of the years followed by El Niño years, monsoon was expected to be normal to above normal (≥96% of LPA). Considering this analogy, the 2016 monsoon was also expected to be normal to above normal with a probability of 71%. On the other hand, the coupled model forecasts available in February and March 2016 also indicated moderate to weak El Niño during June–July and ENSO neutral conditions likely to get established thereafter (August–September). Considering this aspect, most of the statistical and dynamical models had indicated normal to above-normal rainfall for the 2016 southwest monsoon season. Consistent with this, real-time forecast based on the March ensembles of NCEP CFSv2 coupled model valid for 2016 JJAS also indicated stronger monsoon associated with above normal rainfall over most parts of India (Figure 2 *b*), with a quantitative value of 106% from its LPA. Thus, the real-time forecast from NCEP CFSv2 also indicated above-normal monsoon rainfall during JJAS 2016.

Based on the three indices (*Z*850-index, *R*-index and *U*850-index), the final variance inflated/deflated AISMR forecast for the 2016 southwest monsoon rainfall over India was found to be 96.1% of its LPA, which is very close to the observed AISMR of 97% of its LPA. The *Z*850-index, *R*-index and *U*850-index individually indicated AISMR departure for 2016 as +5.6%, –11.1% and –6.8% respectively. Thus, it is the combination of the three parameters that contributed to correct forecast of 2016. Hence the hybrid forecast developed by Pattanaik and Kumar[13] could capture the variability of AISMR for 2016 and correctly predicted the seasonal rainfall departure, when most of the statistical/dynamical models predicted rainfall to be on the positive side of the normal.

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D. R. PATTANAIK

India Meteorological Department, New Delhi 110 003, India
e-mail: drpattanaik@gmail.com

Molecular characterization of *Cucumber mosaic virus* infecting wild betel (*Piper sarmentosum*)

Piper sarmentosum (Roxb.) belongs to the family Piperaceae, and is known by several names, including lolot pepper, la lot and wild betel. The local Indonesian name for this plant species is karuk. In Indonesia, karuk is used to treat asthma[1], abdominal pain, bone and teeth pain, and fungal infections[2]. In Malaysia, *P. sarmentosum* has been widely studied in terms of its antioxidant properties[3,4] and in Thailand, this plant has been investigated as a potential herbal medicine to treat diabetes[5].

Cucumber mosaic virus (CMV) has a wide host range and is capable of infecting more than 1000 species from 85 plant families[6]. The CMV genome consists of three positive-sense single-stranded RNA fragments along with two subgenomic RNA segments. The coat protein (CP) gene is encoded in the subgenomic RNA4 sequence[7,8]. CMV is classified into two major subgroups (I and II) based on serology, nucleotide homology and phylogenetic analysis[9,10].

During a viral disease survey of Piperaceae plant species conducted in Bogor, West Java, Indonesia in March 2005, mosaic symptoms typical of virus infections were observed on karuk leaves (Figure 1). A compound enzyme-linked immunosorbent assay with CMV antiserum (Agdia Inc., Elkhart, USA) was used for the early detection of viruses on symptomatic samples. The assay results indicated a positive reaction with high absorbance values (i.e. 2.8–3.0). Therefore, identification of CMV was confirmed through molecular characterization of the virus infecting karuk leaves. We studied

Figure 1. Mosaic symptoms on a karuk leaf.

Table 1. Comparison of the nucleotide and encoded amino acid sequences of the *Cucumber mosaic virus* (CMV) coat protein gene isolated in the present study with the corresponding sequences of CMV isolates from other countries

Accession	Country	Host	Sub-group	% Identities of	
				Nucleotide	Amino acid
AB070622	Japan	*Prunus mume*	IB	96.6	97.2
FJ268746	China	*Canna* sp.	IB	94.3	97.2
AJ585521	Australia	*Passiflora edulis*	IB	94.2	96.7
EU329007	Taiwan	*Musa* sp.	IB	94.3	97.2
AJ829778	Spain	*Lycopersicon esculentum*	IB	94.0	97.7
AY545924	India	*Piper nigrum*	IB	93.9	97.7
AJ810264	Thailand	*Cucumis sativus*	IB	93.7	97.2
AY690620	India	*Piper betle*	IB	93.1	98.1
AY690621	India	*Piper longum*	IB	93.1	98.1
EU926956	China	*Piper nigrum*	IB	92.9	97.7
AB069971	Indonesia	*Musa sapientum*	IB	92.6	97.2
AY380533	Brazil	*Chrysanthemum* sp.	IA	93.7	96.7
U43888	Israel	*Musa acuminata*	IA	93.4	96.7
AB448696	Syria	*Solanum tuberosum*	IA	93.4	97.2
DQ295914	India	*Gladiolus* sp.	IA	93.3	96.7
AJ810258	USA	*Cucurbita* sp.	IA	93.1	96.3
AJ242585	China	*Nicotiana tabacum*	II	76.1	81.1
EU191025	Poland	*Delphinium* sp.	II	76.1	81.6
KP034963	Serbia	*Calendula officinalis*	II	75.7	80.7
DQ18289	Poland	*Impatiens balsamina*	II	75.7	80.7
FM992672	Hungary	*Robinia pseudoacacia*	PSV	53.8	48.1

