SHORT COMMUNICATION

OKRA ENATION LEAF CURL VIRUS IN PAPAYA FROM IRAN DISPLAYING SEVERE LEAF CURL SYMPTOMS

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SUMMARY

During a survey of the Bahoo-Kalat and Zar-Abad areas of Iran in February 2010, papaya plants were found with severe leaf curling and vein swelling. A begomovirus (family Geminiviridae) was suspected of being responsible for the disease, so begomovirus-specific degenerate primers and PCR on total DNA were used to obtain amplicons. Based on the sequence of these amplicons, abutting primers were designed to recover the complete begomovirus from seven PCR positive papaya plants. Seven distinct begomovirus molecules (2746 nt each) were recovered that share 85.5-91.6% genome-wide sequence identity with Okra enation leaf curl virus (OELCuV) isolates. Phylogenetic analyses with available OELCuV sequences support the identification of the papaya-infecting begomovirus from Iran as an isolate of OELCuV. To our knowledge this is the first report of a begomovirus infecting papaya in Iran and the first record of papaya as host of OELCuV.

Keywords: Papaya, leaf curling, begomovirus.

Papaya (Carica papaya L.) is cultivated commercially throughout tropical and subtropical regions of the world for its edible fruit. Leaf curl disease is a serious threat to papaya cultivation in many papaya growing countries (Singh, 2006). Papaya leaf curl disease was first reported in India (Thomas and Krishanaswamy, 1939). The disease was known to be transmitted by the whitefly B. tabaci in a persistent manner and a begomovirus, Papaya leaf curl virus (PaLCuV), was characterized from papaya originating from India (Saxena et al., 1998). PaLCuV has also been reported from Pakistan (Nadeem et al., 1997) and China (Lubin et al., 2005). The virus is member of the genus Begomovirus of the family Geminiviridae. Other begomoviruses, including Papaya leaf crumple virus (Singh-Pant et al., 2012), Papaya leaf curl Guandong virus (Wang et al., 2004), Papaya leaf curl china virus (Wang et al., 2004), Chili leaf curl virus (Singh-Pant et al., 2012) and Malvastrum leaf curl virus (Wu and Zhou, 2006) have been identified in papaya.

During a survey of papaya in the Bahu-Kalat (near the border with Pakistan) and Zar-Abad (Sistan-Baluchestan, south-east Iran) areas of Iran in February 2010, plants were found exhibiting severe leaf curl symptoms (Fig. 1). In subsequent years the leaf curl symptoms continued to be present in papaya plants in these regions. Samples of leaves showing severe leaf curling and vein swelling symptoms were collected and total DNA was extracted from eleven papaya samples as described by Bendahmane et al. (1995). As a begomovirus was suspected to cause the leaf curl symptoms, a simple PCR assay was performed using begomovirus-specific degenerate primers (Rojas et al., 1993). Begomovirus PCR amplicons with an expected size of ca. 1.1 kb were identified from seven of the symptomatic papaya samples. No begomovirus PCR amplicons were obtained from asymptomatic papaya samples. The reason for the lack of amplification with four symptomatic plants is unclear. This may be due to the presence of other viruses (not a begomovirus) or possibly due to inhibitors in the DNA extracts which prevented amplification. Based on the sequence information of begomovirus amplicons from the 7 symptomatic papaya plants, abutting primers (Pa1: 5’-CAT TAA GAA GCA GAA TCA CAC CAA TGT-3’ and Pa2: 5’-TTG TCA TCC ATC CAT ACC TTA CCA AG-3’) were designed to recover the complete begomovirus genome from the seven PCR positive plants.

The complete sequences of Iranian isolates of OELCuV were submitted in GenBank under the accession numbers KJ397527 to KJ397533. The seven sequences (2746 nt each) share > 97.3% genome-wide pairwise identity calculated
Fig. 1. Left to right: Healthy papaya leaf samples (A and B) and leaf curling symptoms on papaya (C) in Iran.

Fig. 2. Neighbor-joining phylogenetic tree with 1000 bootstrap iterations and pairwise identity comparisons of OELCuV with related begomoviruses and begomoviruses that infect papaya and/or okra. Numbers at nodes of the phylogenetic tree represent percentage bootstrap support and scale bar represent 0.5 nucleotide substitutions per site. EU914819 OMoV-[BR:6328:08], AY751753 CdTV-[MX:Igu] and DQ022611 OYMV-[MX:Maz3:04] are begomoviruses from the New World and the out group for this set of sequences.
using SDT v1.2 (Muhire et al., 2014) and have between 85.5-91.6% identity with the sequences of Okra enation leaf curl virus (OELCuV) isolates but < 82% genome-wide pairwise identity to other papaya infecting begomoviruses (Fig. 2). The current species demarcation criteria for begomoviruses are based on genome-wide pairwise identity and genomes that share > 91% pairwise identity belong to the same species (Brown et al., 2015). Hence the sequences identified in this study are all isolates of the species OELCuV. All sequences of OELCuV and those of closely related begomoviruses, together with representative isolates of other papaya and okra-infecting viruses were aligned using MUSCLE (Edgar, 2004) and a neighbor-joining phylogenetic tree was inferred (Jukes-Cantor model) with 1000 bootstrapped replicates (Fig. 2). The mid-point rooted phylogenetic analysis supports the identification of the papaya-infecting begomovirus virus from Iran as an isolate of OELCuV. To the best of our knowledge this is the first report of a begomovirus infecting papaya in Iran and the first record of papaya as host of OELCuV.

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REFERENCES


