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DISEASE NOTE

First Report of *Celery mosaic virus* Infecting Celery *(Apium graveolens)* in Indonesia

Laporan Pertama *Celery mosaic virus* yang Menginfeksi Seledri (*Apium graveolens*) di Indonesia

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ABSTRACT

Celery mosaic virus (CeMV), member of genus *Potyvirus*, is reported for the first time in Indonesia, from celery plants (*Apium graveolens*) in a vegetable field at Berastagi, North Sumatera Province. The plants possessed mosaic and vein clearing symptoms on the leaves as typical of CeMV infection. Virus incidence was confirmed by RT-PCR using degenerate potyvirus primer which amplified partial coat protein and 3'-UTR of the viral genome. Phylogenetic tree analysis placed Indonesian CeMV isolates in one separated clade within CeMV group and shared 96.5%–96.7% nucleotide identity with exemplar isolate of CeMV.

Keywords: coat protein, mosaic, potyvirus, RT-PCR, vein clearing

ABSTRAK

Celery mosaic virus (CeMV), anggota dari genus *Potyvirus* dilaporkan pertama kali di Indonesia, menginfeksi seledri (*Apium graveolens*) pada lahan pertanaman sayuran di Berastagi, Provinsi Sumatera Utara. Tanaman tersebut memiliki gejala mosaik dan pemucatan tulang daun yang merupakan ciri khas infeksi CeMV. Infeksi virus dikonfirmasi dengan RT-PCR menggunakan *degenerate primer* potyvirus yang mengamplifikasi genom virus, yaitu sebagian protein selubung dan 3'-UTR. Analisis pohon filogenetik menempatkan isolat CeMV Indonesia dalam satu klaster terpisah dalam kelompok CeMV dan memiliki kemiripan nukleotida sebesar 96.5%–96.7% dengan isolat contoh CeMV.

Kata kunci: mosaik, pemucatan tulang daun, potyvirus, protein selubung, RT-PCR

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Celery (*Apium graveolens*) is one of common aromatic and herbs vegetables oftenly found in global culinary recipes, including in Indonesia. Therefore, celery is a vegetable crop with important economic value. Celery mosaic, bacterial leaf spot, and bacterial leaf blight are common main diseases infecting celery that may affect yield (Raid *et al*. 2004). Celery mosaic disease caused by *Celery mosaic virus* (CeMV), also known as *Western celery mosaic virus* and *Celery crinkle-leaf virus*, belongs to the genus *Potyvirus,* family *Potyviridae* (Xu *et al*. 2011). CeMV has a monopartite, positive sense of single-stranded RNA (+ssRNA) genome of 9999 bp nucleotides (nt) in size, excluding the 3'-poly(A) tail (NCBI Acc No. NC_015393.1), and a flexuous filamentous particle about 780 nm in length (Xu *et al*. 2011). CeMV is transmitted by aphid vector in a non-persistent manner with restricted natural host range in the family *Apiaceae* (synonym *Umbelliferae*), including coriander (*Coriandrum sativum*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), parsnip (*Pastinaca sativa*), carrot (*Daucus carota* var. *sativa*), and several weed species (*Conium maculatum, Ptilimnium capillaceum, Apium leptophyllum*) (Bellardi and Rubbies-Autonel 1998; Fox *et al*. 2022; Moran *et al*. 2002; Traicevski *et al.* 2002).

Although it has a worldwide distribution, CeMV is not considered as serious problem in Indonesia. The infection of CeMV was firstly reported in California (US) from celery in early 1922 (Poole 1922). Current geographic distribution of CeMV expands to Europe, Australasia, South America, and Asia (Bos *et al.* 1989; Fernández *et al.* 2007; Fox *et al*. 2022; Iwaki and Komuro 1970; Karanfil *et al.* 2019).

In 2023, a field survey was conducted in a vegetable growing field at Berastagi, North Sumatera Province. The vegetables were planted in inter-cropping system, consisted of chili pepper (*Capsicum annuum*), cauliflower (*Brassica oleracea* var. *botrytis*), and celery (Figure 1). Mosaic and vein clearing symptoms were found on the celery leaves, suggestive of a viral infection (Figure 2). To support this hypothesis, the leaf samples were collected from three different symptomatic celery plants for further detection through RT-PCR (reverse transcription polymerase chain reaction).

