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ORIGINAL ARTICLE

Infection of tomato in Iraq with tomato leaf curl Palampur virus and multiple variants of tomato yellow leaf curl virus

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Abstract

Tomato yellow leaf curl disease (TYLCD) and tomato leaf curl disease (TLCD) cause serious losses in tomato production, especially in tropical and sub-tropical regions. In 2014–2015, tomato samples with TYLCD/TLCD-like symptoms were collected from different provinces of Iraq and infection with tomato yellow leaf curl virus (TYLCV) identified. To study the diversity of TYLCV, DNA of eight positive samples from this survey was used for rolling-circle amplification, cloning and sequencing. Pairwise nucleotide sequence comparisons with complete genomes showed that the Iraqi TYLCV isolates belonged to the strains TYLCV-IL and TYLCV-Mld. In a phylogenetic analysis, the Iraqi TYLCV-IL isolates grouped into three distinct clades, consisting of TYLCV-IL (A) and the two new variants TYLCV-IL (D) and TYLCV-IL (E). The Iraqi isolate of TYLCV-Mld grouped into the newly proposed TYLCV-Mld (D) variant. For one sample, sequencing also revealed co-infection with tomato leaf curl Palampur virus (ToLCPalV). The phylogenetic tree of ToLCPalV DNA-A showed a close relationship between the isolates of different hosts from Iraq and Iran. No evidence of recombination was detected in ToLCPalV DNA-A, but recombination was observed for the TYLCV isolates. The results indicate that there is a high diversity of TYLCV in Iraq, including new variants, that is partly shared with Kuwait and countries in the Eastern Mediterranean Region. Occurrence of multiple TYLCV variants and ToLCPalV can act as a potential threat to tomato production in Iraq.

Keywords Tomato yellow leaf curl disease · Tomato leaf curl disease · *Begomovirus* · TYLCV-IL · TYLCV-Mld · Recombination

Introduction

Tomato yellow leaf curl disease (TYLCD) is one of the most serious and economically important diseases of tomato (*Solanum lycopersicum*). TYLCD was first reported in the Jordan Valley, Israel and has subsequently spread into the Mediterranean Basin and most tropical and subtropical regions of the world (Lefeuvre et al. 2010). The disease is caused by a complex of viruses belonging to at least 13 species of the genus *Begomovirus*, family *Geminiviridae* (Yan et al. 2021). The genus *Begomovirus* is the largest genus of plant-infecting viruses and viruses of this genus have a circular single-stranded monopartite or bipartite DNA genome of 2.8 kb or 5.6 kb, respectively. Among TYLCD-causing viruses, tomato yellow leaf curl virus (TYLCV) has become established worldwide, resulting in economic losses (Rojas et al. 2018). Although TYLCD can be found worldwide, only viruses of two strains, the Israel (TYLCV-IL) and Mild (TYLCV-Mld) strains of TYLCV, are truly global

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TYLCD-causing agents. Other begomoviruses associated with TYLCD have been found only in restricted regions (Navas-Castillo et al. 2011). TYLCD-causing viruses induce similar symptoms in infected tomato plants, but can differ in their molecular and biological (e.g., host range) properties (Navas-Castillo et al. 2011).

TYLCV has many different plant hosts belonging to 15 plant families including vegetables, ornamentals and wild plants (Papayiannis et al. 2011). Like other begomoviruses, TYLCV is transmitted by whiteflies of the *Bemisia tabaci* species complex in a persistent circulative non-propagative manner (Pakkianathan et al. 2015). Transovarial and sexual transmission has been observed for TYLCV (Ghanim et al. 1998; Ghanim and Czosnek 2000).

The analysis of the plausible history of diversification of TYLCV indicated that TYLCV first originated in the Middle East (Jordan Valley, Israel) and then globally spread after the evolution of TYLCV-Mld and TYLCV-IL into the Mediterranean Basin and most tropical and subtropical regions (Lefeuvre et al. 2010; Mabvakure et al. 2016). According to the strain demarcation of 94% pairwise sequence identity, eight TYLCV strains have been identified: TYLCV-IL, TYLCV-Mld, TYLCV-Bou, TYLCV-Gez, TYLCV-IR, TYLCV-Kah, TYLCV-Ker and TYLCV-OM (Idris and Brown 2005; Lefeuvre et al. 2010; Pakniat et al. 2010; Al-Ali et al. 2015). Among the TYLCV strains, while TYLCV-IL and TYLCV-Mld are globally distributed, the distribution of other extant strains is restricted to the Middle East and surrounding regions (Lee et al. 2010; Lefeuvre et al. 2010; Polston et al. 2014; Marchant et al. 2023). The presence of six (TYLCV-IL, -IR, -Bou, -Kah, -Ker and -OM) out of eight TYLCV strains indicate that Iran is a centre of TYLCV diversity and that it is a site for TYLCV evolution (Lefeuvre et al. 2010; Hosseinzadeh et al. 2014; Mabvakure et al. 2016; Marchant et al. 2023). The results of evolutionary analysis have predicted that TYLCV-IL represents the oldest, most recent common ancestor (MRCA), followed by TYLCV-Mld and the two Iranian strains TYLCV-Ker and TYLCV-IR (Marchant et al. 2023). In addition to TYLCD, a complex of different begomoviruses, e.g., tomato leaf curl Palampur virus (ToLCPalV), cause tomato leaf curl disease (ToLCD). ToLCPalV is a bipartite begomovirus, which was first identified in India (Kumar et al. 2008), and then reported from Iran, Pakistan, Iraq, Oman and Saudi Arabia (Heydarnejad et al. 2009; Ali et al. 2010; Mohammed et al. 2021; AlHudaib et al. 2023). In addition to tomato, ToLCPalV naturally infects common beans (*Phaseolus vulgaris*; Heydarnejad et al. 2013), papaya (*Carica papaya*; Shahid and Al-Sadi 2022), *Basella alba* (Kumari et al. 2020), *Rumex* sp. (Sharma et al. 2019) and different plants of the families Cucurbitaceae (Heydarnejad et al. 2009; Ali et al. 2010; Shafiq et al. 2019; Dhkal et al. 2020; AlHudaib et al. 2023; Shahid 2023) and Solanaceae (Venkataravanapa et al. 2018; Sattar et al. 2022; Abass and Lahuf 2023). In Iran, the incidence of ToLCPalV has been gradually increasing since it was first detected in southern Iran and infections could cause up to 100% losses in cucurbit and melon production (Heydarnejad et al. 2009).

