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DNAB AMPLIFICATION CONFIRMED SLCUV INFECTION OF ZUCCHINI SQUASH EXHIBITING SQUASH LEAF CURL DISEASE IN IRAQ

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ABSTRACT

The study was aimed at the characterization of a leaf curl disease on zucchini squash based on molecular approaches. Leaf samples were collected from symptomatic plants in zucchini squash growing area at Baghdad. Total DNA was extracted from collected samples using commercial DNA extraction kit., PCR amplification was performed to screened extracted samples for begomovirus infection using Deng a begomovirus specific primer set. DNA fragment of expected size were sequenced and compared to equivalent Gen Bank sequences. Sequence analyses were performed using MEGA X and SDTv1.2 software packages. Sequence comparison confirmed the detection of *Squash leaf curl virus* (SLCuV), in symptomatic zucchini samples when shared 98-99 and 95-96% maximum nucleotide (nt) identities with coat protein CP gene (DNAA) and DNAB, respectively, from the Gen Bank. Neighbor joining (NJ) Phylogenetic analysis confirmed the relatedness when grouped virus sequences with the equivalent Gen Bank sequences based on partial sequences of CP gene and DNAB. SDT analysis confirmed species demarcation of Iraqi sequences at 99 and 97% identity with the SLCuV partial DNAA and DNAB sequencesm respectively from the Gen Bank. The high (nt) identity suggests SLCuV may recently be introduced to Iraq and could be a serious threatening to squash cultivation

Keywords : Geminiviridae, whitefly, plant viruses, courgetti

Introduction

Zucchini or Courgetti squash *Cucurbita pepo* is cultivated worldwide due to its high nutritional value. It has originated from Mexico about 7000 years ago. In Iraq, zucchini is grown both in protected and open fields, during the year. Based on Iraqi central statistical organization data CSO in (2020), zucchini fruit production decreased up to 79.6 %, in the past few years from 126000 tons in 2014 to 25700 tons. This decline in zucchini production may be attributed to many factors including viral diseases. Zucchini squash is infected by several pathogens including viruses. At least, 61 plant viruses, belonging to 39 different viral groups, were found to infect cucurbits, including zucchini squash (ICTV, 2020). The genus *Begomovirus*, within the family *Geminiviridae,* is the largest amongst the other plant virus genera, as it includes 424 definite species (Brown *et al*., 2015; ICTV, 2020). Begomoviruses comprise either mono- or bipartite DNA genomes encapsidated in geminate particles. They are transmitted by the whitefly *Bemesia tabaci*, the only known vector, in a persistent manner. About 16 begomoviruses were found to infect cucurbits worldwide causing serious diseases including leaf curl diseases (Kurowski *et al*., 2015; Fortes *et al*., 2016; Pandey and Verma, 2017; ICTV, 2020). Leaf curl diseases have threating zucchini squash in many growing areas including the Middle East countries (Lapidot *et al*., 2014; Medina-Hernández *et al*., 2019). These diseases are caused by a number of begomoviruses including *Squash leaf curl virus* (SLCuV). SLCuV comprises a bipartite genome DNAA and DNAB. DNAA includes 5 ORFS encoding AV1 (coat protein CP), AC1 (replication-associated Rep), AC2 (transcriptional activator protein Trap), AC3 (replication enhancer protein REn) and AC4. Whereas, DNAB includes 2 ORFs encoding BV1 (nuclear shuttle protein NSP) and BC1 (movement protein MP). Besides, SLCuV genome includes non-coding intergenic regions (IRs) common regions (CR) A (or IR) and B (long intergenic region LIR) and short intergenic region SIR (ICTV, 2020). Zucchini squash plants infected with SLCuV on exhibiting typical symptoms include a severe chlorotic mosaic or mottle on foliar, curling, malformation and thickened vein-banding of the leaf, stunting, flower drops and fruit set failure (CABI, 2020, Medina-Hernández *et al*., 2019). Beside cucurbits, SLCuV has been reported to infect host plants within the families Solanaceae, Malvaceae, Fabaceae, Euphorbiaceae and Chenopodiaceae (Duffus and Stenger, 1998, Al-Musa *et al*., 2009; Awad *et al*., 2019; CABI, 2020).

