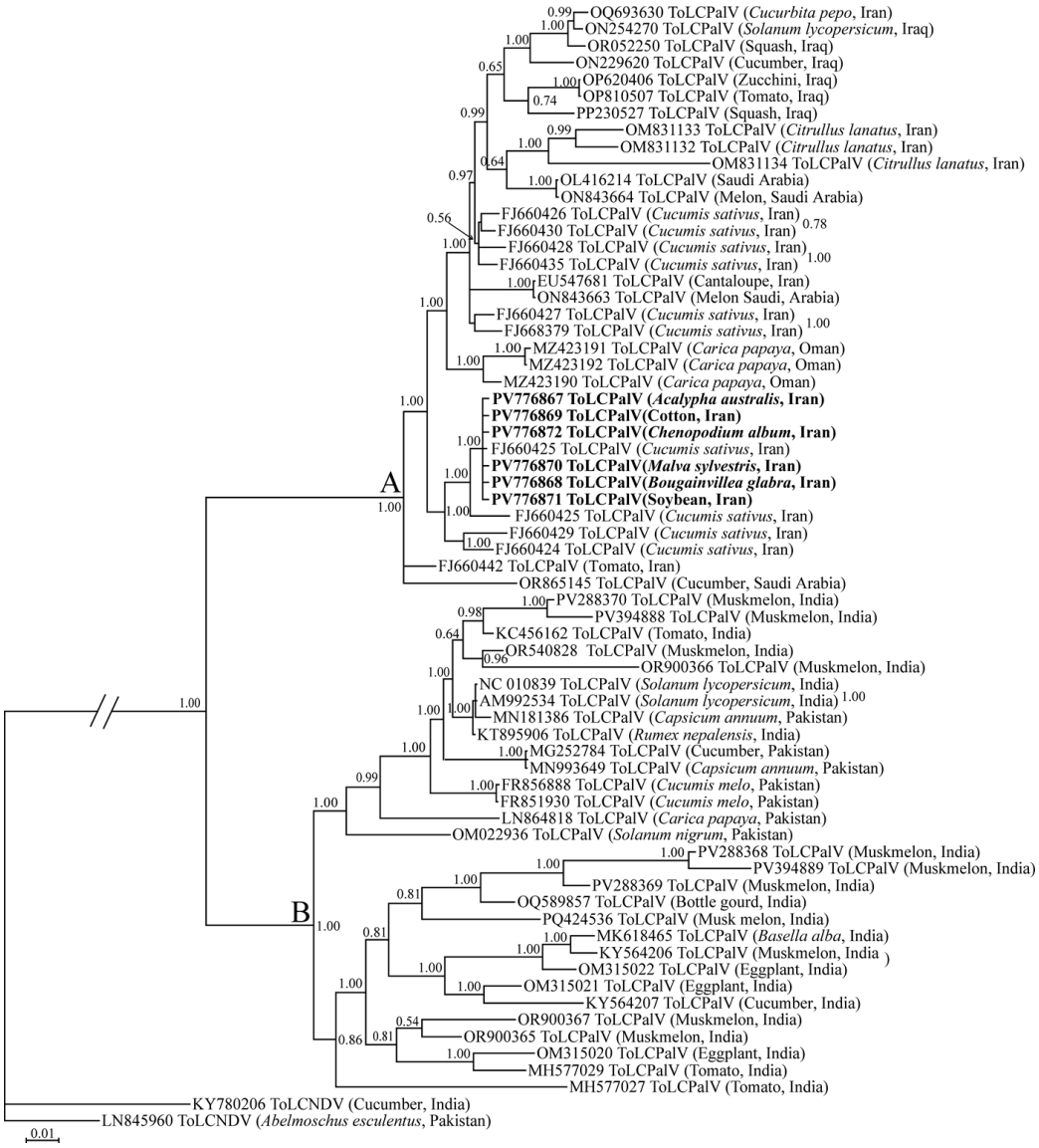


Figure 4 Bayesian 50% majority rule consensus tree inferred using 64 partial sequences of DNA-B (GenBank accession number and species name) under the GTR + G + I model. Bayesian posterior probability values exceeding 0.50 are given for appropriate clades. The newly generated sequences are in bold.