Iraq is located between the subtropical aridity of the Arabian Desert and the subtropical humidity of the Persian Gulf. Except for northern Iraq, the climate is hot and dry with long hot summers and short, cool winters and the rainfall is usually low (Soppe and Saleh 2014). Iraq shares its eastern border with Iran and is one of the most important countries in production of tomato in the Middle East (Anonymous 2007; Soppe and Saleh 2014). Tomato is the most commonly grown vegetable in Iraq, with a total production of 771,000 tonnes in 2018, and high production in Karbala, Basrah and Najaf (FAO 2021). In Iraq, since TYLCV was first detected from tomato plants using serological methods (Makkouk 1978), there are only limited reports about its incidence in this crop based on serological (Al-Ani et al. 2011) or molecular (Al-Kuwaiti et al. 2013; Al-Waeli et al. 2017, 2018; Al-Abedy et al. 2018; Alabde et al. 2021) investigations. So far, only the full genomic sequences of four Iraqi TYLCV isolates have been characterized (Al-Kuwaiti et al. 2013; Alabde et al. 2021). Recently, natural infection of TYLCV in *Malva parviflora* and *Melilotus indicus* and genetic diversity of TYLCV based on the coat protein (*cp*) gene have been reported from Iraq (Al-Waeli et al. 2017, 2018).

More studies are now paying attention to the incidence of begomoviruses in tomatoes in Iraq. Given that the region around Iran is a centre of TYLCV diversity (Lefeuvre et al. 2010), this study focused on the characterization of tomato-infecting begomoviruses in Iraq to reveal the genetic diversity of TYLCV based on full-length genome sequences.

Material and methods

Sampling and DNA extraction

In a previous survey carried out during 2014–2015 (Al-Waeli et al. 2018), leaves of 393 tomato plants with the symptoms of leaf curling, chlorosis, deformation and stunting were collected from major tomato producing regions in Iraq, including Karbala, Babil, Najaf, Qadisiyah, Dhi-Qar and Basrah. Among 21 TYLCV-infected samples identified by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and polymerase chain reaction (PCR) (Al-Waeli et al. 2018), eight samples (Table 1) were selected for the characterization of full-length genome sequences.

Table 1 Characteristics of isolates of tomato yellow leaf curl virus (TYLCV) and tomato leaf curl Palampur virus (ToLCPalV) identified in this study

Isolate	Location	Full genome size (nt)	GenBank accession no.	Species
IQ:Ba-Zu82:Tomato:15	Basrah (Zubair)	2762	OM925532	TYLCV
IQ:Ba-Zu53:Tomato:15	Basrah (Zubair)	2780	OM925531	TYLCV
IQ:Ka-4:Tomato:15	Karbala	2769	OM925533	TYLCV
IQ:Ka-5:Tomato:15	Karbala	2769	OM925534	TYLCV
IQ:Na-4:Tomato:15	Najaf	2770	OM925530	TYLCV
IQ:Na-19:Tomato:15	Najaf	2781	OM925535	TYLCV
IQ:Dq-1:Tomato:15	Dhi-Qar	2762	OM925529	TYLCV
IQ:Dq-A4-12:Tomato:15	Dhi-Qar	2763	OM925536	TYLCV
IQ:Dq-A4-1:Tomato:15	Dhi-Qar	2756	OM925537	ToLCPalV

Total DNA was extracted from leaf samples using a CTAB method (Lodhi et al. 1994) and stored at −80 ºC.

Amplification, cloning and full viral genome sequencing

Circular DNA was amplified from the total DNA extracts using rolling circle amplification (RCA) (TempliPhi RCA kit, GE Healthcare) as described by Shepherd et al. (2008). The RCA product for each sample was digested with a range of restriction enzymes, including *Bam*HI, *Sal*I, *Sac*I and *Xba*I (Thermo Fisher Scientific), to yield the expected fragment of ~2.8 kb. Digested fragments were purified from 1% agarose gel using a gel purification kit (Thermo Fisher Scientific), ligated into digested pBluescript II KS (+) and transformed into competent cells of *Escherichia coli* DH5α. Four recombinant plasmids were sequenced for each sample in both directions using a primer walking strategy (Macrogen Inc.). The presence of betasatellite and DNA-B were tested using PCR and the primer pairs Beta01/Beta02 (Briddon et al. 2002), and PBL1v2040/PCRc1 (Rojas et al. 1993), respectively. For ToLCPalV, the presence of DNA-B was tested by PCR using the primer pair ToLCPMV-1079-F/ToLCPMV-2054-R (Heydarnejad et al. 2013) (Table 2).

Sequence and phylogenetic analyses

Sequences were assembled using SEQMAN from LASERGENE software package (DNAStar Inc.) and compared with previously published sequences available in GenBank using BLASTn. Open reading frames (ORFs) on both sense and antisense strands were predicted using online ORF finder software (www.ncbi.nlm.nih.gov/projects/gorf/).

The complete genome sequences of eight new TYLCV isolates and the complete DNA-A sequence of an isolate of ToLCPalV were aligned separately with 39 cognate sequences retrieved from the GenBank (NCBI) database (Supplementary Tables 1 and 2) using MUSCLE (Edgar 2004) implemented in MEGA 7 (Kumar et al. 2016). Pairwise nucleotide (nt) identity values were determined using SDT v1.2 (Muhire et al. 2013). Maximum likelihood (ML) phylogenetic trees of aligned sequences were constructed using PHYML v3.0 (Guindon et al. 2010) with GTR + G4 as the best substitution model identified by MEGA 7 and with an approximate likelihood ratio test (aLRT) for branch support. Branches with aLRT < 80% were collapsed. Sequences of tomato yellow leaf curl China virus (TYLCCNV; accession no. NC_004044) and tomato leaf curl New Delhi virus (ToLCNDV; NC_004611) were used as outgroups for TYLCV and ToLCPalV DNA-A, respectively.