Total nucleic acid extraction was carried out using GeneJet RNA Purification kit following manufacturer's protocol (Thermo Fisher Scientific, Memphis, USA). Amplification was done using degenerate potyvirus primer, U341 (5'-CCG GAA TTC ATG RTI TGG TGY ATI GAI AAY GG-3') and Poty-1 (5'-GGA TCC CGG GTT TTT TTT TTT TTT V-3') (Gibbs and MacKenzie 1997; Langeveld *et al*. 1991) which amplify partial coat protein and 3-'UTR with expected size of 600–800 bp.

Figure 1 Inter-cropping planting system in a vegetable field in Berastagi, North Sumatera. The celery plants are shown in white arrows.

RT-PCR cocktail was consisted of 12.5 µL of 2 × DreamTaq Green Master Mix PCR (Thermo-Fisher Scientific, Memphis, US), 2 µL of 10 µM for each primer, 0.5 µL of NZY Ribonuclease Inhibitor (40 U μL^{-1}), 0.25 µL of NZY RT-enzyme (200 U μL^{-1}) (NZYtech, Lisboa, Portugal) and added with nuclease-free water up to 25 µL for final volume. Potyvirus infecting garlic (collection of Plant Virology Laboratory, IPB University) and water were used as positive and negative control of RT-PCR, respectively.

One-step RT-PCR conditions were conducted as follows: cDNA synthesis at 45 °C for 1 h and one cycle of initial denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 10 sec, annealing at 50 °C for 10 sec, extension at 72 °C for 1 min and then followed by one cycle of final extension at 72 °C for 10 mins. Amplicons were then electrophoresed on a 1% agarose gel containing 1% FluoroVue™ nucleic acid gel stain (SMOBIO Technology Inc., Hsinchu City, Taiwan). Positive amplicons then were sent to 1st BASE Malaysia for Sanger sequencing.

Each sequence (forward and reverse) was trimmed and assembled to create a contig sequence. Each contig sequence then was checked in BLASTN program to search the highest sequence matches in the NCBI nucleotide database. Selected sequences and samples then were aligned using ClustalW in Geneious software (Biomatters Ltd., New Zealand). Sequence identity matrix was calculated and visualized using SDTv1.3 software (Muhire *et al*. 2014). Phylogenetic tree was inferred using Maximum-Likelihood method based on Tamura-Nei model with 1000 replicates in MEGA 11 software (Tamura *et al*. 2021).

DNA fragments of potyvirus were successfully amplified from three infected celery samples with expected size of 800 bp (Figure 3). Nucleotide sequences from three positive amplicons (celery 1, celery 2, celery 3) have been deposited in GenBank under accession LC801420.1, LC801421.1, and LC801422.1, respectively. Three Indonesian virus isolates shared the highest nucleotide identity (97.7%) with CeMV from Netherlands (AF203534.1), and 96.5%–96.7% with reference

Figure 2 Celery leaf showing vein clearing and mosaic symptoms (left and right with yellow arrow) and asymptomatic leaf (right with white arrow).

