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ORIGINAL ARTICLE

The role of corchorus in spreading of tomato yellow leaf curl virus on tomato in Jeddah, Saudi Arabia

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Abstract Corchorus (*Corchorus capsularis* L. and *Corchorus olitorius* L.) is one of the most important fiber crops grown in tropical and subtropical regions throughout the world. Field survey was conducted and naturally infected leaf samples were collected from corchorus and tomato plants in Jeddah, Saudi Arabia. The causal virus was transmitted by whiteflies to tomato plants and begomovirus infection was confirmed by Polymerase chain reaction. The complete viral genome and associated betasatellites were amplified, cloned and sequenced from both corchorus and tomato samples. The genetic variability and phylogenetic relationships were determined for both isolates (corchorus and tomato). The complete genome sequences showed highest (99.5 % nt) similarity with tomato yellow leaf curl virus (TYLCV) and formed closest cluster with TYLCV-Tomato reported from Jizan and Al-Qasim, Saudi Arabia and betasatellites sequences showed highest similarity (99.8 % nt) with Tomato yellow leaf curl betasatellites-Jeddah followed by Tomato yellow leaf curl Oman betasatellites and formed closed cluster with TYLCV-Tomato. On the basis of results obtained from whiteflies transmission, sequence similarity and phylogenetic relationships; it is concluded that the identified virus could be a variant of TYLCV circulating in the Kingdom. The significance of this study demonstrated that the corchorus is serving as reservoir and alternative host and playing an important role in spreading the begomovirus associated disease in the Kingdom of Saudi Arabia.

Keywords Corchorus · Tomato · Tomato yellow leaf curl virus · Betasatellites · Phylogenetic relationships

Introduction

Corchorus is a native of tropical and subtropical regions throughout the world with genus of about 40–100 species, belongs to family Tiliaceae. The plants are annual herbs, or sub-shrubs or shrubs. *Corchorus capsularis* (jute) and *C. olitorius* (wild jute) are the main source of jutes and significant fiber crops. The young shoots and tender leaves of corchorus are also used as a salad, green leafy vegetables (mulukhiyah) and bast (phloem) fiber production in rural belts of Asian, African and European countries [8, 34]. Begomoviruses belongs to the family *Geminiviridae* are single stranded circular DNA viruses with either monopartite (DNA- A genome of about 2.7 kb, encoding six ORFs) or bipartite genomes (DNA- A and DNA- B genomes of 2.5–2.6 kb) encapsulated in twinned particles [35]. They are efficiently transmitted by whitefly (*Bemisia tabaci*) to dicotyledonous plants. The DNA A component of the bipartite begomoviruses is involved in replication and production of virion, but requires the DNA B component for nuclear localization, systemic infection, host range determination and symptom expression [33, 35]. The quality and yield of jute is seriously affected by many diseases [12] and among them yellow mosaic disease is the most important limiting factor and causes significant loss to jute production. Earlier; only two viruses were identified

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causing disease in jute; Corchorus yellow vein virus (CoYVV) and Corchorus golden mosaic virus (CoGMV) [18, 19] from diseased plants in Vietnam, but recently, CoGMV also reported from India and Bangladesh which provided strong evidence of wide distribution in Asia [5, 13–17, 20]. The naturally infected plants develop symptoms like small yellow flakes on the lamina during the initial stage which gradually increases in size to form green and chlorotic intermingled patches producing a yellow mosaic appearance.