Maximum likelihood phylogenetic trees were also constructed from the aligned nt sequences of the genes encoding replication-associated protein (Rep), movement protein (MP) and CP of TYLCV as well as *rep* and *cp* genes of ToLCPalV. The trees were inferred using PHYML v3.0 (Guindon et al. 2010) with T92 + G for the *mp* and *cp* genes and HKY + G + I for *rep* as best nt substitution models determined by MEGA 7 (Kumar et al. 2016) with 1,000 replicates of bootstrap support. The ML phylogenetic tree of each gene was rooted with the corresponding gene of TYLCCNV and ToLCNDV for TYLCV and ToLCPalV, respectively. Branches with bootstrap support < 70% were collapsed. The pairwise identity of nt sequences of each gene were determined using SDT (Muhire et al. 2013).

Recombination analysis

Constructed alignments of the TYLCV genome and ToLCPalV DNA-A were also used for intra-species

Table 2 Sequences of the oligonucleotide primers

Primer	Nucleotide sequence	Reference
Beta01	5′-GGTACCACTACGCTACGCAGCAGCC-3′	Briddon et al. (2002)
Beta02	5′-GGTACCTACCCTCCCAGGGGTACAC-3′	
PBL1v2040	5′-GCCTCTGCAGCARTGRTCKATCTTCATACA-3′	Rojas et al. (1993)
PCRc1	5′-CTAGCTGCAGCATATTTACRARWATGCCA-3′	
ToLCPMV-1079-F	5′-TTGGGTCACGTTCCGCGACGAAGA-3′	Heydarnejad et al. (2013)
ToLCPMV-2054-R	5′-TACGCGCTCACAAACGATGCTGCA-3′	

recombination analysis with Recombination Detection Program (RDP v. 4.95) (Martin et al. 2015) and default settings. RDP implements analysis of recombination using seven different detection algorithms, including RDP (Martin and Rybicki 2000), GENECONV (Padidam et al. 1999), Bootscan (Martin et al. 2005), Maxchi (Smith 1992), Chimera (Posada and Crandall 2001), Siscan (Gibbs et al. 2000) and 3seq (Boni et al. 2007). The events which were detected by at least three or more implemented algorithms with *p-values* of $<10^{-3}$ were considered as positive/possible recombination events. Interspecies recombination analysis was performed among Iraqi begomovirus isolates and other isolates of closely related species using RDP (Martin et al. 2015).

Results

Sequencing of begomovirus isolates

Among TYLCV-infected samples, showing leaf curling, chlorosis and deformation symptoms (Fig.S1), the complete genomes of eight selected Iraqi TYLCV isolates from Basrah, Karbala, Najaf and Dhi-Qar were amplified, sequenced and assembled to yield full-length sequences of 2762–2781 nt (Table 1). Nucleotide BLAST searches for genome sequences of the Iraqi TYLCV isolates revealed the highest identity (93–98%) with previously reported TYLCV isolates worldwide. The RCA product of sample Tomato-12 (from Dhi-Qar, Iraq) was digested with *Sal*I and sequencing of different clones showed the presence of ToLCPalV-[IQ] in mixed infection with TYLCV. The DNA-A component of this Iraqi ToLCPalV isolate (IQ:Dq-A4-1:Tomato:15; accession No. OM925537) consisted of 2756 nt that shared > 99% identity with previously reported ToLCPalV isolates from Saudi Arabia and Iraq (OL416211, OL416213, ON229618, OQ693629). An analysis of the predicted gene content revealed that the TYLCV genome and ToLCPalV DNA-A contained six ORFs, two on virion sense (CP and V2) and four on complementary sense strand (Rep, TrAP, REn and C4). Recently, six additional ORFs encoding small proteins with specific subcellular localization and virulence function have been identified in geminiviruses (Gong et al. 2021). These ORFs were identified in Iraqi TYLCV sequences, including ORF1 corresponding to nt 862 – 659/850 – 647 (67 aa), ORF2 nt 512 – 321/524 – 333 (63 aa), ORF3 nt 456 – 268/444 – 256 (62 aa), ORF4 nt 498–662/486–650 (54 aa), ORF5 nt 678–818/666–806 (46 aa), and ORF6 nt 2338–2571/2350–2583 (77 aa). Comparative analysis of predicted Rep and CP amino acid sequences of the identified isolates from Iraq with other isolates from GenBank showed no difference in sequences of Rep (RCR1, RCR2 and RCR3, binding to retinoblastoma-related protein) (Fondong 2013) or CP motifs for nuclear localization signals, DNA binding, nuclear export signal and cell wall targeting (Fondong 2013).

Amplification of DNA-B and betasatellite using PBL1v2040/PCRc and Beta01\Beta02 primers, respectively, was not successful for any of the TYLCV-infected samples, which indicated the absence of these molecules in this study. However, for the ToLCPalV-infected plant, PCR amplification of DNA-B using specific primers yielded a band of the expected size of approximately 1 kb, confirming the presence of DNA-B. Previously, betasatellites have been found in association with ToLCPalV (Namrata et al. 2011; Sharma et al. 2019), but no betasatellite was detected from the tomato plant harbouring ToLCPalV-[IQ].

Sequence and phylogenetic analyses

Using SDT software, genome-wide nt sequence pairwise comparisons of the eight Iraqi TYLCV isolates identified in this study with 39 previously reported isolates belonging to different strains, variants and regions (Supplementary Table 1) showed that they shared 89.7–98.6% identity (Fig. 1A). Following taxonomic criteria for strain demarcation currently established for begomoviruses (94% sequence threshold) (Brown et al. 2015), all isolates, except IQ:Na-4:Tomato:15 (OM925530) and IQ:Dq-A4-12:Tomato:15 (OM925536), shared the highest nt identity (> 94%) with isolates of the TYLCV-IL strain (Fig. 1A). The nt identity among the Iraqi TYLCV-IL isolates in this study ($n=6$) was 94.2–98.6%. IQ:Na-4:Tomato:15 shared < 94% nt identity with isolates of previously reported TYLCV strains, and showed the highest identity of 92.3–93.6% and 92.5–94.0% with TYLCV-IL and TYLCV-Mld, respectively. IQ:Dq-A4-12:Tomato:15 shared 94.0-95.8% and 94.0-94.1% pairwise nt identity with isolates of the TYLCV-Mld and TYLCV-Kah strains, respectively (Fig. 1A). This isolate shared highest identity at 95.7–95.8% with two isolates previously reported from Iraq (MT583814 and OP771625; Alabde et al. 2021) (Fig. 1A).

