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Short Communication
Molecular Cloning and Sequencing of Telosma Mosaic Virus (TeMV) Causing Mosaic Disease on Patchouli

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Abstract

Patchouli belonging to the family Lamiaceae is one of the important aromatic plants that originated from the Philippines. Telosma mosaic virus (TeMV) is one of the dominant types of viruses which attack Indonesian patchouli causing mosaic symptoms on the leaves. Only few research publications were conducted in Indonesia, hence this study was conceptualized in the information on the genome structure of the virus was not enough. The nucleotides sequence encoding the Coat Protein (CP) of TeMV-I (Indonesia isolate) was cloned and determined to complete information about this virus and designed an original primers for sequencing of the genes of NIb and NIa of TeMV-I. A part of genome structure of TeMV was sequenced from patchouli plants showing mosaic symptoms by using universal primer from the genus Potyvirus, then cloned to pGEM-T vector. Sequence analysis was done with program Sequence Scanner and CLC Sequence Viewer. The result showed that newly primer set for NIa and NIb region was design and cloned to TeMV-I. It was determined a sequence of 3805 nucleotides (nt) or a polypeptide chain of 1193 amino acid (aa) at the 3'-terminal region of the genome of TeMV. This contained the 3'-terminal part of the NIa (nts 1-1182), the NIb (nts 1183-2733), the coat protein (nts 2734-3549) and the 3'-UTR (nts 3550-3805). The stop codon (TAA) was followed by a 3'-untranslated region (3'-UTR) of 253 nt. The sequence analysis and development of specific primers for TeMV will be of great help in the detection and protection of this virus.

Key words: Genus potyvirus, plant virus, patchouli, RT-PCR, TeMV

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Patchouli (*Pogostemon cablin*) belonging to the family Lamiaceae is one of the important aromatic and medicinal plants that originated from the Philippines (Sreedevi *et al*., 2009) but also reported a native from Indonesia (Hasegawa *et al*., 1992). The dry leaves of patchouli on steam distillation yield an essential oil called the patchouli oil (Sreedevi *et al*., 2009) which is used as a natural fixative in the aromatic industry (Hasegawa *et al*., 1992) and as main active ingredients of more than 30 common traditional Chinese medicines. Studies on the main active constituents of patchouli oil namely patchouli alcohol and pogostone, showed that they can be used as medicine (Zhang *et al*., 2006). They also possess anti-insecticidal, antifungal and bacteriostatic properties (Zhang *et al*., 2002) and were proved to inhibit neurotoxic activityof β-amyloid peptide (Huang *et al*., 2008) and to act as Reactive Oxygen Species (ROS) scavenger in oxidant-induced cell death of human neuroglioma cells (Kim *et al*., 2010).

The TeMV is one of the dominant types of viruses which attack Indonesian patchouli causing mosaic symptoms on the leaves of patchouli. This virus infection leads to decrease production of patchouli leaves and oil levels in the leaves. Nucleotides of coat protein this virus are submitted to Gene Bank with accession number AB699343.1, AB699342.1, AB699341.1, AB699340.1, AB699339.1, AB699338.1 and AB699131.1 (Noveriza *et al*., 2012a, b). As patchouli plants can only be propagated by stem cuttings, this method of propagation can accelerate the spread of the virus in patchouli production (Hartono and Subandiyah, 2006). Only few studies were conducted in Indonesia, hence this study was conceptualized in the information on the genome structure of the virus was not enough, the nucleotides sequence encoding the Coat Protein (CP) of TeMV-I (Indonesia isolate) was cloned and determined to complete information about this virus and designed an original primers for sequencing of the genes of NIb and NIa of TeMV-I.

MATERIALS AND METHODS

Virus isolates were obtained from patchouli plants collected in Indonesia and directly detected using a set of Poty Universal primers (Table 1). The NIa and NIb specific primers (Table 2) were designed based on the alignment of complete sequence of TeMV from Vietnam (ABI34612.1). Total plant RNAs were extracted from samples using Trizol® Reagent (Invitrogen, USA) as recommended by the manufacturer. First-strand cDNA were synthesized using ReverTra Ace-alpha-® kit (TOYOBO, Japan) according to the manufacturer's instructions and the oligo d(T) primer M4T (5′-GTT TTC CCA GTC ACG AC $(T)_{15}$-3′) as initial primer.

