Diseases Caused by Viruses

Cucurbit Leaf Crumple Virus Associated to Watermelon Disease in Campeche State, Mexico

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An annual recurrent disease causing yield reduction in cultivated watermelon (*Citrullus lanatus*) was documented by the growers in different farms of Campeche state, Mexico. In April 2019 and March 2020, open field grown watermelon plants showed symptoms such as leaf curling, crumpling, and leaf basal or apical necrosis, with an incidence ranging from 30 up to 80%. These plants also presented high populations of whitefly, especially in the most affected fields. To identify the causal agent of the disease, a total of 22 symptomatic watermelon plants were collected in four locations from Campeche state. Total nucleic acids (DNA and RNA) were extracted from these leaf samples. Initially, RT-PCR analysis was performed with specific primers for cucurbit-infecting crinivirus transmitted by whitefly, but the expected size PCR product for those viruses was not amplified in any of these samples. To investigate the presence of cucurbit-infecting begomoviruses, PCR was performed by using specific primers for those begomoviruses reported in Mexico and north/central America, including squash leaf curl virus (SLCV), watermelon chlorotic stunt virus, melon chlorotic leaf curl virus, and cucurbit leaf crumple virus (CuLCrV). Only the expected amplicon size of ~1,089 bp for CuLCrV was amplified from DNA extracts from all 22 watermelon samples, suggesting a single cucurbit-associated virus. The putative complete genome of the CuLCrV Campeche isolate was amplified

by circular DNA enrichment using a rolling circle amplification procedure from two representative samples, followed by enzymatic digestion using *Bam*HI, *Eco*RI, *Kpn*I, and *Sac*I enzymes (Inoue-Nagata et al. 2004). Expected linearized full-length viral components (~2.7 kb) were obtained with *Eco*RI and *Sac*I, and both products, from one selected sample, were cloned into pGreen0029 vector and were fully sequenced. Sequence analysis of the *Eco*RI clone, designated as LV2019Camp_A (deposited in GenBank as accession no. MW273384) revealed the highest identity of 97.52% to CuLCrV DNA-A Baja California Sur isolate (GenBank accession no. MN625831.1), whereas the *Kpn*I clone, designated as LV2019Camp_B (deposited in GenBank as accession no. MW273385), shared 94.87% identity with DNA B of CuLCrV isolate Arizona (GenBank accession no. AF327559.1). Subsequently, a CuLCrV isolate Campeche-derived agro-infectious clone was obtained by constructing a partial dimeric tandem repeat of both DNA-A and DNA-B components (Bang et al. 2014). Twelve watermelon plants were agroinfiltrated with the infectious clone at the fourth true leaf stage, resulting in symptomatic plants (11/12) exhibiting leaf yellowing, curling, and crumpling 15 days after agroinfiltration, and CuLCrV infection was confirmed by PCR specific detection using DNA extract from noninoculated leaves. Previously, CuLCrV has been detected in the United States (Arizona, Texas, California, Florida, South Carolina, and Georgia) and north Mexico (Coahuila) infecting cucurbits including squash, cucumber, cantaloupe, pumpkin, and watermelon (Brown et al. 2002; Keinath et al. 2018), in both single and mixed infection with other whitefly-transmitted RNA viruses (cucurbit yellow stunting disorder virus, genus *Crinivirus*) and DNA viruses (SLCV, genus *Begomovirus*) (Kuo et al. 2007). To our knowledge, this is the first report of CuLCrV infecting a cucurbit crop in the Campeche state from the Yucatán peninsula in Mexico.

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