Squash leaf curl disease (SLCD), caused by SLCuV, was confirmed in Iraq based on molecular approach (Al-Kuwaity. 2017). Since then, this disease was threatening zucchini squash in recent growing seasons. In the previous study SLCD was observed in three plants found in Zucchini growing area near Baghdad. Severe SLCD symptoms were observed on zucchini plants in the same growing area with a high disease incidence (ca. 100%). Thus, this study was initiated to investigate whether those symptoms is caused by SLCuV or another begomovirus.

Materials and Methods

Two leaf samples (referred to as D8 and D12) were collected from zucchini squash exhibiting leaf curl disease symptoms (fig 1). Total DNA was extracted using commercial DNA extraction kit (Bioneer, South Korea) following the manufacturer`s instructions. PCR amplification was performed using AccuPower PCR PreMix commercial kit (Bioneer, South Korea) and a bigomovirus specific primer set (Deng *et al*., 1994) following the protocol described by (Al-Kuwaiti, 2017). PCR products were visualized by ethidium bromide agarose gel electrophoresis following Sam brook and Russell (2006) standard protocol, then PCR products were sent to (Macrogen, South Korea) for sequencing. Sequence analysis was performed using MEGAX (Kumar *et al*., 2018) and Sequence Demarcation Tool Version 1.2 (SDTv1.2) (Muhire *et al*., 2014) software packages. The Gen Bank accession numbers (MT248392, MT248400) and (MW027023-MW027024) were assigned to the DNAA and DNAB partial sequences obtained, respectively.

Fig. 1: Naturally infected zucchini squash plant showing leaf curl disease symptoms including leaf yellowing and curling, vein thickness and stunting

Results and discussion

Sequence comparison confirmed the detection when all DNAA fragments obtained were amplified from SLCuV AV1 (CP) genome region located on DNAA. Sequences isolated shared 98-99 % maximum nucleotide (nt) identities with the equivalent Gen Bank sequences form Israel (KT099131- KM595115), Jordan (JX444577- KM595211), Palestine (KM595230) and Egypt (KM595154). Furthermore, an amplification of SLCuV DNAB fragments was confirmed based on sequence analysis. Sequences obtained scored 95-96% maximum (nt) sequence identities to equivalent SLCuV DNAB sequences from the Gen Bank. The maximum identities shared were with SLCuV DNAB DNAB sequences from Egypt (DQ285020- MG763921), Palestine (KC441466), Jordan (JX131282) and Israel (HQ184437). Phylogenetic analyses of partial DNAA and DNAB nt sequences, confirmed the relatedness when grouped Iraqi sequences obtained to the equivalent SLCuV Gen Bank sequences (Fig2 A & B).

