Viruses

First Report of Cassava Mosaic Disease and Sri Lankan Cassava Mosaic Virus in Laos

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Cassava (*Manihot esculenta* Crantz) has been traditionally grown as a subsistence crop in Laos, but in recent years cassava cultivation in this country has expanded, and it is becoming a “cash crop” for farmers (Malik et al. 2020). This also means that cassava vegetative seed (stakes) is rapidly multiplied and distributed. One of the most important diseases affecting cassava in the world is cassava mosaic disease (CMD), caused by several species of begomoviruses and disseminated by infected stakes or vectored by the whitefly *Bemisia tabaci* (Legg et al. 2015). Sri Lankan cassava mosaic virus (SLCMV), a bipartite begomovirus, is the virus species causing CMD in Southeast Asia (SEA) and is widespread in Cambodia, Vietnam, Thailand, and south China (Siriwan et al. 2020). During field surveys on July 12 to 14, 2020, the team in south Laos surveyed eight fields along the border with Cambodia, in the southern provinces of Attapeu and Champasack, and identified CMD symptoms in only one of the fields, located at Kong District of the Champasack province (GPS coordinates 13.94325, 105.99102). From these eight fields, samples were collected from every third plant in an X pattern. Photographs from each sampled plant were taken and uploaded into CIAT's PestDisPlace platform (Cuellar et al. 2018) (<https://pestdisplace.org>), for CMD symptom confirmation. Leaf samples were sent to the laboratory for PCR using primers SLCMV-F (5'-ATGTCGAAGCGACCAGCAGATATAAT-3') and SLCMV-R

(5'-TTAATTGCTGACCGAATCGTAGAAG-3') targeting the AV1 gene (Dutt et al. 2005), following the protocol described in Siriwan et al. (2020), and primers SLCMV-B-F1 (5'-ACCGATGGCCGCGCCCCCTCT-3') and SLCMV-B-606R (5'-CACCTACCCTGTTATCGCTAAG-3') targeting part of the BV1 gene. Out of 60 samples collected for the field in Kong District, 11 (18.3%) were PCR positive to SLCMV (to DNA-A and DNA-B), but only four plants (6.7%) showed symptoms of CMD. None of the samples in the other seven fields had CMD symptoms, nor was SLCMV detected in any of these plants. Furthermore, the presence of CMD symptoms in the older leaves of the plants suggests that the virus was introduced with contaminated stakes. The complete bipartite genome of one isolate (Champ1), was amplified by rolling circle amplification and sequenced with the nanopore MinION technology as described by Leiva et al. (2020). The sequences were submitted to GenBank under accession numbers MT946533 (DNA-A) and MT946534 (DNA-B). A phylogenetic tree for SLCMV and a link to the open SLCMV Nextstrain map (Hadfield et al. 2018) are included in the supplementary materials. The sequences of the DNA-A and DNA-B components of the Champ1 isolate were nearly identical to those of an isolate of SLCMV from Ratanakiri, Cambodia (99.72% for DNA-A and 99.82% for DNA-B; Wang et al. 2016). Phylogenetic analysis grouped isolate Champ1 with those that form the cluster of SEA isolates that contain the shorter version of the *rep* gene (Siriwan et al. 2020). This short version of *rep* presents a deletion of seven amino acids at the C-terminus, which is involved in host responses to SLCMV (Wang et al. 2020). The confirmation of CMD and SLCMV in the border between Laos and Cambodia should be followed by disease containment and management strategies, particularly given that the majority of cassava varieties grown in Laos are from neighbor countries, most of which have already reported the presence of CMD.

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