



## Molecular characterisation and first complete genome sequence of *Tomato yellow leaf curl virus* (TYLCV) infecting tomato in Iraq

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Tomato yellow leaf curl disease (TYLCD, genus *Begomovirus*, family *Geminiviridae*) is one of the factors that severely limits tomato production in Iraq, as it attacks tomato cultivated in both protected and open fields (Al-Fadhil, 2012). In Iraq, TYLCD incidence may reach up to 100% and can cause economic losses between 50-90%, especially when the virus infects tomato plants in the early growing stages (Al-Ani *et al.*, 2011). In April 2010 leaf curling, yellowing and a reduction in leaf size symptoms were observed on tomato in fields in Baghdad, Iraq. A total of 49 tomato samples were collected and total nucleic acids were extracted from samples using the CTAB protocol described by Abarshi *et al.* (2010). PCR amplifications of extracted nucleic acids were performed using Red Hot DNA polymerase (Thermo Scientific Inc., USA) and begomovirus specific primers (Deng *et al.*, 1994). Rolling circle amplification (RCA) was performed using an Illustra TempliPhi 100 Amplification Kit (GE Healthcare Limited, UK) according to the manufacturer's protocol. Restriction digestion of RCA products with *NcoI* was performed to release the full-length fragment of the amplified viral DNAs, which were gel purified, cloned and sequenced (Bioscience Gene Service, UK). Based on PCR and sequence analyses using MEGA 5 software (Tamura *et al.*, 2011), six out of 49 tomato samples (12%) were found to be infected by TYLCV. MEGA BLAST search of sequences amplified by Deng primers revealed that sequences isolated showed 98% maximum identity with *Tomato yellow leaf curl virus* GenBank sequences.

The full length sequence referred to as TYLCV IRQ is 2780 bp in length (GenBank Accession No. JQ354991). Sequence comparison of full length TYLCV-Iraq1 using MEGA BLAST search was performed against available GenBank sequences. TYLCV IRQ shared 99% identity with TYLCV isolates Mauritius (HM448447) from Mauritius, RE4 (AM409201) from Reunion Island and Almeria (AJ489258) from Spain. The primer pair 'TYLCV-F/TYLCV-R' (TYLCV-F, 5'-GGATAAGCACATGGAGATGTG-3' and TYLCV-R, 5'-CAAGATAACAGAACAC AGCCA-3') was designed from the TYLCV IRQ isolate to amplify a 1.7 kb fragment from the TYLCV genome regions between 1394-2463 nucleotide positions. This primer could detect TYLCV in all six tomato samples previously tested using the Deng primer set (Fig. 1). Phylogenetic (neighbour-joining) analysis of full sequence of TYLCV IRQ and those sequences from GenBank grouped

TYLCV IRQ within the TYLCV cluster but diverged this sequence into a separate clade from the TYLCV isolates from Spain, Reunion Island, Morocco, New Caledonia, and The Netherlands (Fig. 2). This separate grouping of TYLCV- Iraq1 from other GenBank TYLCV sequences is supported by a bootstrap value of 89%, which may indicate a distinct origin for TYLCV IRQ. This study provides the first molecular evidence of TYLCD in Iraq.

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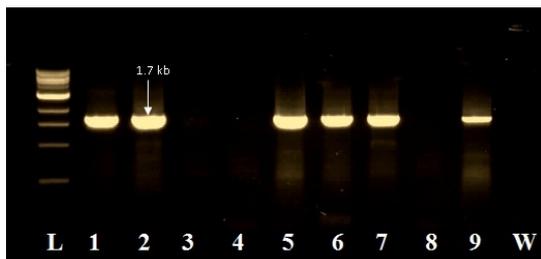


Figure 1

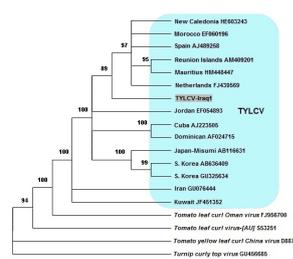


Figure 2

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