Based on nt pairwise identity analyses, ToLCPalV-[IQ] DNA-A showed highest identity of 97.4–99.2% with previously reported Iranian and Iraqi ToLCPalV isolates (Fig. 1B).

A maximum likelihood phylogenetic tree based on complete genome nt sequences of TYLCV revealed that the Iraqi TYLCV isolates identified in this study belonged to the strains TYLCV-IL and–Mld. The sequences of eight Iraqi TYLCV isolates (including seven isolates identified in this study (Table 1) and one previously reported isolate, JQ354991) fell into three clusters of TYLCV-IL, while only IQ:Dq-A4-12:Tomato:15 along with three

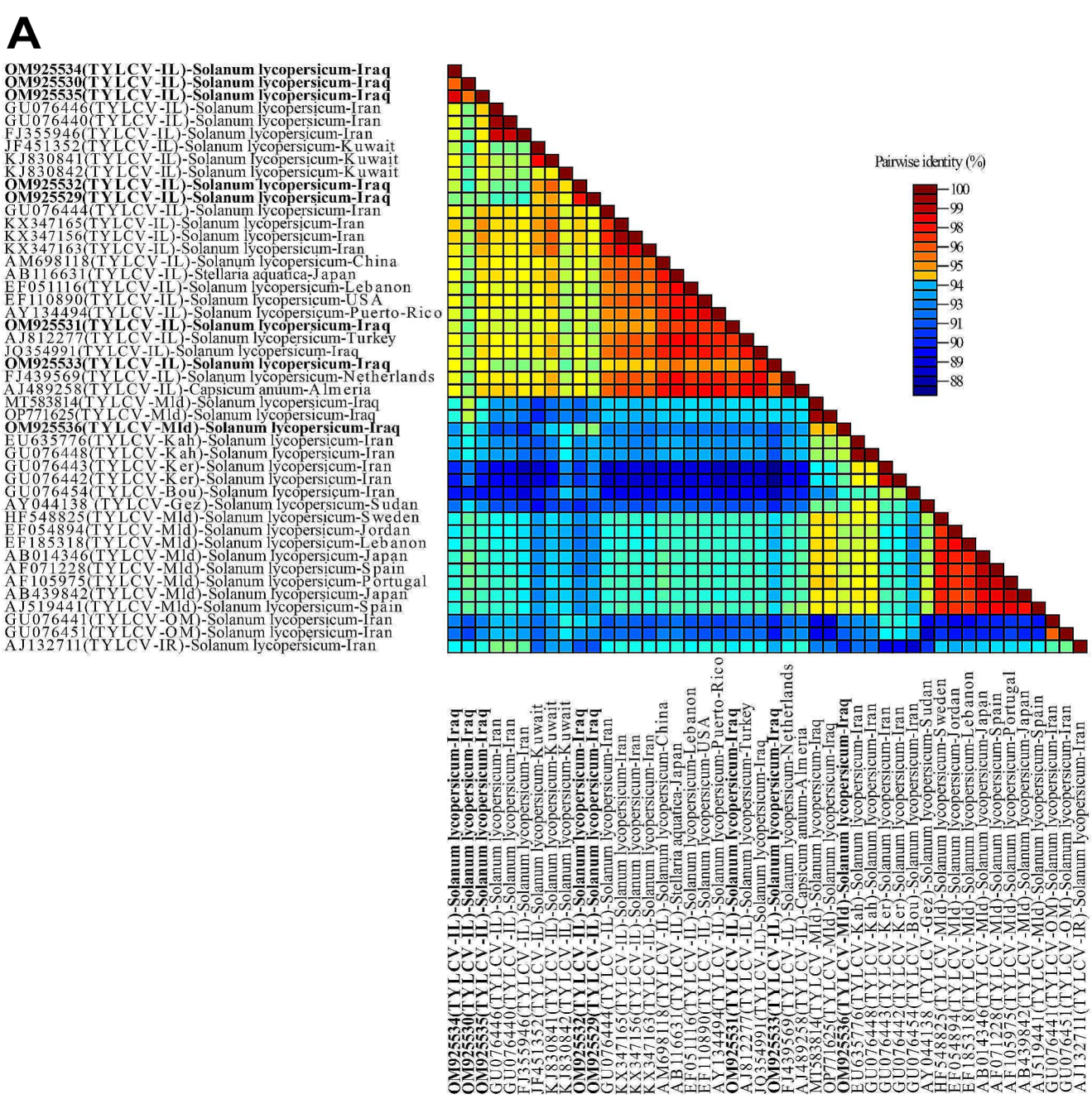

Fig. 1 Pairwise nucleotide identity colour matrix for complete genome sequences of TYLCV isolates (**A**) and DNA-A sequences of ToLCPalV isolates (**B**) calculated by SDT v1.2. Variants of TYLCV-IL and TYLCV-Mld identified in this study are in bold

previously reported Iraqi isolates (MT583814, ON254272 and OP771625) formed a distinct cluster belonging to TYLCV-Mld (Fig. 2A).

Both TYLCV-IL and TYLCV-Mld have been classified into three variants based on an identity of 93.0-100% (Lefeuvre et al. 2010). In the phylogenetic analysis, most of the TYLCV-IL isolates along with three Iraqi isolates, including previously published TYLCV-IRQ (JQ354991) as well as the two new ones IQ:Ba-Zu53:Tomato:15 (OM925531) and IQ:Ka-4:Tomato:15 (OM925533), grouped into the TYLCV-IL (A) variant (Fig. 2A). In addition to TYLCV-IL (B) and (C), both including Iranian TYLCV isolates, two new variants named TYLCV-IL (D) and TYLCV-IL (E) were now identified. TYLCV-IL (D) included the Iraqi isolates IQ:Ba-Zu82:Tomato:15 (OM925532) and IQ:Dq-1:Tomato:15 (OM925529) along with three TYLCV-IL isolates identified from Kuwait (Al-Ali et al. 2015). TYLCV-IL (E) included only Iraqi isolates identified in this study:

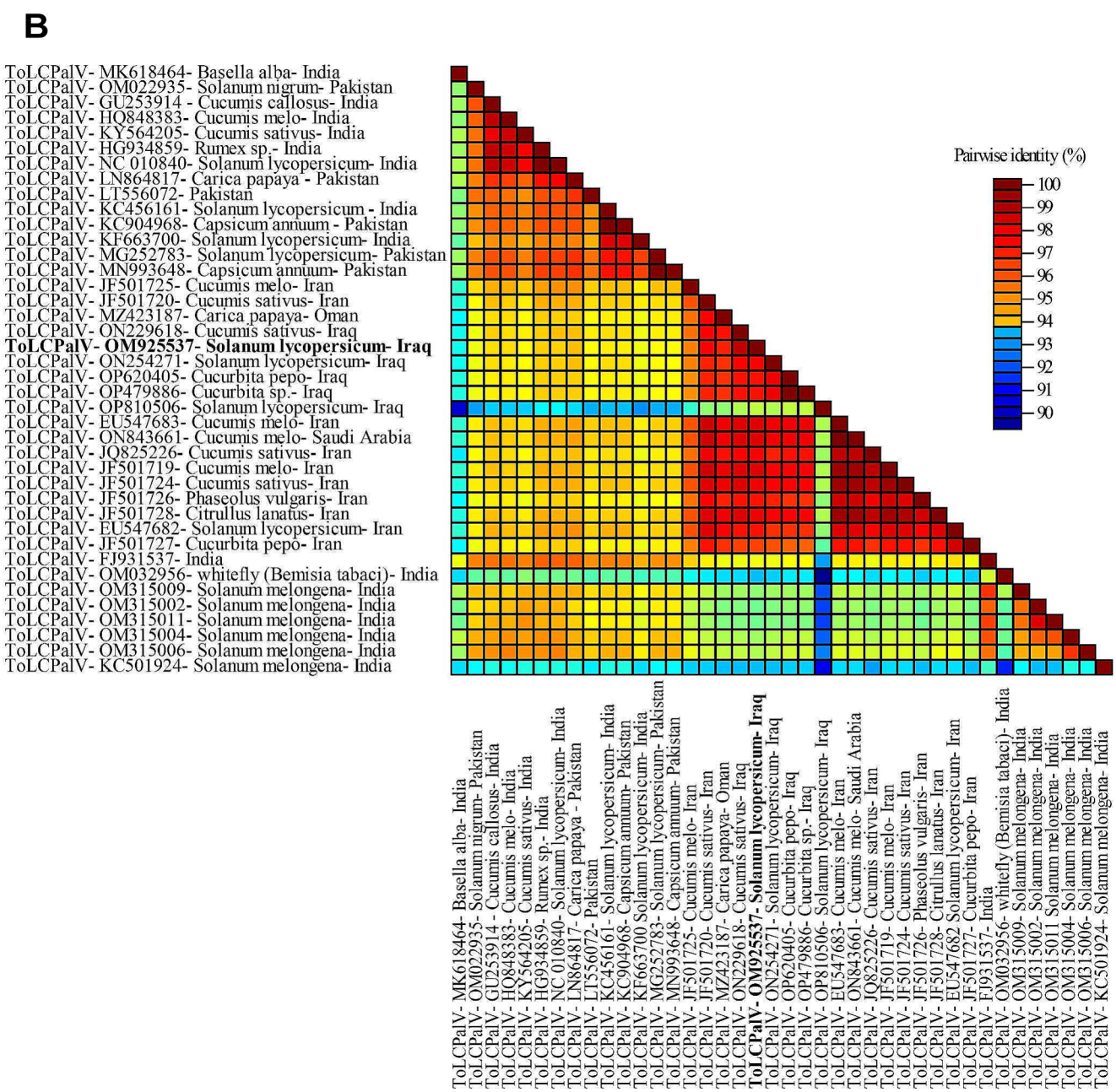

Fig. 1 (continued)

IQ:Na-19:Tomato:15 (OM925535), IQ:Ka-5:Tomato:15 (OM925534) and IQ:Na-4:Tomato:15 (Fig. 2A). The nt identity between Iraqi isolates of different TYLCV–IL variants was 92.5–94.8%. For TYLCV-Mld, four phylogroups (TYLCV-Mld A - D) were identified. TYLCV-Mld (A) included most of the isolates from Japan, Spain and Portugal, TYLCV-Mld (B) included isolates from Spain and Sweden. TYLCV-Mld (C) also included isolates from Jordan and Lebanon. The four Iraqi isolates IQ:Dq-A4-12:Tomato:15 (identified in this study) and the previously reported isolates Najaf (MT583814), Karbala-1 (ON254272) and Kufa (OP771625) formed TYLCV-Mld (D) as a new variant (Fig. 1A).

In the ML phylogenetic analysis of full-length nt sequences of DNA-A of 40 ToLCPalV isolates (Supplementary Table 2), ToLCPalV isolates from Iraq, Iran, Oman, and Saudi Arabia formed a distinct Middle East (ME) clade (Fig. 2B). In this clade, ToLCPalV-[IQ] along with other isolates from Iraq grouped into a distinct subclade. Isolates identified from India and Pakistan fell into Indo-Pakistan (IN-PAK) clade, while isolates identified from *Solanum melongena* and *Basella alba* in India fell into a distinct India