In Jeddah, Saudi Arabia, during field survey, corchorus plants were growing in and around the tomato field exhibiting yellow vein mosaic disease. The leaf curl and yellow vein mosaic with stunted plant growth disease symptoms were also observed in tomato crops. There are many reports published about the role of weeds as an alternative hosts and play an important role in perpetuation and spread of many begomoviral diseases [4, 32, 37–39, 41, 42]. *Tomato yellow leaf curl virus* (TYLCV) poses a serious threat to tomato production throughout the temperate regions of the world. The spread of TYLCV from Middle East to other parts of world has been reported recently and it is suspected that Iran could be the center for TYLCV diversity and display complex inter and intra-species recombination pattern [36]. Recently, the association of begomovirus species infecting tomato, tobacco and okra; like *Tomato leaf curl Sudan virus* (ToLCSDV), ToLCSDV-Oman isolate, *Tomato leaf curl Al-Batinah virus* (ToLCABV) *Tomato yellow leaf curl virus* (TYLCV), TYLCV-Oman isolate, *Chili leaf curl virus* (ChiLCV) and Okra *leaf curl Oman virus* (OkLCOMV) has been reported from Nile Basin and Southern region of the Arabian Peninsula like Oman and Yemen [1, 2, 10, 22–24, 27–31]. Collectively, these begomoviruses cause significant loss to tomato, okra and chili crops in the Nile Basin, arid and semi-arid southern part of the Arabian Peninsula [1, 22, 28]. Currently, there is no report published about the aasociation of begomovirus on weeds from Jeddah, Saudi Arabia. So, the present study was undertaken to identify the causal virus and the role of corchorus in spreading of TYLCV on tomato in Jeddah, Saudi Arabia.

Materials and methods

Field survey and virus detection

Field survey was conducted in 2013–2014 farmer's and experimental plots of King Abdulaziz University, Jeddah, Saudi Arabia. During field survey, leaf curl and yellow vein mosaic symptoms were observed on corchorus and tomato plants. At the same time the presence of whiteflies were also observed on the lower side of the tomato and corchorus leaves. Total 15 samples were randomly collected from corchorus and tomato plants exhibiting leaf curling and yellow vein mosaic symptom. The collected samples were brought to the lab and further processed. Total genomic DNA was isolated from 100 mg leaf tissue using DNAeasy plant mini kit (Qiagen Inc.) following the manufacturer's instructions and about 100 ng of DNA was used for PCR amplification.Begomovirus infection was confirmed by using specific primers; TYC1F (5′-GGGCCTAGAGACCTGGCCCAC-3′) and TYC1R (5′-CCGGTAATATTATACGGATGGC-3′) [21] which amplified 856 bp amplicon. The PCR reaction mixture consisted of 2.5 units of *Taq* DNA polymerase (MBI; Fermentas, Glen Burnie, MD, USA), 5 µl of 10 × PCR buffer, 0.5 µl of 10 mM dNTPs, and 0.5 µl (10 pmol) of forward and reverse primers. Total reaction volume was made up of 50 µl using sterile distilled water. PCR products were analyzed on 1 % Agarose gel stained with ethidium bromide (0.5 µg ml^{-1}) and visualized on an Ultraviolet transilluminator. The presence of betasatellites was also confirmed by amplifying the full-length betasatellites (~1350 bp) using specific primers; beta01: 5′ GGTACCACTACGCTACGCAGCAGCC 3′/beta02: 5′ GGTACCTACCCTCCCAGGGGTACAC 3′ [6]. Only, the PCR positive samples were used for further analyses.

For whiteflies transmission, fresh culture of non-viruliferous whitefly was raised from the whitefly eggs and maintained on eggplant (*Solanum melongena*) in insect-proof cages. Adult whiteflies were given an acquisition access period (AAP) of 24 h on infected plants. After the required AAP, the viruliferous whiteflies were given for an inoculation access period (IAP) of 24 h on healthy tomato seedlings (15 whiteflies/plant) and the inoculated plants were kept and observed in insect-proof cages for symptom development for up to five weeks. The virus isolates were maintained on the respective hosts by whitefly transmission.