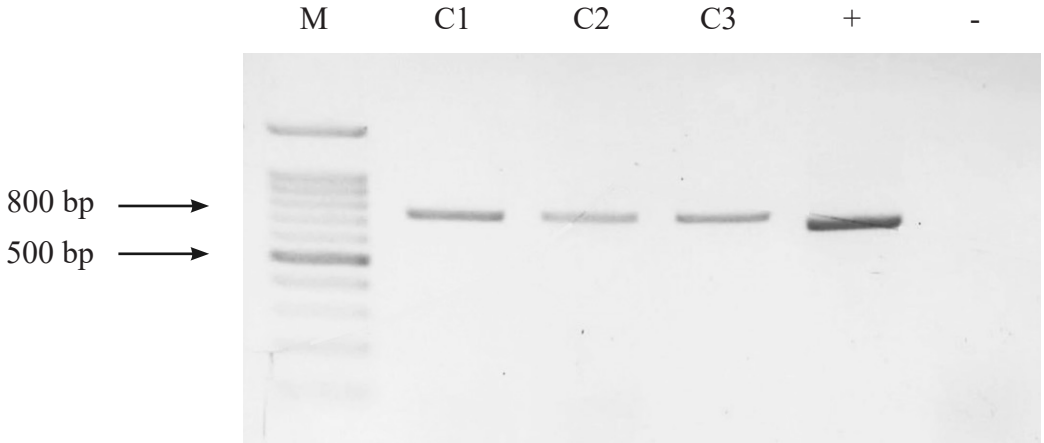

Figure 3 Reverse transcription-PCR detection of *Celery mosaic virus* (CeMV) using degenerate potyvirus primers (U341/Poty1) to amplify partial coat protein and 3'-UTR of the viral genome. M, 100 bp DNA marker (Thermo-Fisher Scientific, Waltham, US); C1 – C3, celery sample 1, 2, 3, respectively; +, positive control; -, negative control.

isolate of CeMV (NC_015393.1) (Figure 4). According to ICTV demarcation, the thresholds for different species using nucleotide or amino acid identity values for coat protein is < 76% and < 80%, respectively (Inoue-Nagata *et al*. 2022).

Maximum-Likelihood phylogenetic tree analysis placed three Indonesian CeMV isolates in one separated sub-clade within CeMV group (Figure 5). Two closely related potyvirus species to CeMV, *Carrot virus* Y (CarVY) and *Apium virus* Y (ApVY) were employed as outgroups to confirm the identification of Indonesian CeMV isolates. The data indicates that Indonesian CeMV isolates have variations and place in different group from previously known CeMV isolates in GenBank. Further identity analysis of CeMV strain for Indonesian viral isolates is needed as virus strains may determine the severity of the disease (Koike *et al*. 2008). To date, there have been only 18 CeMV isolates that lodged in GenBank. There was no available sequence from Asian isolates even though the incidence of CeMV infecting carrot has been recorded in Japan by Iwaki and Komuro (1970). The sequences information from this study might gave an insight of the possibility of CeMV variants which present in Asia.

To our knowledge, this is the first report of CeMV infecting celery in Indonesia. Further biological study and disease monitoring for the possibility of CeMV and another closely related potyvirus infection on carrot and other celeriac in the field should be considered as they commonly present in mix infection.

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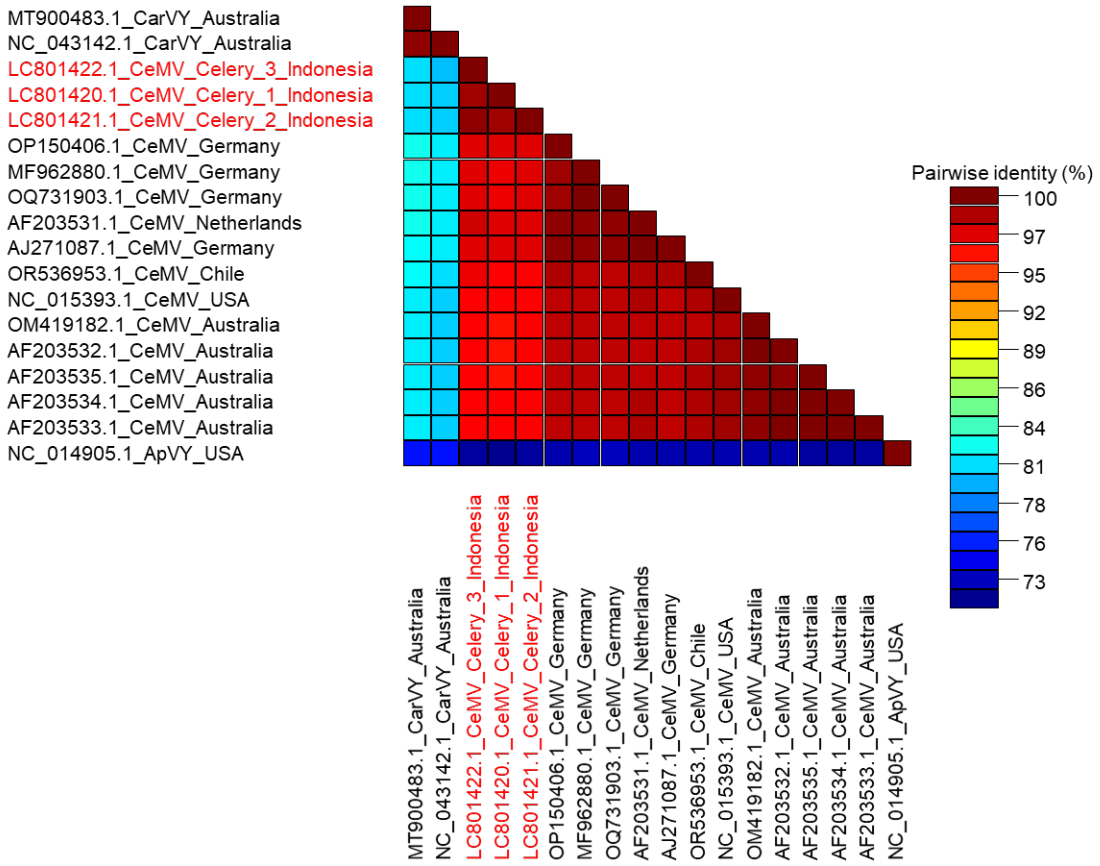