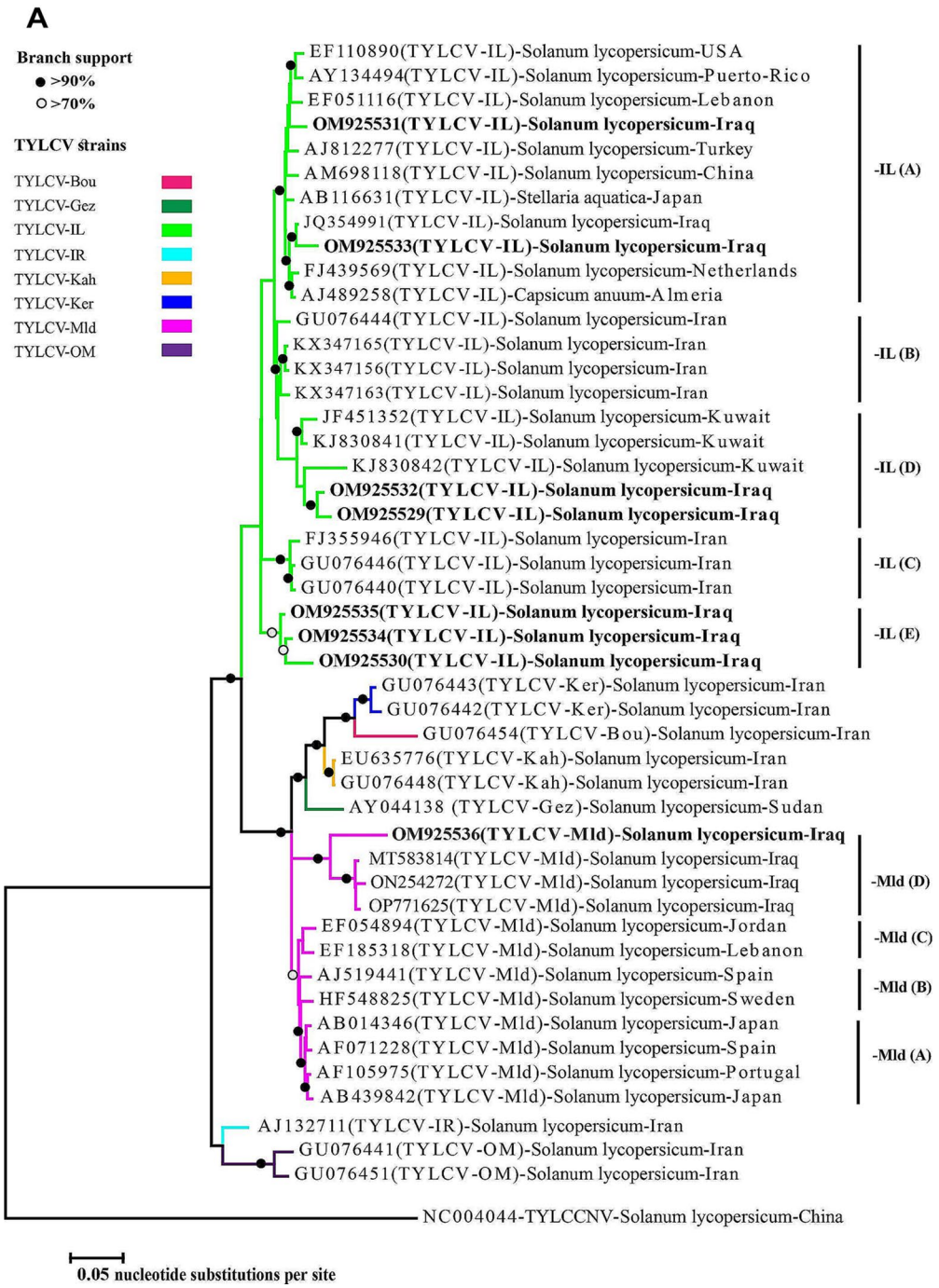

Fig. 2 Maximum likelihood phylogenetic tree for complete genome sequences of TYLCV isolates (**A**) and DNA-A sequences of ToLCPalV isolates (**B**), constructed using PHYML v3.0. The sequences of tomato yellow leaf curl China virus (TYLCCV) and tomato leaf curl New Delhi virus (ToLCNDV) isolates were used as outgroups for TYLCV and ToLCPalV, respectively. Bootstrap values are indicated by open (70–90%) and closed (> 90%) circles, while branches supported by < 70% bootstrap have been collapsed. Isolates identified in this study are in bold

(IN) clade. A newly identified ToLCPalV sequence from *B. tabaci* (accession No. OM032956) was separate from the other ToLCPalV sequences in the ML tree (Fig. 2B) and it shared 92.9–95.2% nt identity with sequences of other ToLCPalV isolates (Fig. 1B).

An ML phylogenetic analysis with nt sequences of the *rep* gene identified most of the TYLCV strains, while the ML trees based on the *mp* and *cp* genes did not. This inconsistency could be related with the occurrence of recombination (Fig. S2), which is in agreement with previously published