Cloning and complete genome sequencing

The full length genome of begomovirus were amplified from isolated DNA from symptomatic tomato and corchorus plants by using TempliPhi 100 Amplification Kit (GE Healthcare, Life Sciences, Piscataway, NJ, USA) following the manufacturer's instructions. The amplified products were digested with *Eco*RI restriction enzymes to obtain full length (~2.7 kb) begomovirus genome and a betasatellite (~1.4 kb) fragment was PCR amplified and cloned. Putative begomovirus genomes obtained by *Eco*RI digestion of the rolling circle amplified products were cloned into pUC19 vector while betasatellites fragment were cloned into pGEMT-easy vector. Two clones of full length viral genome (~2.7 kb) and betasatellites

(~1.4 kb) from corchorus (cor-1 & cor-2) and tomato (tom-1 & tom-2) were selected and sequenced in both directions using a primer walking strategy. The DNA sequence was determined for full length begomoviral genomic clones and analyzed using BLASTn algorithm to query the GenBank database (NCBI).

Sequence and phylogenetic analysis

The full length sequences of complete genome and betasatellites were assembled from two clones each without any errors and analyzed for determination of percentage similarity matrix by using BioEdit software programme, (version 5.0.9) and begomovirus genes were predicted using ORF Finder (NCBI) and multiple sequence alignments were performed by using CLUSTALW program (http://www.ebi.ac.uk/clustalw) using nucleotides sequences of selected begomoviruses from the GenBank. A phylogenetic tree was constructed using MEGA6 program from the aligned nucleotide sequences with neighbor joining and maximum parsimony methods using maximum composite likelihood for DNA substitution test [43].

Results

Field survey and virus detection

During field survey, approximately 70–80 % plants were observed with yellow vein and leaf curl disease and begomovirus infection was confirmed by visualizing an amplicon of 856 bp on 1 % Agarose gel from infected corchorus and tomato leaf samples (Fig. 1a–c). The causal virus was efficiently transmitted to young tomato seedlings and developed characteristic yellow vein mosaic symptoms in 80 % plants by 6–18 days after inoculation (DAI) and the disease symptoms produced in the experimental plants were similar to those observed in the field. These results demonstrated that the virus causing yellow vein mosaic disease in corchorus can be efficiently transmitted by whiteflies and also cause yellow vein mosaic and leaf curl disease in tomato in Jeddah, Saudi Arabia.

Cloning and sequencing

The sequences of complete genome had 2790 nt (corchorus) and 2789 nt from tomato plants samples and betasatellites 1370 nt (corchorus) 1368 nt from tomato. The full genome and betasatellites sequence have been submitted to GenBank under accession numbers KT350023 (full genome-corchorus) and KT033715 (full genome - tomato) and betasatellites (KT355022-corchorus, KT355021-tomato).

Sequence and phylogenetic analysis

The complete genome sequence comparison revealed that TYLCV-Jeddah-tomato isolate shared 99.5–71.6 % nt identity with other selected TYLCV isolates. The highest identity (99.9 % nt) was found with TYLCV-Tomato-Jeddah (KT033715) followed by (99.5 % nt) with TYLCV-Jizan103 (KC845301) and 92.8 %nt with TYLCV-Al-Qasim (KF561125) while the lowest (71.6 % nt) with TYLCV-Egypt (EF107520). Based on the ICTV guidelines for species demarcation, at <91 % nt [7] the TYLCV-corchorus-Jeddah isolate could be considered as a variant of TYLCV-Jizan 103. Even so, the TYLCV-Oman-tomato isolate showed a range of diversity from 78 to 79 % nt and the TYLCV isolates from Iran ranged from 79 to 82 % nt along with isolates from Jordan varied from 78 to 82 % nt similarity. The highest amino acid sequence identities was observed with TYLCV-KT033715 in all the 6 proteins (V2-99.5 %, V1-99.7 %, C3-99.4 %, C2-99.2 %, C1-99.8 %, and C4-99.0 %) respectively, with respective sequences of selected begomovirus isolates (Table 1).