Discussion

The worldwide spread of ToLCPalV is primarily attributed to the dispersal of its insect vector, silverleaf whitefly, *Bemisia tabaci* (B biotype), and the transcontinental movement of plants infected with the virus (Lefeuvre *et al*., 2010; Shahmohammadi *et al*., 2022). The proximity of greenhouses and plastic tunnels likely facilitates the rapid proliferation and spread of whitefly populations in the open areas of the faculty campus in the present study.

Based on currently available data, the distribution of ToLCPalV is limited to Asia (including Iraq, Iran, Oman, Saudi Arabia, India and Pakistan), while its occurrence is progressively increasing in Iran (Kumar *et al*., 2008; Heydarnejad *et al*., 2009, 2013; Ali *et al*., 2010; Shahid and Al-Sadi, 2022; AlHudaib *et*

al., 2023). Previously, high infection rates of 50–100% in greenhouse-grown cucurbits have been reported (Heydarnejad *et al*., 2013). In recent years, due to climate change, the population of whiteflies, which are vectors of begomoviruses, has increased (Aregbesola *et al*., 2019; Thompson *et al*., 2024). The virus was first detected in southern and southeastern Iran in tomato and some weed species and later identified in cucumber and melon fields and greenhouses in these regions (Fazeli *et al*., 2009; Heydarnejad *et al*., 2009). It was later reported in other eastern regions on various cucurbits, beans, and weeds, such as *Chenopodium* sp. and *Heliotropium europaeum* (Heydarnejad *et al*., 2013). In recent years, the virus has been documented in beans grown in the western regions of Iran (Astaraki and Shams-Bakhsh, 2023). It has also been documented in watermelon fields in the southwest (Jelavi *et al*., 2022). The studied plants may act as virus reservoirs, maintaining ToLCPalV between seasons and facilitating transmission via whitefly (*Bemisia tabaci*) (Yazdkhasti *et al*., 2021). Understanding infection in these plants is important for assessing outbreak dynamics and cross-species risk (Sattar et al., 2025). In cucurbits, ToLCPalV causes symptoms resembling iron deficiency, characterized by chlorotic young leaves with green veins; this presentation may contribute to the virus being under-recognized by researchers and growers.

A previous phylogenetic analysis using DNA-A sequences revealed that ToLCPalV is closely related to ToLCNDV, another highly destructive begomovirus in the region (Heydarnejad *et al*., 2009). Al-Waeli *et al*. (2024) and Sattar *et al*. (2025) used DNA-B sequences. The results of these two studies showed close relationships between isolates from the Middle East, including Iran, Iraq, Oman, and Saudi Arabia, and those from India and Pakistan. Although the Bayesian phylogenetic tree clearly distinguishes two major clades of ToLCPalV isolates corresponding to West Asia (Iran, Iraq, Oman, Saudi Arabia) and South Asia (India, Pakistan), the evolutionary interpretation remains preliminary. The phylogenetic insights are limited, as clustering appears to be driven primarily by geographic origin rather than host species, indicating the need for more comprehensive analyses to elucidate host adaptation and viral evolution. In the present study, we detected ToLCPalV in six additional plant species. While PCR detection and sequencing confirmed the presence of viral DNA, experimental inoculation was not performed; therefore, these plant species should be considered putatively associated with ToLCPalV rather than confirmed hosts. Viral sequence clustering was not associated with host species but with geographic origin, suggesting potential adaptation of ToLCPalV to local plant populations.

ToLCPalV is rapidly spreading from the southern and southeastern provinces to new areas with subtropical climate conditions in Iran. Detection in additional plant species highlights the potential for expanded natural occurrence, which may increase the epidemiological significance of the virus. More surveys and detailed studies are needed to detect and characterize other hosts prone to begomovirus infection in Iran using new techniques, such as high-throughput sequencing (HTS), and monitoring and control measures should be adopted to manage the spread of this virus.

