First Report of Chilli leaf curl virus and Associated Alpha- and Beta-satellite DNAs Infecting Nettle Weed (Urtica dioica) in Pakistan

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April 2016, Volume 100, Number 4
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http://dx.doi.org/10.1094/PDIS-10-15-1132-PDN

DISEASE NOTES

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- Citation

- Print: 23 Mar 2016
- Ahead of Print: 16 Feb 2016
- First Look: 17 Nov 2015
- Accepted: 12 Nov 2015

ABSTRACT

Weeds act as reservoirs of begomoviruses and ‘‘mixing vessels’’ leading to emergence of new viruses that may infect crops once a susceptible host plant is introduced into an agro-ecological system. Owing to the importance of weed hosts in the emergence of begomoviruses, we evaluated “nettle weed” (*Urtica dioica* L.) showing leaf curl and vein-yellowing symptoms, suspected of begomovirus infection in Lahore, Pakistan, during 2013. *Urtica dioica* is an herbaceous,

perennial, flowering plant of the dicotyledonous family *Urticaceae.* Total DNA was extracted and the presence of begomovirus was confirmed by PCR using coat protein-specific primers from all five symptomatic plants (Haider et al. 2005). To amplify the full-length genome of the begomovirus(es), total DNA was subjected to rolling-circle amplification (RCA) using TempliPhi TM DNA Amplification Kit (GE Healthcare, USA). The RCA product of all samples was digested with *Eco*RI and a DNA fragment of ~2.8 kb was cloned in pGEM-3Zf+ (Promega, Madison, WI). One full-length betasatellite and two alphasatellites were amplified using universal primers (Zia-Ur-Rehman et al. 2013). As sequences of all five clones of helper viruses and betasatellites were 99.5 to 100% similar, only one sequence from each was reported and used in the analysis. Twenty closely related sequence hits were downloaded from NCBI GenBank for each helper virus, betasatellite, and alphasatellite sequence. These sequences were aligned using MUSCLE, and homology of pairwise sequence comparisons was calculated using SDTv1.2. Results of pairwise sequence comparison showed that the helper virus (GenBank Accession No. KT699194) shares maximum pairwise nucleotide identity with *Chilli leaf curl virus* (ChiLCV; AF336806) at 94.4%, reported to infect pepper plants (Shih et al. 2003). Based on begomovirus species demarcation criteria (Brown et al. 2015), this is the first isolate of ChiLCV infecting nettle weed. The betasatellite (KT716083) shared a maximum of 94.5% nucleotide identity with Ageratum yellow leaf curl betasatellite (AYLCB; AJ316028), while one alphasatellite (KT716082) shared 97.5% nucleotide identity with Ageratum yellow vein Pakistan alphasatellite (AYVPKA; FR772085) and the second alphasatellite (KT716081) was an isolate of Bhendi yellow vein alphasatellite (BYVA) with 94.1% pairwise nucleotide identity to KJ843306. The presence of ChiLCV was also confirmed by Southern blot hybridization of RCA enriched DNA extracts of the five symptomatic plants using a digoxygenin (DIG)-labeled probe based on nucleotides 1733 to 2511 of the viral replication-associated gene. ChiLCV associated with different betasatellites has been reported to infect important vegetables including chili, tomato, and potato in India and Pakistan. The discovery of ChiLCV, AYLCB, AYVPKA, and BYVA shows that nettle weed harbors this disease complex and may act as a reservoir of this complex to hamper the production of vegetables in the area.

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