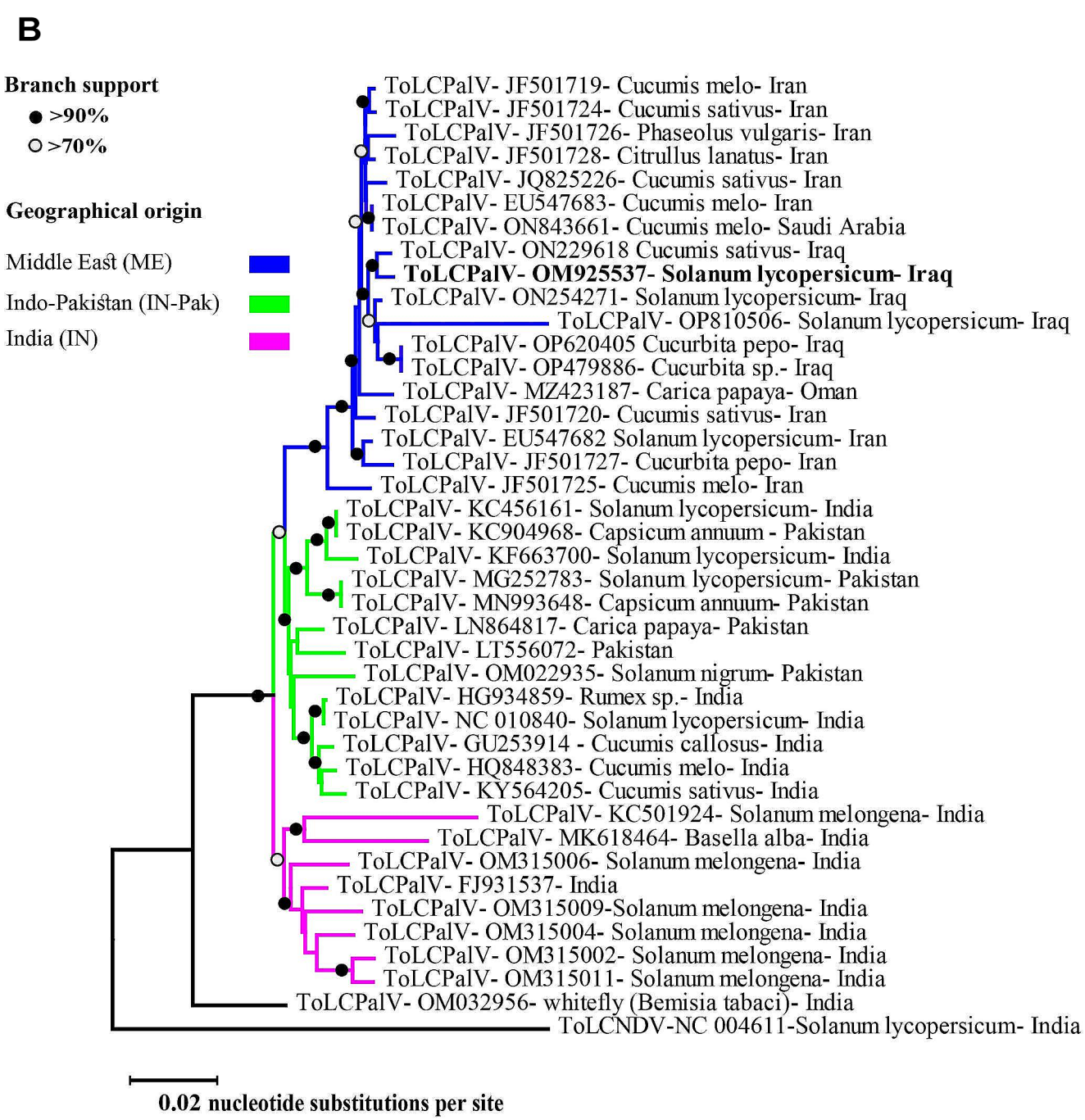

Fig. 2 (continued)

analyses (Lefeuvre et al. 2010). The ML tree of the *cp* and *mp* genes revealed a close relationship between IQ:Dq-A4-12:Tomato:15 and TYLCV-IL isolates from Kuwait, but in phylogenetic analyses using *rep* and complete genome sequences, IQ:Dq-A4-12:Tomato:15 was closely related to isolates of the TYLCV-Mld strain (Fig. 2A; Fig. S2). The results of the phylogenetic analyses of different genes urged us to analyse potential recombination events in the sequences of the Iraqi isolates. Therefore, in the next step, we analysed the evidence for intra- (within and between the strains) and inter-species recombination.

In the phylogenetic analyses of *cp* and *rep* genes of ToLCPalV, the Iraqi isolates of ToLCPalV grouped with Iranian isolates (Fig. S3). The grouping obtained for the *cp* gene was more similar to that in the tree of DNA-A.

Among the TYLCV strains, pairwise identity matrices using SDT software indicated that the *cp* and *mp* genes were more conserved (identities of 94.0 and 93.0%, respectively)

Table 3 Possible recombination events within the genome of Iraqi TYLCV isolates

Event	Recombinant sequence(s)	Recombinant breakpoint		Minor parent(s)	Major parent(s)	Detection methods	*p*-value
		begin	end				
1	IQ:Dq-1:Tomato:15	1880	2629	TYLCV-Mld	TYLCV-IL (GU076444)	RGBMCST	8.64×10^{-7}-3.32×10^{-53}
2	IQ:Ba-Zu82:Tomato:15	1880	2762	TYLCV-Mld	TYLCV-IL (GU076444)	RGBMST	8.64×10^{-19}-3.32×10^{-45}
3	IQ:Na-4:Tomato:15	2103	2410	TYLCV-Mld	TYLCV-IL (GU076444)	RGBMC	2.11×10^{-12}-6.01×10^{-19}
4	IQ:Dq-A4-12:Tomato:15	118	1289	TYLCV-IL (JF451352, KJ830841, KJ830842)	TYLCV-Mld	RGBMCST	2.48×10^{-9}-3.62×10^{-27}

than the *rep* gene (80%) (Fig. S4). The pairwise analysis of all ToLCPalV isolates showed a slightly higher identity for the *cp* gene (> 91.0%) than the *rep* gene (> 90.0%) (Fig. S5).

Recombination analysis

Recombination analysis of aligned TYLCV genome sequences using RDP4 software (Martin et al. 2015) revealed that four isolates in this study (IQ:Dq-1:Tomato:15, IQ:Ba-Zu82:Tomato:15, IQ:Na-4:Tomato:15 and IQ:Dq-A4-12:Tomato:15) were of recombinant origin (Table 3). Evidence of recombination was found in the *rep* gene and in IR (position 1880–2629/2762) for IQ:Dq-1:Tomato:15 and IQ:Ba-Zu82:Tomato:15, and C4/*rep* region (position 2103–2410) for IQ:Na-4:Tomato:15. In these events, TYLCV-IL (Shiraz-Iran, GU076444) and all TYLCV-Mld isolates served as putative major and minor parents, respectively (Table 3). Almost the same recombination event has been reported for GU076444 and 12 additional TYLCV-IL isolates from Asia, Australia, Africa, Europe, and America (Hosseinzadeh et al. 2014). IQ:Dq-A4-12:Tomato:15 also harboured recombinant *mp* and *cp* regions (position 118–1289) with TYLCV-Mld and TYLCV-IL (isolates from Kuwait) as putative major and minor parents, respectively (Table 3). Interspecies recombination analyses between Iraqi isolates of TYLCV and ToLCPalV (identified in this study) as well as closely related species showed no reliable events (data not shown).

Discussion

TYLCD and ToLCD, the most economically important and wide spread viral diseases of tomato, are caused by a number of begomoviruses. The global distribution of these diseases is mainly due to the dissemination of the insect vector *B. tabaci* (both B and Q biotypes) and intercontinental transport of virus-infected plants (Lefeuvre et al. 2010; Shahmohammadi et al. 2022).

Despite the intensive cultivation and economic importance of tomato in Iraq, only limited data is available about the incidence and prevalence of tomato-infecting begomoviruses. Until now, only the presence of TYLCV (Al-Kuwaiti et al. 2013; Al-Waeli et al. 2017, 2018; Al-Abedy et al. 2018; Alabde et al. 2021) and ToLCPalV (Abass and Lahuf 2023) have been reported on tomato in Iraq. In this study, the genetic diversity of TYLCV and ToLCPalV in Iraq was studied after sequencing the complete genomes of eight TYLCV isolates and DNA-A of a ToLCPalV isolate from tomato.

Prior to this study, full-length sequences had been determined for four TYLCV isolates from tomato plants of Iraq: Najaf (MT583814; Alabde et al. 2021), IRQ (JQ354991; Al-Kuwaiti 2013), Kufa (OP771625) and Karbala-1 (ON254272). A phylogenetic analysis of the Iraqi TYLCV isolates identified in this study and those previously sequenced grouped them into clades representing the strains TYLCV-IL and TYLCV-Mld, which are the most prevalent strains globally (Lefeuvre et al. 2010; Mabvakure et al. 2016). In this study, the ML tree and pairwise identity matrix supported the classification of isolate Najaf (MT583814) as belonging to the strain TYLCV-Mld, which differs to the analyses of Alabde et al. (2021), where it was classified as an isolate of the strain TYLCV–Kah.