Since association of a betasatellites molecule with TYLCV isolates from tomato and other crops has been published earlier in many reports [2, 13, 24, 29, 31], separate PCR was performed to amplify the betasatellites using betasatellites specific primers [6]. An amplicon of ~1.4 kb was amplified from infected samples by PCR, indicating the presence of betasatellites. Comparative sequence analysis of the betasatellites of the TYLCB-Jeddah-corchorus isolate (KT355022) with previously reported isolates showed highest similarity (99.8 % nt) with TYLCB-Tom-Jeddah (KT355021) followed by TYLCOB-Oman (NC010126 & DQ644566) and the lowest (46.4 % nt) was found with TYLCB-Mali isolate (NC007485) (Table 2). The phylogenetic analysis results based on complete genome aligned with selected begomoviral sequences indicated that many isolates identified here and previously reported begomoviruses isolates or variants of TYLCV formed two separate clades. The TYLCV-corchorus-Jeddah isolate (KT355023) formed closest cluster with TYLCV-Jizan 103 isolate (KC845301), TYLCV-Tomato-Jeddah isolates (KT033715) and TYLCV-tomato-Al-Qasim isolates (KF561125). Most of the TYLCV isolates from Oman formed separate cluster while isolates from Iran, Egypt and Jordan clustered together. One more cluster was formed with mixed isolates from Saudi Arabia, Sudan, Iran and Jordan (Fig. 2). The phylogenetic analysis results based on selected betasatellites demonstrated that the isolate from TYLCV-corchorus (KT355022) formed closed cluster with TYLCV-Tomato (KT355021) while TYLCB isolates from Oman and Yemen formed separate cluster (Fig. 3).

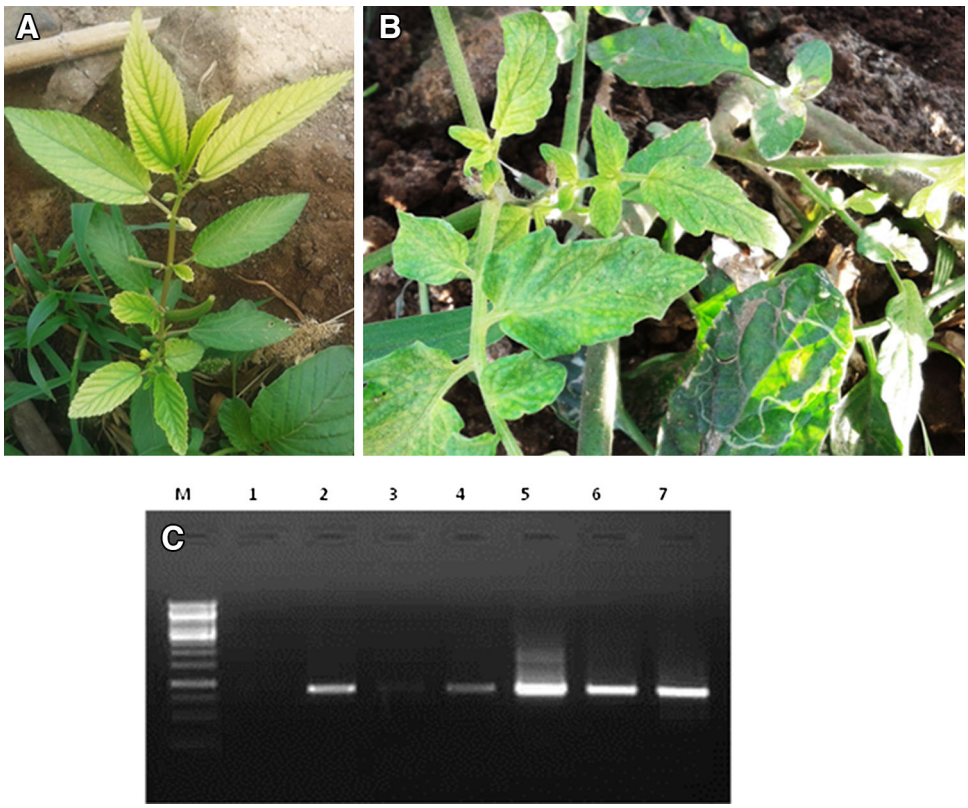

Fig. 1 **a** Naturally infected corchorus plant with yellow vein mosaic symptoms. **b** Naturally infected tomato plant with yellowing symptoms. **c** Detection of begomovirus by PCR from naturally infected samples. M: 1 Kb ladder, 1; Healthy, 2–4; infected corchorus leaf; 5–7- infected Tomato leaf