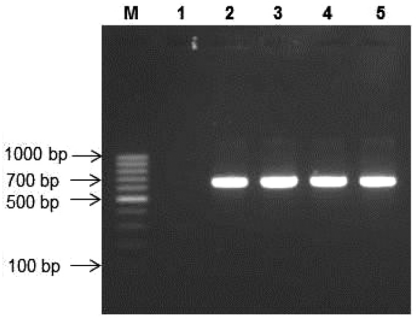

Figure 2. Visualization of the *Cucumber mosaic virus* coat protein gene fragments amplified by a reverse transcription polymerase chain reaction. Amplified fragments were analysed by 1.5% agarose gel electrophoresis. Lane M, 100-bp DNA marker; lane 1, Negative control; lanes 2–5, Infected samples.

the genetic relatedness between the CP gene of the analysed CMV and the corresponding genes of other CMV isolates in GenBank.

Karuk leaves exhibiting mosaic symptoms were collected from Bogor. Total RNA was isolated using the ATP Plant Total RNA Mini Kit (ATP Biotech Inc., Taipei, Taiwan), and then treated with DNase (Thermo Scientific) to eliminate any contaminating genomic DNA. We synthesized cDNA for a subsequent one-step reverse transcription polymerase chain reaction (RT–PCR) using the following primers: CMV-F (5′-ATGGACAAATCTGAATCAAC-3′) and CMV-R (5′-TCAAACTGGGAGCACCC-3′)[11]. The primers were designed to amplify the CMV CP gene (i.e. approximately 650 bp). The one-step RT-PCR mixture (25 µl) contained 1× KAPA *Taq* Extra HotStart ReadyMix PCR buffer (KAPA Biosystems, Missouri, USA), 5 U RNase inhibitor (Thermo Scientific), 0.2 mM dithiothreitol, 10 U Revert Aid reverse transcriptase (Thermo Scientific, Waltham, MA, USA), 0.4 µM CMV-F and CMV-R primers, 2 µl RNA template, and nuclease-free water for a final volume of 25 µl. The PCR programme was as follows: 42°C for 45 min; 40 cycles of 95°C for 30 sec, 50°C for 1 min and 72°C for 1 min; 72°C for 10 min. Nuclease-free water was used instead of RNA template in negative controls. The RT–PCR products were separated on a 1.5% agarose gel, stained with FluoroVue™ Nucleic Acid Gel Stain, and visualized using a gel documentation fire reader V4 system (Uvitec Cambridge, Cambridge, UK). The expected amplicon was sequenced (PT Genetika Science, Indonesia). The resulting sequence was compared with the CP gene sequences of CMV isolates belonging to subgroups I and II that were available in GenBank. Nucleotide and amino acid homologies were analysed using the Bioedit Sequence Alignment Editor, while a phylogenetic tree was constructed using MEGA software (version 6.06).

Gel electrophoresis results indicated the PCR-amplified fragment was of the expected size (i.e. approximately 650 bp) (Figure 2). The sequenced region was submitted to GenBank (accession number LC168754), and included 657 nucleotides that potentially encoded 219 amino acids. The nucleotide sequence of the examined CMV CP gene was most homologous (i.e. 96.6%) to that of a Japanese apricot gene (accession number AB070622). The homology between the CMV CP gene and those of the subgroup I, subgroup II and *Peanut stunt virus* (PSV) (i.e. outgroup) was 92.6–94.3%, 75.7–76.1% and 53.8% respectively. The homology between the amino acid sequence of the CMV CP and those of the subgroup I, subgroup II and PSV proteins was 96.3–98.1%, 80.7–81.6%, and 48.1% respectively (Table 1). The phylogenetic tree constructed based on nucleotide sequences revealed four clusters that clearly separated the CP genes from the CMV isolates into subgroups IA, IB and II, with PSV as an outgroup. The CP gene from the CMV isolate was clustered with the genes from subgroup IB isolates, which were closely related to the Japanese isolates (Figure 3). The results of the homology analysis and the

