

First Report of *Alfalfa mosaic virus* Associated with Severe Mosaic and Mottling of Pepper (*Capsicum annuum*) and White Clover (*Trifolium repens*) in Oklahoma

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Alfalfa mosaic virus (AMV), a member of the genus *Alfavirus*, family *Bromoviridae* (1), has been reported in 44 states in the United States excluding Oklahoma. During a cucurbit survey in the summer of 2010, severe mosaic and mottling symptoms were observed on many peppers (*Capsicum annuum*) and white clover (*Trifolium repens*) plants in Tulsa, Oklahoma. Symptomatic leaf samples from 15 pepper and two white clover plants were collected in the Bixby area and analyzed serologically by dot-immunobinding assay (DIBA) using specific polyclonal antibodies against AMV (Agdia, Inc). Seven out of 15 pepper samples and both white clover samples were tested positive by DIBA to AMV. The remaining symptomatic samples were positive to *Cucumber mosaic virus* (CMV). Total RNA was extracted from DIBA positive AMV samples by Tri-reagent method. A small aliquot of total RNA was tested by reverse transcription (RT)-PCR using specific primers: AMV-F 5' GTCCGCGATCTCTTAAAT 3' and AMV-R 5' GAAGTTTGGGTCGAGAGA 3' that were designed to amplify 900 bp of the AMV-RNA 3. Analysis of the PCR products on agarose gel electrophoreses showed that all tested samples showed a band of the expected size while DIBA negative AMV samples did not produce any band. The amplified PCR product (900 bp) obtained from pepper and white clover were cleaned with PCR purification kit (Qiagen, Germantown, MD) and directly sequenced bi-directionally using the above primers. Sequence analysis confirmed that this virus shared 97% identity at nucleotide sequence with RNA 3 of AMV isolate from Madison-USA (GenBank Accession No. K02703). For biological and morphological characterization of the virus, eight pepper plants were mechanically inoculated using 0.1 M K₂HPO₄ buffer (pH 7.2) with total RNA extracted from AMV positive pepper or white clover plant samples. One to two weeks post-inoculation, all inoculated plants produced severe mosaic, mottling, and stunting. Virus-like particles preparations were obtained from these symptomatic plants according to our previously described method (2) and electron microcopy examination showed typical AMV particles. These biological and morphological data further confirmed the presence of AMV infecting pepper and clover in Oklahoma. AMV is a significant pathogen worldwide and infects more than 600 species in 70 families, especially alfalfa, pepper, soybean, and tobacco (3). AMV has a worldwide distribution, including the United States, and particularly the Midwestern U.S. where the incidence of the virus is on the rise recently because of the presence of its vector (*Aphis glycines*) (4). To our knowledge, this is the first report of AMV infecting crops in Oklahoma, which could pose a threat to other economic crops grown in Oklahoma, especially soybean.

References: (1) E. E. Mueller et al. *Plant Dis.* 91:266, 2007. (2) A. Ali et al. *Plant Dis.* 96:243, 2012. (3) J. F. Bol. *Mol. Plant Path.* 4:1, 2003. (4) M. Malapi-Nelson et al. *Plant Dis.* 93:1259, 2009.