Occurrence of mixed infection of lettuce chlorosis virus and cowpea aphid-borne mosaic virus in *Passiflora* spp. in Brazil

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Virus infection in *Passiflora* spp. can result in low fruit yield and reduced longevity of orchards in Brazil and other countries. One of the largest *Passiflora* germplasm banks in the world (Germplasm bank "Flor da Paixão", BAG-FP) is maintained at Embrapa Cerrados, in Brasília, DF, Brazil. BAG-FP is intended for the conservation, characterization, and diversified use of *Passiflora* genetic resources and is used in *Passiflora* breeding programs aiming at agronomic improvement. In June 2017, diverse virus-like symptoms (mosaic, yellowing, yellow spots, blisters, and leaf deformation) were observed in the *Passiflora* accessions from the BAG-FP. Therefore, the present work aimed to investigate the viral diversity in *Passiflora* accesses from BAG-FP. Symptomatic leaves of 21 *Passiflora* spp. were collected in the BAG-FP. dsRNA was extracted, pooled, and sequenced by Illumina HiSeq2500 technology (Macrogen, South Korea). *De novo* assembly of the raw reads was undertaken using the SPAdes assembler (*k-mer*=66). BLASTn and BLASTx search revealed several contigs with the highest identities to RNA1 and RNA2 of lettuce chlorosis virus (LCV, *Crinivirus*, *Closteroviridae*) and cowpea aphid-borne mosaic virus (CABMV, *Potyvirus*, *Potyviridae*). To validate the HTS results, virus-specific primers based on the contigs were designed. To verify the presence of LCV in individual samples, primers Bag1226lt317LCV16F(5'-GACGGGAGATTCCAATTGTGTAAAA-3')/ Bag1226lt317LCV260R (5'TGAACCATTTTGAAGCAATTCTCCA3') that amplify the partial RNA1 p8/p23 genes (244 nt), and LCVRNA22793F (5'-AAGGTTCAGATCCGTTCATCTTGTA3')/ LCVRNA23997R (5'CTTCCACGCATTCTCTGAATAAGTC-3') that amplifies the partial RNA2-HSP70h/p6.4/p60 (1,199 nt) were used in RT-PCR assays. Two of the 21 BAG-FP samples (*P. auriculata* and *P. alata*) were positive for LCV. The Sanger sequencing of the amplicons confirmed their identity as LCV-RNA1 and LCV-RNA2 (GenBank MN564795-MN564798). CABMV presence in these two samples was also evaluated. For the detection of CABMV, RT-PCR with the primers CABMVLNJP2492F (5'-GGTTCGTGATGTTTTGGTGCC-3')/ CABMVLNJP3373R(5'-CAAAAAGCACGCACTCACAAATC-3') that amplify the partial HC-Pro/p3 genes (900 nt) was performed. The mixed infection of LCV and CABMV was confirmed in these two samples. All CABMV RT-PCR amplicons were sequenced, and their identity confirmed. The occurrence of LCV was also investigated in the samples collected in

2015 from passion fruit commercial fields in Bahia state. LCV was detected in one *P. edulis* plant from Dom Basílio (*n*=11) in mixed infection with CABMV. CABMV is responsible for passion fruit woodiness disease (PWD), the main viral disease that affects passion fruit in Brazil. LCV has been reported in natural infections in lettuce, beets, papaya, cilantro, and cannabis (USA); bean (Spain), tomato and tobacco (China); and periwinkle (China and Brazil). This is the first report of the natural occurrence of LCV in *Passiflora* spp. and the first report of CABMV infection in *P. auriculata*. Additional studies will be necessary to evaluate the epidemiology of LCV in Brazil and determine the impact of this virus in the passion fruit crop.

Keywords: HTS; LCV; CABMV; passion fruit

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