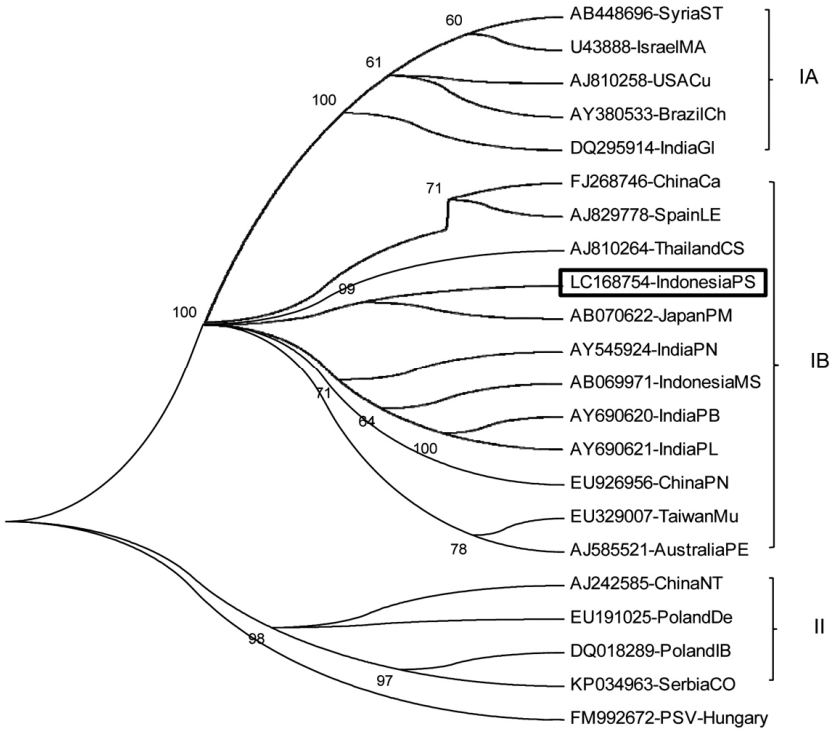

Figure 3. Phylogenetic tree including CP gene from a CMV isolated from karuk plants in Indonesia and CP genes of CMV isolates from several countries. The phylogenetic tree was constructed using the neighbour-joining method of MEGA 5.0 software (1000 bootstrap replicates). The boxed accession indicates the CP gene from the CMV isolated in the present study.

constructed phylogenetic tree indicated that the CMV isolated in the present study belongs to subgroup IB. Additionally, the highly homologous sequences and close phylogenetic relationships between the CMV isolate and Japanese isolates suggest that the viruses share a similar origin, and migrated between countries in infected plant materials.

In Indonesia, a serological analysis indicated that among the Piperaceae plants, CMV was detected only on *Piper nigrum* from Bangka, Lampung, and West Java, and *Piper colubrinum* from West Java[12]. However, the isolated viruses were not molecularly characterized. Other studies involving CMV isolated from *P. nigrum*[11], *Piper betle* L. and *Piper longum* L.[13] included the molecular characterization of the viral isolates with primers that we used in this study. Additionally, a CMV isolated from *Piper methysticum* was studied at the molecular level using another primer pair[14]. Viral diseases on *P. sarmentosum* plants in India were associated with *Piper yellow mottle virus*[15]. To the best of our knowledge, there are no earlier studies describing the molecular characterization of CMV isolated from *P. sarmentosum* plants in Indonesia.

The detection of CMV on *P. sarmentosum* plants in Indonesia suggests that other Piperaceae plant species should be tested for the presence of CMV. Furthermore, future studies should focus on the genetic diversity of CMV, and its ability to infect other hosts. The resulting information will likely be relevant for preventing the spread of CMV to other agriculturally important Piperaceae plants, especially black pepper.

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MIFTAKHUROHMAH*
MAYA MARIANA
DONO WAHYUNO

Indonesian Spice and Medicinal Crops Research Institute,
Indonesian Agency for Agricultural Research and Development (IAARD),
Jalan Tentara Pelajar, No. 3 Bogor, Indonesia, 16111
**For correspondence.*
e-mail: miftah_tia05@yahoo.co.id