Discussion

Corchorus is an important plant used for jute and tender leaves are used for salad and leafy vegetables. Corchorus are infected with many viruses and serve as an alternative hosts for many viruses including begomoviruses. This study describes the molecular detection, genetic variability, phylogenetic relationship and the role of corchorus as an alternative host and spreading of TYLCV on tomato by serving as reservoir of begomovirus in the Kingdom of Saudi Arabia. During field survey, naturally infected corchorus plants showing yellow vein mosaic symptoms was found in and around the tomato field it was suspected that this weed is serving as reservoir of begomovirus causing leaf curl and yellow vein mosaic disease in tomato grown in Jeddah, Saudi Arabia. Tomato is an important agricultural crop grown globally as well as in Saudi Arabia for local consumption. Severe leaf curl and yellow mosaic disease is responsible for serious threat to tomato cultivation in many tropical and subtropical areas of the world. The global emergence and spread of many begomoviruses, including TYLCV and TYLCSV, has been associated with the spread and increase of a more fecund and polyphagous whitefly biotype, referred to as the B-biotype [40]. The emergence and diversity of begomovirus infecting Solanaceous crops in the east and south East Asia has been reported recently [26, 46] but it is reported that TYLCV arose in between 1930 and 1950 in Middle East and their global spread starts in 1980 after the emergence of two strains; TYLCV-Mld and-IL [36]. It is suspected that Iran is the center for TYLCV diversity and display complex inter- and intra-species recombination patterns. This means that novel pathogenic TYLCV variants that arise in this region will probably be less of a threat to global agriculture than those arising closer to more internationally connected regions such as the Mediterranean basin [36]. For the past two decades, tomato production in Arabian Peninsula and Nile Basin has been affected by yellow mosaic and severe leaf curl disease. Currently, many begomovirus have been reported to be associated with leaf curl disease of tomato in Arabian Peninsula and Nile Basin. These included ToLCSDV, TYLCV and TYLV-Om, OkLCOMV as well as associated betasatellite (Tomato leaf curl betasatellite) with origins on the Indian subcontinent [1–3, 21, 23, 25, 28, 29].

The TYLCV-Jeddah-corchorus isolate analyzed herein represents a variant that have been spread throughout the western region and Arabian Peninsula either on plant material moved by human activities and/or by the endemic whitefly vectors. The analysis included field isolates collected from Jeddah, Saudi Arabia together with selected full-length begomovirus genome sequences available in the GenBank database that includes TYLCV and ToLCV respectively from various regions. Analysis of the full-

