Diseases Caused by Viruses

First Report of Cowpea Polerovirus 2 and Southern Cowpea Mosaic Virus Infecting Cowpea in Nepal

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Cowpea (*Vigna unguiculata* L. Walp), one of the important legume crops of Nepal, is consumed as a green vegetable or a dried pulse. In recent years, virus diseases have caused significant yield and quality losses in cowpea. In September 2019, five cowpea plants showing virus-like symptoms of mosaic, yellow mosaic, vein clearing, chlorotic spots, and puckering were collected in Chitwan, Nepal. The incidence of symptomatic plants in the three kitchen gardens was about 10 to 20%. To identify the viruses associated with the disease, a pooled sample from all five plants was screened initially by next generation sequencing (NGS). Total RNA was extracted from the symptomatic leaves using RNeasy Plant Mini Kit (Qiagen, Germany) and a transcriptome library was generated using the TruSeq Stranded Total RNA LT Sample Prep kit (Illumina, San Diego, CA) according to the standard protocol. NGS was performed using an Illumina NovaSeq 6000 system (Macrogen, Korea). A total of 324,807 contigs in the range of 201 to 14,645 nucleotides (nt) were obtained and analyzed against the viral reference genome database in GenBank by BLASTn and BLASTx search. Among the analyzed contigs, two large contigs showed homologies to cowpea polerovirus 2 (CPPV2) and southern cowpea mosaic virus (SCPMV). The CPPV2 contig (361,121 mapped reads, mean read coverage of 9,206.4 times) had a nearly complete genome sequence of 5,923 nt and showed 96% identity (99% coverage) with CPPV2 isolate BE179 (GenBank acc. no. KY364847) isolated from cowpea in Burkina Faso (Palanga et al. 2017). The SCPMV contig (10,612 mapped reads, mean read coverage of 384.1 times) had

a nearly complete genome sequence of 4,172 nt and showed 90% identity (100% coverage) with SCPMV isolate C (GenBank acc. no. M23021) isolated from cowpea in the United States (Wu et al. 1987). Additionally, mungbean yellow mosaic India virus (MYMIV, genus *Begomovirus*) and bean common mosaic virus (BCMV, genus *Potyvirus*) were detected at very low read depths. To confirm the presence of these viruses, total RNA was extracted from individual leaf samples, and reverse transcription PCR (RT-PCR) was performed using specific primers for each virus. Three of five cowpea samples were positive for CPPV2, and they were coinfecting with one other virus: SCPMV, MYMIV, or BCMV. One cowpea sample was positive for only MYMIV, and the remaining sample with symptoms of overall chlorosis was negative for all four viruses. The amplified products of 1,205 bp for CPPV2 isolates CPPV2-NP10, -NP12, and -NP24 were sequenced and deposited in GenBank under accession nos. MZ318692, MZ318693, and MZ318694. These Sanger sequences shared 99% nt identity with the NGS-derived sequence. The amplified product of 1,394 bp for SCPMV isolate SCPMV-NP12 (GenBank acc. no. MZ355623) shared 100% nt identity with the NGS-derived sequence. CPPV2 is a member of the genus *Polerovirus* and it was first identified and characterized by molecular assays in Burkina Faso (Palanga et al. 2016, 2017). SCPMV is a member of the genus *Sobemovirus* and it has been reported in the United States, China, and Burkina Faso (Hull et al. 2000; Lee et al. 2001; Wu et al. 1987). In Nepal, MYMIV has been reported in legumes, such as kidney bean, black gram, and mungbean, and BCMV in common bean (Acharya and Regmi 2020). To our knowledge, this is the first report of CPPV2 and SCPMV in cowpea in Nepal. Further work is required to determine the distribution, pathological properties, and economic impact of these two viruses.

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