Diseases Caused by Viruses

The Occurrence of Pea Enation Mosaic Virus 1 and Pea Enation Mosaic Virus 2 from Disease-Affected Pea Fields in China

Xiaojiao Chen,¹ Kehua Li,¹ Henming Luo,¹ Shu Han,² Qin Hai Liu,¹ Guanlin Tan,³ Ruhui Li,⁴ and Fan Li^{1,†}

¹ State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming 650201, China

² College of Landscape Architecture and Horticulture, Yunnan Agricultural University, Kunming 650201, China

³ Modern Education Technology Center, Yunnan Agricultural University, Kunming 650201, China

⁴ USDA-ARS, National Germplasm Resources Laboratory, Beltsville, MD 20705, U.S.A.

Funding: This research was supported by the Natural Science Foundation of Yunnan Province, China (2018FD024) and the Program for Innovative Research Team (in Science and Technology) in University of Yunnan Province (Yunjiaoke 2014-22). Plant Dis. 105:518, 2021; published online as <https://doi.org/10.1094/PDIS-05-20-1123-PDN>. Accepted for publication 25 August 2020.

Pea (*Pisum sativum* L.) is an economically important legume crop that is commonly used as dried beans, fresh peas, pods, and shoots (Guo et al. 2009). Pea enation mosaic is an important virus disease of pea caused by two viruses in an obligate symbiosis, *Pea enation mosaic virus 1* (*Enamovirus*, *Luteoviridae*) and *Pea enation mosaic virus 2* (*Umbravirus*, *Tombusviridae*) (Hema et al. 2014). In November 2019, foliar yellow mosaic and vein enation symptoms were observed from pea plants in five fields of Honghe autonomous prefecture, Yunnan province, China. Incidence of symptomatic plants ranged from 20 to 40% and was distributed in both small and large fields. Leaves with typical virus-like symptoms were collected from five symptomatic pea plants in two fields and used for total RNA extraction. The five extracts of equimolar quantities were pooled into a sample and subjected to high-throughput sequencing (HTS) by the Illumina HiSeq system. Analyses of raw RNA reads were performed using CLC Genomics Workbench 12 (Qiagen). A total of 60,009,746 RNA reads were obtained from the sample, and de novo assembly of the reads using the CLC Genomics generated 88,105 contigs. BLASTN searches revealed the presence of contigs with high similarities to pea enation mosaic virus 1 (PEMV1), pea enation mosaic virus 2

(PEMV2), pea seed-borne mosaic virus, and bean yellow mosaic virus. To confirm the presence of PEMV-1 and PEMV-2 in the samples, two virus-specific primer pairs were designed based on the contig sequences obtained by HTS in this study. Primer pairs PEMV-1F/PEMV-1R (5'-ATGCCGACTAGATCGAAATC-3'/5'-TCAGAGGGAGGCATTCATT A-3') that flank the *cp* gene of PEMV-1 and PEMV-2F/PEMV-2R (5'-ATGACGATAATCATTAAATG-3'/5'-TCACCCGTAGTGAGAGGCA-3') that target the ORF3 region of PEMV-2 were used to amplify the two viruses by reverse transcription polymerase chain reaction (RT-PCR). DNA fragments of the expected sizes (PEMV-1, 570 bp; PEMV-2, 693 bp) were amplified from all five samples. The RT-PCR products were cloned and sequenced. Sequence analysis showed that the 570-bp amplicon (MT481989) shared the highest nucleotide sequence identity of 98.95% with PEMV-1 (Z48507), whereas the 693-bp fragment (MT481990) had the highest nucleotide sequence identity of 97.4% with PEMV-2 isolate JKI (MK948534). One gram of the symptomatic leaves from each of the five plants was homogenized with 5 ml of 0.01 M phosphate-buffered saline (PBS buffer), pH 7.0. Each of the resulting saps was used to inoculate onto five healthy pea seedlings. A total of 25 healthy pea seedlings were inoculated, and 16 inoculated plants developed yellowing and mottling at 10 days postinoculation (dpi); no symptoms were observed on control plants inoculated only with PBS buffer. The formation of typical enation was observed along the veins of the lower side of the symptomatic leaves of the inoculated plants at 30 dpi. PEMV-1 and PEMV-2 infection were confirmed by RT-PCR assays using the specific primer pairs described above. Although the presence of the pea enation mosaic virus complex was suspected in China based on symptomatology (Brunt et al. 1997), to our knowledge this is the first molecular and biological confirmation of PEMV-1 and PEMV-2 occurrence in China. The coinfection of PEMV-1 and PEMV-2 usually causes severe yield losses; therefore, integration of detection and control measures is important in pea production regions where the two viruses occurred.

References:

- Brunt, A. A., et al., eds. 1997. Plant Viruses Online: Description and Lists from the VIDE Database. CAB International, Cambridge, U.K.
- Guo, P. Y., et al. 2009. Anhui Agric. Sci. Bull. 15(8):87 (in Chinese).
- Hema, M., et al. 2014. Adv. Virus Res. 90:431.

The author(s) declare no conflict of interest.

Keywords: viruses and viroids, PEMV-1 and PEMV-2, oilseeds and legumes, pathogen detection

[†]Indicates the corresponding author.
E-mail: F. Li; fanlikm@126.com