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DISEASE NOTES

First Report of Alfalfa Leaf Curl Virus from Alfalfa in Iran

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The family Geminiviridae comprises a large group of plant ssDNA viruses with geminate particles that are classified in nine genera, including the newly established Capulavirus genus (Zerbini et al. 2017). Aphid-transmitted alfalfa leaf curl virus (ALCV) is a capulavirus that was first isolated from alfalfa (Medicago sativa L.) associated with leaf curling symptoms in France, Spain, and Argentina (Bejerman et al. 2018; Bernardo et al. 2016). From 2015 to 2017, 137 leaf samples of alfalfa exhibiting leaf curling, marginal leaf chlorosis, and leaf malformation were collected from nine provinces of Iran. Total DNAs of these alfalfa samples were extracted using the cetyltrimethylammonium bromide method, and the presence of ALCV was tested by polymerase chain reaction (PCR) using Tag DNA Polymerase (Thermo Fisher Scientific, U.S.A.) and a specific primer pair (Gemini F1, 5'-ATGATGGATAATTCAAACCC-3'; and Gemini R2, 5'-CACCTCCACTGTCTTGTCCA-3') designed based on 28 available ALCV genomes and amplifying a 1,380-nt-long fragment encompassing parts of the cp and the repA genes. Amplification conditions consisted of 95°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, and a final extension for 10 min at 72°C. A total of 22/137 alfalfa samples tested positive for ALCV, including 1 alfalfa sample from Fars, 1 from Kuzestan, 2 from Sistan and Baluchestan, 7 from Kerman, and 11 from Isfahan. These 22 PCR amplicons that were Sanger sequenced at Macrogen (South Korea) shared high levels of similarity with ALCV. The total number of samples that tested positive was unexpectedly low, suggesting that either the observed symptoms of collected plants were caused by other biotic or abiotic stresses or that the efficiency of the PCR-mediated detection of ALCV was not optimal. Total DNAs from 3 out of the 22 ALCV-infected samples from Isfahan (ME8), Kerman (12UK), and Western Azerbaijan (CO7) provinces were further amplified using Phi29 DNA polymerase (TempliPhi, GE Healthcare) by rolling circle amplification (RCA) as described by Shepherd et al. (2008). RCA products were then digested using EcoRI or Bg/II restriction enzymes, which yielded for each of the three alfalfa samples products of about 2.8 kb. These products were each cloned into the pBluescript plasmid and Sanger sequenced by

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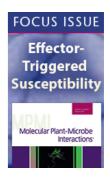
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primer walking at either Bioneer or Macrogen (South Korea). The three complete genome sequences that were obtained (GenBank accession nos.: MH085201 for isolate ME8 from Isfahan province, 2,727 nt in length; MH085199 for isolate 12UK from Kerman province, 2,712 nt in length; and MH085200 for isolate CO7 from Western Azerbaijan province, 2,764 nt in length) shared 81.9% (ALCV isolate LUZ076, GenBank accession no. KT214370) to 95.3% (ALCV isolate LUZ075, GenBank accession no. KT214353) genomewide pairwise identity with ALCV isolates from France and phylogenetically clustered with ALCV strain A (ME8 and CO7) and ALCV strain B (12UK). These three novel ALCV genomes have a classical ALCV genome organization (Bernardo et al. 2016), including seven (ME8 and CO7) and six (12UK) open reading frames and the nonanucleotide stem-loop sequence TAATATTAC in the intergenic region. These results confirm that the geographical range of ALCV extends beyond the Western Mediterranean basin (France and Spain) and Argentina and that ALCV is probably freely moving across the Mediterranean basin and beyond. To our knowledge, this is the first report of ALCV from alfalfa in Iran.



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