Phylogenetic analysis further revealed clear regional differentiation in ToLCPalV populations, with distinct clades and sub-clades corresponding to South Asia (India and Pakistan) and West Asia (Iran, Iraq, Oman, and Saudi Arabia). This regional clustering, evident in both phylogenetic and MJ network analyses, suggests limited gene flow between these two major populations, likely due to geographical barriers and localized vector transmission dynamics (Sattar *et al*., 2025). The whitefly *Bemisia tabaci* (B biotype) largely drives ToLCPalV spread and efficiently transmits the virus among crops and weeds. High vector density, proximity to greenhouses, and closely planted crops accelerate transmission. Climate change and human-mediated plant movement can

significantly increase whitefly populations, promoting the rapid expansion of begomovirus in Asia and the Middle East (Lefeuvre *et al*., 2010; Thompson *et al*., 2024).

Conclusion

Phylogenetic analyses of ToLCPalV DNA-B sequences indicate that viral populations are structured mainly by geographic origin rather than host species, indicating localized gene flow mediated by vector activity. Regional clustering between West Asia (Iran, Iraq, Oman, Saudi Arabia) and South Asia (India, Pakistan) likely reflects environmental barriers, vector behavior, and host availability (Al-Waeli *et al*., 2024; Sattar *et al*., 2025). Moreover, the genomic flexibility of ToLCPalV enables adaptation to new host species, increasing the epidemiological risk in areas where new hosts emerge. Integrating vector dynamics with population genetics is crucial for predicting outbreaks, implementing targeted surveillance, and designing region-specific control strategies.

Acknowledgments

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ردیابی ویروس پالامپور پیچیدگی برگ گوجه فرنگی در گونه‌های گیاهی جدید از ایران

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چکیده: ویروس پالامپور پیچیدگی برگ گوجه‌فرنگی (Tomato leaf curl Palampur virus: ToLCPalV) گونه *Begomovirus solanumpalampurense* یک بگوموویروس دوبخشی از خانواده *Geminiviridae* است که موجب بیماری پیچیدگی برگ گوجه‌فرنگی می‌شود. هدف این مطالعه ردیابی ToLCPalV در گونه‌های گیاهی جدید با آلوده طبیعی در ایران و بررسی روابط فیلوژنتیکی آن‌ها بود. نمونه‌های برگی از گیاهان دارای علائم مشکوک از استان تهران جمع‌آوری شد. حضور ToLCPalV از طریق واکنش زنجیره‌ای پلیمراز (PCR) و سپس تعیین توالی قطعات تکثیر شده در گیاهان سویا از تیره Fabaceae، سلمه تره از تیره Amaranthaceae، آکالیفا استرالیس از تیره Euphorbiaceae، پنبه از تیره Malvaceae، پنیرک از تیره Malvaceae، گل کاغذی از تیره Nyctaginaceae تأیید شد. در این بررسی، برای نخستین بار ToLCPalV از تیره‌های Euphorbiaceae، Malvaceae و Nyctaginaceae در شرایط طبیعی ردیابی شده است. در درخت فیلوژنتیکی بیزی بازسازی شده براساس توالی‌های جزئی DNA-B موجود، دو دودمان اصلی شکل گرفت. یک دودمان شامل جدایه‌های رایج در ایران و نیز توالی‌های به‌دست آمده در مطالعه حاضر، کشورهای عراق، عمان و عربستان سعودی بود؛ دودمان دوم شامل جدایه‌هایی از هند و پاکستان از میزبان‌های مختلف بود. گسترش ToLCPalV از جنوب‌شرقی ایران به مناطق مرکزی و شمال‌شرقی، تهدیدی جدی برای کشت کدوئیان و گوجه‌فرنگی در این مناطق محسوب می‌شود و ضرورت اجرای برنامه‌های پایش و کنترل را برجسته می‌سازد، زیرا این مناطق از قطب‌های اصلی تولید این محصولات هستند. با توجه به خسارت قابل‌توجه مشاهده شده در مزارع و گلخانه‌ها، آشکار است که ToLCPalV با گسترش دامنه میزبانی خود درحال تبدیل شدن به تهدیدی عمده برای کدوئیان، گوجه‌فرنگی و سایر محصولات کشاورزی است.

واژگان کلیدی: بگوموویروس، جمینی ویروس، ایران، فیلوژنتیکی