Figure 4 Pairwise identity matrix of *Celery mosaic virus* (CeMV) inferred using SDT matrix in 'two-color' mode. Indonesian CeMV isolates are indicated by red letter. The percentage of pairwise identity is visualized by the color-coded boxes.

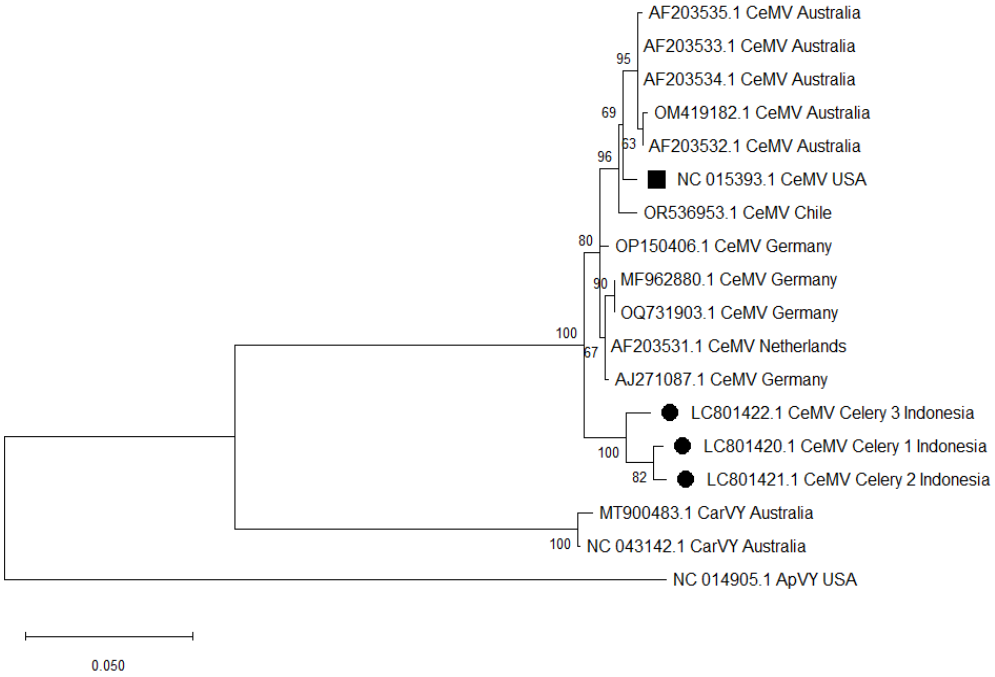

Figure 5 A Maximum-Likelihood phylogenetic tree of *Celery mosaic virus* (CeMV) implemented in MEGA 11 with 1000 bootstrap replicates. The symbol ● indicates Indonesian CeMV isolates and ■ indicates reference isolate of CeMV (NC_0153953.1). Another two closely related potyviruses to CeMV, i.e. *Carrot virus Y* (CarVY) and *Apium virus Y* (ApVY) are used for outgroups.

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