Middle East in general, and the region surrounding Iran in particular, is probably the centre of ongoing TYLCV diversification (Lefeuvre et al. 2010; Hosseinzadeh et al. 2014; Mabvakure et al. 2016; Marchant et al. 2023). Considering this fact, it was expected that isolates from Iraq would have a closer relationship with Iranian isolates. However, in the phylogenetic analysis, TYLCV-IL isolates from Iraq and Iran grouped into different variants. Besides the globally distributed variant TYLCV-IL (A), the TYLCV-IL isolates from Iraq grouped into a clade with only Iraqi isolates (variant E) and into a clade with isolates from both Iraq and Kuwait (variant D). This indicates the migration of the variant TYLCV-IL (A) and the regional occurrence in Iraq and Kuwait of TYLCV-IL variants (D) and (E). Furthermore, TYLCV-Mld, which not yet has been reported from

Iran, was identified in Iraq. Also in this case, the isolates from Iraq were members of a new variant, TYLCV-Mld (D). Based on the results, it seems that there is a high diversity of TYLCV in Iraq that is partly shared with Kuwait and countries in the Eastern Mediterranean Region. However, with the trade that has developed between Iraq and Iran during the recent decade, it is likely with the occurrence of additional TYLCV strains in Iraq. Therefore, it is important with additional surveys and molecular studies on tomato and other cultivated plants as well as reservoir weeds.

Recombination is a major driving force in determining genetic variability and evolution of TYLCV and other begomoviruses, leading to the adaptation and emergence of new recombinant strains and invasive virus species (Lefeuvre et al. 2010). Previous studies have shown that recombination mostly has involved the TYLCV IR and *rep* regions making them highly divergent (Padidam et al. 1999; Lefeuvre et al. 2010). In the current study, similar recombination events were evident for two TYLCV-IL isolates (IQ:Dq-1:Tomato:15 and IQ:Ba-Zu82:Tomato:15). Two other isolates of recombinant origin (IQ:Na-4:Tomato:15 and IQ:Dq-A4-12:Tomato:15) are examples of conflicting situations for classification of begomovirus isolates. While TYLCV-[IQ:Na-4:Tomato:15] grouped into TYLCV-IL in the ML phylogenetic tree, it shared less than 94% identity with isolates of all known TYLCV strains and could potentially be considered as a member of a new strain. On the other hand, IQ:Dq-A4-12:Tomato:15 shared > 94% pairwise nt identity with isolates of both TYLCV-Mld and TYLCV-Kah strains. Following criteria described by Brown et al. (2015), isolate IQ:Dq-A4-12:Tomato:15 should be classified as belonging to the TYLCV-Mld strain. This classification was verified by the phylogenetic analysis of complete genome sequences, where it grouped into the newly proposed TYLCV-Mld (D) variant. The detected recombination event in IQ:Dq-A4-12:Tomato:15, involving Kuwaiti TYLCV-IL isolates as minor parents, was confirmed by ML phylogenetic trees of *cp* and *mp* genes showing its close relationship to isolates of TYLCV-IL (D) from Kuwait. Similarly, several recombinant TYLCV isolates have been identified in Kuwait, which is a neighbouring country to Iraq (Al-Ali et al. 2015, 2023).

Mixed infection of monopartite and bipartite begomoviruses may cause more severe symptoms and crop losses (Roye et al. 1999). In the present study, a tomato plant was found to have a mixed infection of monopartite TYLCV (IQ:Dq-A4-12:Tomato:15) and bipartite ToLCPalV (IQ:Dq-A4-1:Tomato:15) with yellowing and leaf curling symptoms, which were not more severe than for other symptomatic plants. Although the occurrence of mixed infections of ToLCD-causing viruses is frequent (Garcia-Andres et al. 2007), there is no previous report on the association of TYLCV with ToLCPalV. 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; AlHudaib et al. 2023; Shahid 2023). In the phylogenetic analysis, the new isolate of ToLCPalV (IQ:Dq-A4-1:Tomato:15) showed a close relationship with isolates from Middle East including Iran, Iraq, Oman and Saudi Arabia. These results support the idea that ToLCPalV is probably circulating in different hosts between these four countries, which are geographically close and share common borders. Mixed infections of different species could also result in recombination (Garcia-Andres et al. 2007), but no evidence for recombination between TYLCV and ToLCPalV was obtained in this study, in accordance with previous study (Heydarnejad et al. 2013). High infection rates of 50–100% in greenhouse grown cucurbits have been found for ToLCPalV in Iran (Heydarnejad et al. 2013) indicating that there is a need to pay attention to the risk of ToLCPalV infections not only in tomato, but also in other crops in Iraq.

The results presented here extend our knowledge of the diversity of begomoviruses infecting tomato in Iraq. Furthermore, two additional begomoviruses, squash leaf curl virus and cotton leaf curl Gezira virus, have been previously reported on squash and *Malva parviflora*, respectively, in Iraq (Mohammed et al. 2021; Shahmohammadi et al. 2023). As Iran has been hypothesized as the centre of TYLCV divergence (Lefeuvre et al. 2010; Hosseinzadeh et al. 2014; Mabvakure et al. 2016; Marchant et al. 2023) and considering that Iran and Iraq are neighbouring countries with developed trade, the occurrence of other begomoviruses and TYLCV strains is likely on this crop in Iraq. More surveys and detailed studies are required to detect and characterize other tomato-infecting begomoviruses in Iraq using new techniques, e.g., high-throughput sequencing (HTS).

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Author contributions MAW and AD conceived and designed the experiments; MAW performed sample collections; MAW and NS performed the laboratory bench experiments; AD and AK provided the financial funds; NS performed the computational analysis of the data; AD, NS and AK wrote draft of the paper; all the authors reviewed and approved the manuscript.

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Data availability Data availability The complete genome sequences of the TYLCV isolates and the DNA-A sequence of the ToLCPalV isolate have been deposited in GenBank under the accession numbers OM925529 - OM925537.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest All the authors declare that they have no conflict of interest.

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