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DISEASE NOTES

Pepper huasteco yellow vein virus Associated to Sweet Pepper Disease in Sinaloa, Mexico

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Pepper (Capsicum annum L.) is an economically important vegetable crop in Mexico. In June 2013, symptoms of yellow mosaics, intervenal chlorosis, wrinkling, and stunting were observed in sweet pepper plants in a commercial greenhouse from Guasave Valley in northern Sinaloa. The symptomatic plants were scattered with an incidence of about 20%. Pepper plants with the same symptoms also were observed in 2014 and 2015. In addition, whiteflies (Bemisia tabaci Genn.) were present in the greenhouse with the symptomatic plants. During 2013 to 2015, 24 leaf samples of symptomatic sweet pepper plants were collected and processed to identify the causal agent. Total DNA and RNA were extracted. Initially, samples from symptomatic plants were analyzed by RT-PCR for the presence of whitefly-transmitted viruses of genus Torradovirus and Crinivirus and tested negative. Then, the leaf samples were tested for the presence of begomoviruses, using the PCR test with degenerated primers (Ascencio-Ibáñez et al. 2002). The expected amplicons size of ~600 bp were obtained from 17 of 24 plant samples tested. In order to clone the full circular DNA genome of the begomovirus, a rolling circle amplification (RCA) was performed on three selected positive samples. The RCA products were digested with different restriction enzymes, including EcoRI, BamHI, XbaI, KpnI, and XhoI (Inoue-Nagata et al. 2004). The expected full-length components of \sim 2.6 kb were obtained by using EcoRI and BamHI enzymes and both of them from a single plant were cloned into the pBluescript SK⁻ phagemid and fully sequenced. Sequence analyses of the EcoRI clone, LV2014GveCap9 (deposited in GenBank under accession no. KP890827) showed 94.3% nucleotide sequence identity with Pepper huasteco yellow vein virus (PHYVV) DNA A (GenBank accession no. X704181.1). The BamHI clone LV2014GveCap10 (deposited as KP890828) showed only 83.6% nucleotide sequence identity with PHYVV DNA B (X70419.1). The infectivity of PHYVV Sinaloa isolate (DNA A and B) was tested by biolistic inoculation into five plants, at the third true-leaf stage, of Capsicum annuum cv. mini bell and Nicotiana benthamiana. At 15 days post inoculation, interveinal chlorosis and wrinkling were observed in 5/5 C. annuum and 5/5 N. benthamiana in vitro seedlings. PCR analyses with specific primers confirmed the presence of PHYVV. The low sequence identity of DNA-B component with the previous reported PHYVV Tamaulipas suggest that it may have evolved independently than DNA-A and the biological significance of DNA B have to be

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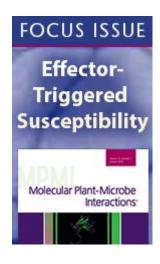
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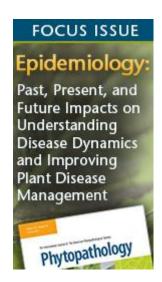
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determined for PHYYV Sinaloa isolate. Previous studies carried out by swapping DNA B component in cassava begomoviruses indicate that DNA B could contribute significantly to the symptom severity (Patil et al. 2015). To our knowledge, this is the first report of natural infection of a new PHYVV isolate affecting sweet pepper in Sinaloa, Mexico.

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