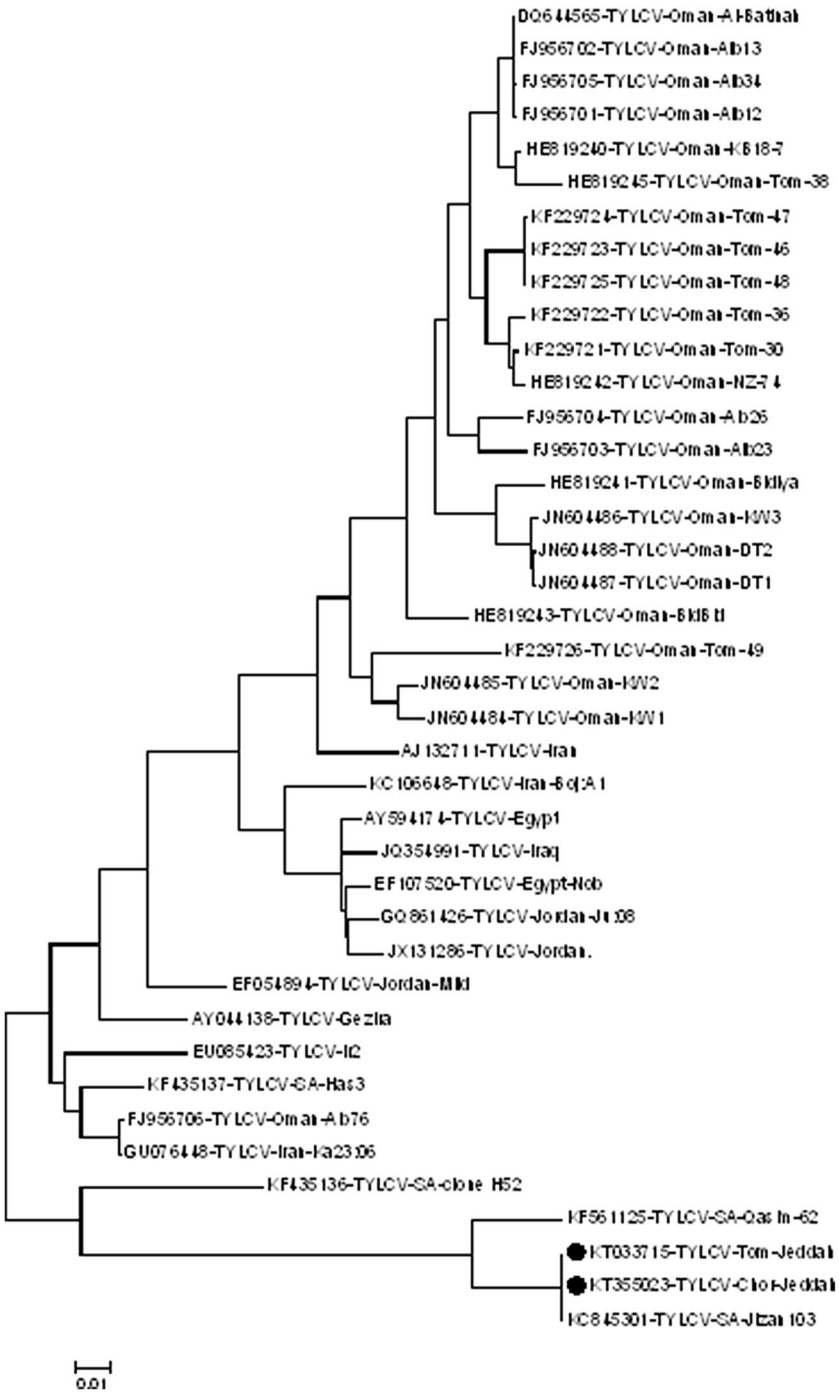

Fig. 2 Phylogenetic dendrograms based upon the alignments of the sequences of TYLCV-corchorus-Jeddah with selected other begomovirus sequences available in the nucleotide sequence databases

length genomic DNA sequences revealed that the level of genetic variation observed in the natural population of TYLCV. It is more than likely that TYLCV isolate present in Saudi Arabia has originated either from Yemen or from Oman as of this virus being the closest relative to the Oman and Yemen isolates. It has been speculated that the high mutation rate of geminivirus genomes is in part due to improper methylation patterns occurring during their replication [9, 11]. In Yemen, tomato crops are being grown in the far west. Between the major agricultural areas of Yemen and the southern agricultural area of Oman lies a desert, which would pose a significant barrier to the spread of the whitefly vector and consequently the virus. However, recent studies have shown that geminivirus genomes are subjected to DNA methylation in infected host plants [45, 47]. Thus, it is possible that base-excision repair may not act on geminivirus genomes because the double-stranded state lasts for a very short time during rolling circle replication [44]. In Oman, TYLCV is associated with a betasatellites that has only been identified in Oman