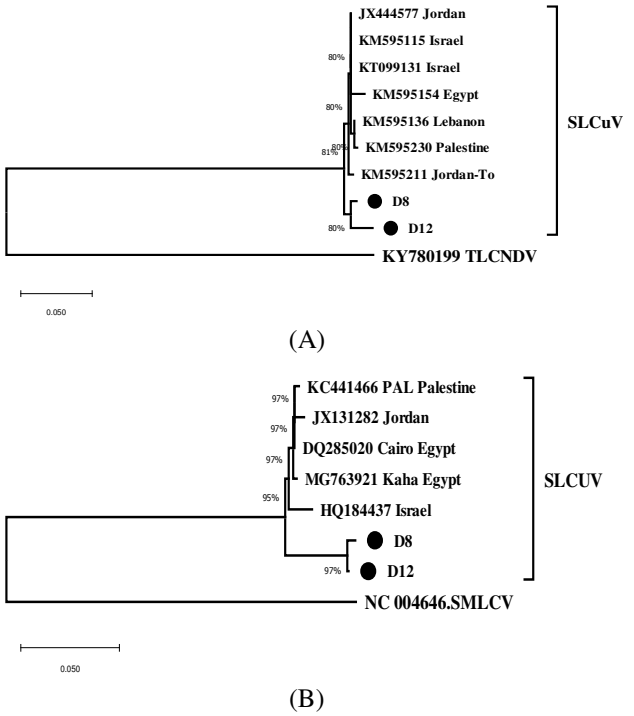
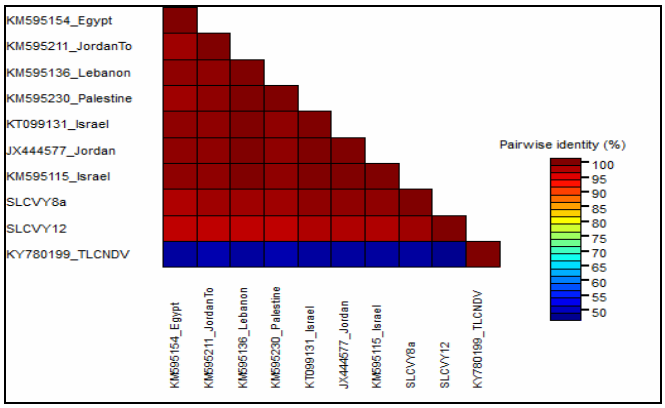
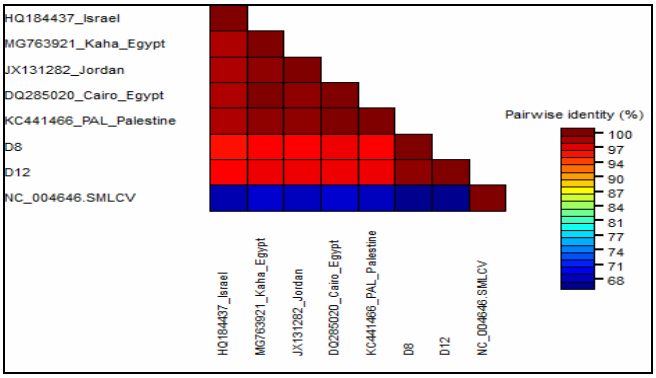


Fig. 2: Neighbor-joining phylogenetic trees constructed from partial DNA A (AV1) (A) and DNAB (B) nucleotide sequences of Iraqi *Squash leaf curl virus* SLCuV isolates D8 and D12 (marked with ●) and equivalent Gen Bank sequences. *Tomato leaf curl New Delhi virus* (ToLCNDV) DNAA and *Squash mild leaf curl virus* DNAB were used as an out-group comparison.

Besides, SDT analysis confirmed species demarcation of Iraqi sequences at ca. 99 and 97% identity with the SLCuV partial DNAA and DNAB sequences from the Gen Bank (Fig 3 A&B).The high identity percentages confirmed that D8 and D12 sequences are isolates belonging to SLCuV according to ICTV criteria for the genus *Begomovirus* species classification and demarcation (Muhire *et al*., 2014; Brown *et al*., 2015; ICTV, 2020). *Squash leaf curl virus* has become one of the most devastating virus diseases infecting cucurbits in the Middle Eastern countries (Lapidot *et al*., 2014). SLCuV is a New World begomovirus and may have been introduced from the USA to the Old World, the Middle Eastern countries in the year 2000 (Antignus *et al*., 2003; Lapidot *et al*., 2014). This virus could be originated from Mexico, the origin of zucchini squash, due to the isolate high variability (Medina-Hernández, 2019) then moved to USA in 1970s later on (Flock & Mayhew, 1981). Based on results presented in this study, this virus has become endemic in Iraq since the first time reported in 2017 (Al-Kuwaiti, 2017). The high activity of the vector *B. tabaci*, alternative hosts availability and agriculture intensification in Iraq and the Middle Eastern countries may have been involved in SLCuV re-settlement (Al-Musa *et al*., 2009; Lapidot *et al*., 2014; Awad *et al*., 2019). The high nt identity suggests SLCuV may recently be introduced to Iraq and could be a serious threatening to squash cultivation. However, molecular data about full length genome are required to investigate the possible origin of Iraqi SLCuV (Vargas-Salinas *et al*., 2019). Rapid action and precaution procedures must be taken to protect zucchini and other crops against SLCuV in Iraq, both in protected and open field cultures



(A)



(B)

Fig. 3: Sequence comparison of SLCuV partial DNAA(A) and DNAB (B) sequences from Iraq (D8 and D12) and the Gene Bank performed by (SDTv1.2). *Tomato leaf curl New Delhi virus* (ToLCNDV) DNAA and *Squash mild leaf curl virus* DNAB were used as an out-group comparison.

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