First Report of Tobacco Mosaic Virus Infecting Papaya in China

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Papaya (*Carica papaya*, L.) is widely cultivated in tropical and subtropical regions of China and has high nutritional and medicinal values. More than 11 species of papaya viruses have been recorded in the world, but the most destructive one for papaya production in China is papaya ringspot virus (PRSV) (Li 2019). In order to control PRSV, a transgenic papaya cultivar, designated as ‘Huanong No.1’, carrying the nuclear inclusion b (*Nib*) cistron of PRSV Ys isolate, was successfully commercialized in 2006 and has shown a wide range of resistance to PRSV in China (Li et al. 2007). However, more than 10% of Huanong No.1 plants developed different virus-like symptoms on leaves, including mosaic, yellow mottle, and deformation in some plantations in Guangdong Province, China, in 2020. Based on observation of the symptomatic phenotypes, field surveys indicated that the disease incidence ranged from 10 to 40%, resulting in significant loss of papaya fruit. The virus particles were purified from symptomatic papaya plants following Gooding and Hebert (1967) and rigid filamentous particles resembling tobacco mosaic virus (TMV) were observed by transmission electron microscopy. Purified virus samples were further utilized to mechanically inoculate healthy seedlings of papaya, *Nicotiana glutinosa*, and *N. tabacum* K326. At 15 days after inoculation, obvious symptoms of virus infection were observed on different plants. The diseased plants showed systemic mottling and mosaic in the papaya leaves, necrotic spots on the leaves of *N. glutinosa*, and mosaic

and mottling spots on leaves of *N. tabacum* K326. These symptoms produced on the hosts were exactly the same as those caused by TMV. In order to reconfirm the species of the infected virus, total RNA was extracted from a single leaf of 30 diseased papaya plants using RNAiso Plus kit (Takara, Japan) and reverse transcription-PCR was performed using TMV coat protein cistron specific primers (TMV-CP-R: 5'-TCAAGTTGCAG GACCAGA-3' and TMV-CP-F: 5'-ATGTCTTACAGTATCACTAC-3'), as described previously (Srivastava et al. 2015). An expected 480-bp fragment was amplified from all of the samples. Sequence analysis revealed that the diseased papaya was infected with TMV, designated as Cpa-TMV. In order to understand the difference among TMV isolates on papaya and other host plants, the whole genomic sequence of TMV from papaya was obtained and analyzed. The total length of the genome of Cpa-TMV was 6,395 bp, and the sequence was submitted to the NCBI database (GenBank no. OK149218). A neighbor-joining phylogenetic tree of 19 TMV isolates was constructed using MEGA X software. Phylogenetic analysis indicated that the 19 TMV isolates were divided into Clades I, II, and III. Interestingly, Clade I was composed of 12 Chinese mainland isolates, which further were grouped into IA (Northern China) and IB (Southern China), while 6 isolates from other countries and 1 isolate (pet-TMV) from China Taiwan belonged to Clade II and III. It is inferred that the TMV isolates from the Chinese mainland are quite different from other countries and China Taiwan. This suggests that geographical differences between Northern and Southern China may lead to the gradual differentiation of TMV isolates and eventually induce those isolates to evolve into two subclades. To our knowledge, this is the first report of TMV infection on papaya under natural conditions. It is necessary to find effective methods to control TMV in transgenic papaya.

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