

Disease Note

Diseases Caused by Viruses

First Report of Pepper Yellow Leaf Curl Thailand Virus Infecting Hot Chili in Central Vietnam and Laos

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Hot chili pepper (*Capsicum annuum*) cultivation has been on the rise in Southeast Asia to meet export demands. In Thailand, the top chili exporter in Southeast Asia, chili production has been severely hampered by pepper yellow leaf curl disease (YLCD) caused by the begomovirus pepper yellow leaf curl Thailand virus (PepYLCThV) (Chiemsoombat et al. 2018; Suwor et al. 2021). In the neighboring countries Laos and Vietnam, a limited survey of chili fields (200 plants in total) in Savannakhet (Savannakhet University campus, $n = 150$), Laos, and Quang Nam Province (Ka Dang commune, Dong Giang district, $n = 50$), central Vietnam, in 2023 led to the finding of eight plants (5 in Laos and 3 in Vietnam) exhibiting YLCD-like symptoms, which included bright yellowing of young leaves and leaf curl and mosaic chlorosis in mature leaves. Total DNA was extracted from leaves of two symptomatic plants (one from Savannakhet and one from Quang Nam) using a cetyltrimethylammonium bromide-based DNA extraction protocol (Doyle and Doyle 1987; Nguyen et al. 2023). Next, PCR was performed using newly designed PepYLCThV-specific primers based on PepYLCThV sequences in GenBank. PCR products of expected sizes were observed in samples with disease symptoms, but not from DNA extracted from *C. annuum* (cv. VA.99999) grown at the Institute of Biotechnology in Thua Thien Hue, Vietnam. The amplicons were Sanger sequenced (Apical Scientific, Selangor, Malaysia), and the complete bipartite genome sequences of two isolates (Sava01 from Laos and QNam01 from Vietnam)

were obtained. The sequences of the DNA-A component from the isolates Sava01 (GenBank PP437580) and QNam01 (GenBank PP437581) exhibited the highest sequence identity of 99.2 and 94.7% with the PepYLCThV isolate ChiangDaoS1 (GenBank OM677627), respectively. Conversely, the sequences of the DNA-B component from the isolates Sava01 (GenBank PP437579) and QNam01 (GenBank PP437582) exhibited the highest similarity of 91.8 and 90.9% with the PepYLCThV isolate KKN601 (GenBank MW715820), respectively. These results confirmed the presence of PepYLCThV in hot chili pepper plants exhibiting YLCD-like symptoms in central Vietnam and Laos. Infectious clones of PepYLCThV DNA-A and DNA-B (isolate QNam01) were created based on the pLX-AS vector as described by Pasin (2022) and transformed into *Agrobacterium tumefaciens* EHA105. The resulting bacteria were cultured in lysogeny broth containing rifampicin (25 µg/ml) and kanamycin (50 µg/ml) at 28°C and used for agroinoculation of *Nicotiana benthamiana* ($n = 6$) and *C. annuum* (cv. VA.99999, $n = 6$) (4- to 6-leaf plants) as described by Pasin (2022). In all *N. benthamiana* plants, agroinoculation with both DNA-A and DNA-B infectious clones caused stunted growth and severe leaf curl, with yellow and white patches 21 days postinoculation. In *C. annuum* plants, symptom expression, which included leaf curl and stunted leaves with yellow mosaic patterns, was observed in two out of six inoculated plants 6 weeks postinoculation. PCR assays confirmed the presence of PepYLCThV DNA in *N. benthamiana* and *C. annuum* symptomatic leaves. To our knowledge, this is the first report of pepper yellow leaf curl Thailand virus in hot chili pepper in Laos and central Vietnam. Appropriate containment and management strategies should be developed and implemented to control the spread of this disease in hot chili pepper crops in both countries.

References:

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