First Report of Tomato Yellow Leaf Curl Virus Infecting *Mikania micrantha* in China

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Tomato yellow leaf curl virus (TYLCV), a monopartite begomovirus (genus *Begomovirus*, family *Geminiviridae*), is one of the major virus species causing tomato yellow leaf curl disease and a major limiting factor in tomato production worldwide. In China, TYLCV was first reported in 2006 in Shanghai (Wu et al. 2006). *Mikania micrantha* Kunth is a perennial vine that is native to Central and South America and has become an important invasive weed widely distributed in South China. In July 2013, *M. micrantha* plants exhibiting mild yellow vein symptoms were observed around farm of Guangzhou, Guangdong Province, China. Total DNA was extracted from seven leaf samples collected from five symptomatic and two asymptomatic *M. micrantha* plants with the EasyPure Plant DNA kit (TransGen Biotech, Beijing, China) and was used as template for PCR amplification using degenerate begomovirus primer AV₄₉₄ (Wyatt and Brown 1996) with the reverse CoPR primer (He et al. 2004) to amplify a ~570-bp fragment of the viral coat protein gene. The amplicon of the expected size was obtained in all five symptomatic samples and sequenced, but no amplicon was obtained in asymptomatic samples. The BLASTn results showed that the ~570-bp sequences had similarity with Israel strain of TYLCV (TYLCV-IL) in GenBank, indicating that these *M. micrantha* plants were infected by TYLCV-IL. Total DNA of one symptomatic

sample was subjected to amplification using rolling circle amplification (TempliPhi kit; GE Healthcare, Buckinghamshire, U.K.), followed by digestion with *Bam*HI and *Eco*RI endonucleases (Fermentas, Glen Burnie, MD). One ~2.7-kbp putative begomoviral fragment was obtained following digestion with *Bam*HI, gel purified, ligated into the plasmid vector pGEM-3Z (Promega Co., Madison, WI) previously digested with *Bam*HI, transformed into *Escherichia coli* DH5α, and sequenced (Invitrogen Co., Shanghai, China). Pairwise nucleotide (nt) sequence analysis was performed with MUSCLE alignment using the Sequence Demarcation Tool (SDT 1.2) (Muhire et al. 2014). The genome of isolate WGJ (GenBank accession no. KF990604) was characterized, revealing a full-length begomoviral DNA-A component of 2,781 nt, encoding six ORFs, two ORFs (AV1 and AV2) in the virion sense strand, and four ORFs (AC1, AC2, AC3, and AC4) in the complementary sense strand, which shared >98% nt identities with all available TYLCV-IL isolates from China, with the highest nt identity at 99.9% to TYLCV-[CN-HBLF4-09] (GenBank accession no. HM208334). Based on the sequence, abutting primers, TYF (5'-TGCAGAGGAAGATAGTGGGAATTC-3') and TYR (5'-ATCCAGGACCTACCTCCTCATA-3'), were designed and used to detect the presence of TYLCV-IL in *M. micrantha* plant samples; a ~2.7- to 2.8-kbp full-length genomic DNA fragment was amplified from all five samples and sequenced. The sequences of full-length genome of TYLCV-IL obtained from each symptomatic *M. micrantha* plant sample (GenBank accession nos. MT671425, MT671426, MT671427, MT671428, and MT671429) shared >99.0% nt identities with TYLCV-[CN-WGJ-13], indicating that the samples were infected by TYLCV-IL. To our knowledge, this is the first report of TYLCV-IL infecting *M. micrantha*. Weeds serving as reservoirs of begomoviruses can have an important role in disease epidemiology and spread to cultivated crops. Thus, *M. micrantha* may serve as an important TYLCV-IL reservoir for tomato crops in China.

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