Diseases Caused by Viruses

First Report of Tomato Spotted Wilt Virus Isolated from *Nasturtium* (*Tropaeolum majus*) with a Serious Leaf Mosaic Disease in China

M. C. Yu,¹ C. X. Yang,^{1,†} J. Z. Wang,¹ Q. S. Hou,¹ S. Zhang,² and M. J. Cao²

¹ Liaoning Key Laboratory of Urban Integrated Pest Management and Ecological Security, College of Life Science and Engineering, Shenyang University, Shenyang 110044, China

² National Citrus Engineering and Technology Research Center, Citrus Research Institute, Southwest University, Chongqing 400712, China

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Tomato spotted wilt virus (TSWV, genus *Orthotospovirus*, family *Tospoviridae*) is a thrips-transmitted negative-stranded RNA virus with a large host range. Major economic losses caused by TSWV have been recorded in various crops such as tomato, pepper, and lettuce (Adams et al. 2017; Pappu et al. 2009). *Nasturtium* (*Tropaeolum majus* L.), a common flowering plant species native to Peru, is generally cultivated as a garden and greenhouse ornamental plant in China. It is also known for its medical, edible, and cosmetic values (Jakubczyk et al. 2018). In June 2019, a serious leaf mosaic disease was observed in ~77% of *T. majus* plants in Beiling Park in Shenyang City, Liaoning, China. To reveal the possible viral agent associated with the disease, leaf tissue was collected from nine symptomatic and two asymptomatic plants, followed by total RNA extraction from each of the samples using an RNASimple Total RNA Kit (Tiangen, Beijing, China). The RNA from one representative symptomatic sample was used to construct an rRNA-depleted library using the Ribo-Zero rRNA Removal Kit (Plant Leaf) (Illumina, San Diego, CA). The library was subjected to RNA-Seq using a BGISEQ platform (Shenzhen Huada Gene Science and Technology Service Co., Shenzhen, China). A total of 109,279,540 quality-filtered reads were obtained using CLC Genomics Workbench 9.5 software (Qiagen, Valencia, CA). Clean reads were assembled into 88,091 contigs ranging in length from 200 to 19,695 bp using Velvet (Zerbino and Birney 2008). Among the contigs,

three sized at 8,801, 4,617, and 2,909 bp were found to share a sequence identity of 98.74 to 99.35% with the RNA segments (L, M, and S) of TSWV. No sequences of other viruses, viroids included, were detected. Ten primer pairs were designed based on the TSWV contigs to obtain the full genome sequence of the virus. Five, three, and two amplicons were obtained for the TSWV RNA segments L, M, and S, respectively, from the same RNA sample used in the RNA-Seq process. These amplicons were then cloned into the pMD18T vector (TaKaRa, Dalian, China) and Sanger sequenced. The resulting sequences were assembled and analyzed using DNAMAN version 8.0 (Lynnon Biosoft, Vaudreuil, QC, Canada) and DNASTar version 6.0 software (DNASTar, Madison, WI). The RNA segments were determined to be 8,914 nt (L, accession no. MT241883), 4,791 nt (M, MT241884), and 2,922 nt (S, MT241885) in length. They shared a sequence identity of >99% with the LL-N.05 (segment L, KP008128) and SPAIN-1 (segment S, AY744479) isolates from tomatoes in Spain and the Beijing isolate (segment M, MH717046) from chrysanthemum in China. These results, together with phylogenetic analysis, suggest that the isolate (designated as isolate LN-HJL) likely resulted from genome reassortment between other isolates. No reliable recombination events were detected in the RNA segments of LN-HJL by the RDP4 program (Martin et al. 2015). RT-PCR assay with primer pair S-1F/1R on all samples led to positive detection of TSWV only in the symptomatic samples, indicating that the virus was likely responsible for the disease symptoms observed in *T. majus*. To the best of our knowledge, this is the first report of TSWV infection in *T. majus* in China and the second in the world since the first report in the United States (Ie 1964). TSWV-infected *T. majus* plants not only lose their aesthetic and economic value but also may act as reservoirs for the spread of TSWV to other important crops. *T. majus* should therefore be monitored regularly for the virus and managed accordingly in China.

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[†]Indicates the corresponding author.

E-mail: C. X. Yang; xueyang27@126.com