First report of cherry virus A infecting *Prunus mume* in South Korea

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Abstract We report the detection of cherry virus A (CVA) in a *Prunus mume* sample using Illumina HiSeq 2500 paired-end RNA sequencing. CVA isolate OC shared highest sequence identity with CVA isolate J, which was recently identified in *P. armeniaca* in Japan. To the best of our knowledge, this is the first report of CVA infecting *P. mume* in Korea and the first complete genome sequence of CVA isolated from *P. mume* in the world.

Keywords CVA · *Capillovirus* · *Prunus mume* · Next-generation sequencing

In this study, cherry virus A (CVA; genus *Capillovirus* in the family *Betaflexiviridae*) was detected in a weak *Prunus mume* sample collected in Okcheon County, Korea, in May 2014. To verify the presence of the virus(es) in the sample,

Illumina HiSeq 2500 (San Diego, CA, USA) paired-end RNA sequencing was performed as described in a previous report [5]. Seven CVA-like contigs were obtained from the *P. mume* sample (Fig. 1a) and were subsequently used to design primers to determine the complete genomic sequence of this Korean isolate of CVA (Supplementary Table S1). Total RNA was extracted from leaves using the BIOPURE® Plant RNA Mini Kit (HANAKOREA, Seoul, Korea), and cDNA was synthesized with a random N25 primer and RevertAid Reverse Transcriptase (Thermo Scientific, Waltham, MA, USA). Six overlapping fragments, amplified by PCR with AccuPower ProFi Taq PCR PreMix (Bioneer, Daejeon, Korea), were cloned into the RBC T&A Cloning Vector (RBC Bioscience, Taipei, Taiwan) for Sanger sequencing by Macrogen (Daejeon, Korea). Both ends of the CVA genome were confirmed with rapid amplification of cDNA ends (RACE), using the 5'/3' RACE Systems (Invitrogen, Carlsbad, CA, USA). Eight individual PCR products comprising the entire CVA genome were assembled and analyzed with the DNAMAN 5.0 program (Lynnon Biosoft, Quebec, Canada). Thus, the complete 7431-nucleotide (nt) genomic sequence, excluding the 3'-terminal poly(A) tail, of CVA isolate OC (GenBank accession no. KY286055) was determined, and two predicted open reading frames (ORFs; ORF1 [nt 106–7134] and ORF2 [nt 5451–6842]) were identified (Fig. 1a). Comparison with the Pfam protein families database (<http://pfam.xfam.org/>) [1] showed that ORF1 encodes a large polyprotein with conserved domains, including a methyltransferase, RNA helicase, RNA-dependent RNA polymerase, and coat protein, and nested ORF2 encodes a movement protein.

CVA is distributed widely in various *Prunus* species throughout the world, including *P. armeniaca*, *P. avium*, *P. cerasifera*, *P. cerasus*, and *P. mume* [2–4, 6]. A National

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Fig. 1 Genome organization and phylogenetic analysis of CVA isolate OC (GenBank accession no. KY286055). **a** Complete genome of CVA-OC is composed of two ORFs with conserved domains. Seven open boxes and eight filled boxes below the genome map indicate the CVA-like contigs obtained from *P. mume* and the PCR

Center for Biotechnology Information BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed that CVA-OC shares highest full-length nucleotide sequence identity (91%) with CVA isolate J (GenBank accession no. LC125634), sampled from *P. armeniaca* in Japan [4]. To understand the molecular relationships among the CVA isolates, phylogenetic analysis and pairwise comparison based on an alignment of the complete polyprotein amino acid sequences of CVA-OC and other CVA isolates were performed with CLC Main Workbench 6.1.1 (CLC Bio, Cambridge, MA, USA). The phylogenetic tree showed that the polyprotein of CVA-OC is most closely related to that of CVA-J (Fig. 1b). The pairwise comparison showed that the polyprotein of CVA-OC has 84.01–92.87% identity to the corresponding proteins of other isolates (Supplementary Fig. S1). ORF2 also shares the highest amino acid identity (95%) with that of the CVA isolate J. To the best of our knowledge, this is the first report of CVA infecting *P. mume* in Korea. The complete genomic sequence of CVA isolate OC sampled from *P. mume* should help us understand the sequence divergence among CVA isolates in future research.

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fragments used for complete genome assembly, respectively. **b** Phylogenetic analysis based on an alignment of polyprotein amino acid sequences of CVA-OC and other CVA isolates was performed. *Apple stem grooving virus* (ASGV) was used as the outgroup. The lengths of the branches and percentage bootstrap frequencies are shown

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