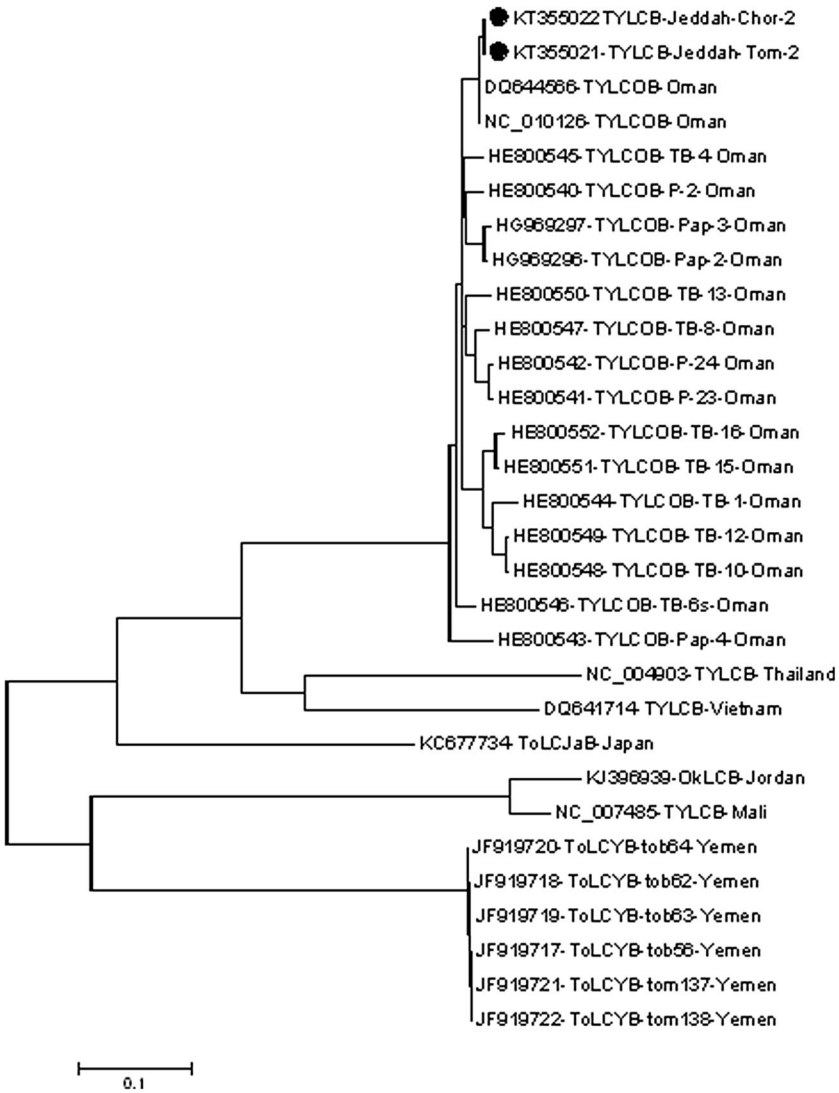

Fig. 3 Phylogenetic dendrograms based upon the alignments of the sequences of TYLC betasatellites-corchorus-Jeddah with selected other begomovirus sequences available in the nucleotide sequence databases

(Tomato yellow leaf curl Oman betasatellite) [28]. On the other side Yemen is separated from Oman and Saudi Arabia by a vast harsh desert, collectively constituting potential barriers to virus and whitefly movement. It is well known that weeds serves as alternative hosts for many viruses and play an important role in spread of many viral disease globally [4, 32, 37–39, 41, 42]. The sequences similarity and phylogenetic analysis based on full genome and betasatellites results itself indicates that the identified virus strain causing yellow vein mosaic disease of corchorus can efficiently transmitted by whiteflies and causes yellow vein mosaic and leaf curl disease tomato in Jeddah, Saudi Arabia could be a variant strain of TYLCV reported from Arabian peninsula. Based on these observations, it is suspected that the TYLCY-corchorus isolate is likely to very close with TYLCV-Tomato and it could be a variant of TYLCV-isolate from Jizan. It is concluded that virus causing yellow vein mosaic disease in corchorus can be transmitted to tomato by whiteflies and cause yellow mosaic and leaf curl disease of tomato in the kingdom of Saudi Arabia.

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