A degenerate primer (Sprimer: 5′-GGX AAY AAY AGY CGX CAZ CC-3′, x = A, G, C or T; Y = T or C; Z = A or G) was designed based on the amino acid sequence motif GNNSGQP according to Chen *et al*. (2001). Second-strand cDNAs of Coat Protein (CP) of TeMV were synthesized by polymerase chain reaction using TaKaRa Ex Taq™ (Takara Biomedicals, Japan) as recommended by the manufacturer. The PCR contained 5 μL template cDNA, 25 pmol of each amplification primer (M4: 5′-GTT TTC CCA GTC ACG AC-3′ and sprimer) (Table 2), 2 μL dNTP mixture, 2.5 μL 10xEx Taq™ Buffer and 0.1 μL TaKaRa Ex Taq™ in a total of 50 μL. Amplifications were for 30 cycles each of 0.5 min at 94°C, 1 min at 47°C and 2 min at 72°C with final extension 10 min at 72°C. The PCR products were examined by electrophoresis in 1.5% agarose gel. Gel extraction of PCR product was done using a Wizard@SV Gel and PCR clean up system following the manufacturer's protocols.

The PCR fragments were cloned into the pGEM-T vector (Promega) following the manufacturer's protocols and then

Table 1: Sequences of primers used to amplify second-strand cDNA of telosma mosaic virus (TeMV)

Primer code	Sequence (5′ to 3′)	Size (bp)
PotyUniversal	Sprimer: GGX AAY AAY AGY CGX CAZ CC	1,700 Chen *et al*. (2001)
	M4: GTT TTC CCA GTC ACG AC	
TeMV-NIb	F: ACC CGA GAG GAT TGC ATG G	1,176
	R: CCT CTA TGT CCT TCA TAT CCC	
TeMV-NIa	F: GGA AGC ACT CAC ACA AAA GG	1,117
	R: CCT TGA ACT CCT CCT TCG G	

Table 2: DNA amplification of patchouli plant samples from Indonesia

		DNA amplification		
Sample code	Origin	PotyUniversal	TeMV-NIb	TeMV-NIa
P3	Cimanggu, West Java, Indonesia	+	+	0
P5	Jambi, Sumatera, Indonesia	+	+	0
P9	Cijeruk, West Java, Indonesia	+	+	0

isolated and purified by Labopass Plasmid Mini Kit (Hokkaido System Science, Japan). Additional primers were designed to internal sequences for sequencing. At least three independent PCR clones of each virus isolates were sequenced with the DNA analysis using Automate Sequencer (Applied Biosystems 3130 xl Genetic Analyzer, Japan). Sequence analysis was done with program Sequence Scanner version 1.0 and CLC Sequence Viewer version 6.6.2 (http://www.clcbio.com/products/latest-improvements-sequence-viewer/).

RESULTS

The DNA fragments of the expected size of 3'terminal of RNA genomes of telosma mosaic virus (TeMV) were amplified from 3 tested Indonesian samples. They were amplified using primers TeMV-NIb and TeMV-NIa (Table 2).

A sequence of 3,805 nucleotides (nt) at the 3'-terminal region of the genome of Indonesian TeMV (TeMV-I) was determined (Fig. 1). This contained the 3'-terminal part of the NIa (nts 1-1182), the NIb (nts 1183-2733), the coat protein (nts 2734-3549) and the 3'-UTR (nts 3550-3805). Analysis showed that the sequence was very similar (85% identical nucleotides) to only one reported data of Telosma mosaic virus (ABI34612.1) (hereafter TeMV-V), genus Potyvirus from *Telosma cordata* in Vietnam.

The aa sequence homologies of the TeMV-I among TeMV-V and the previously reported species in the genus Potyvirus suggest that the relationship among them represent similar (91.1% identical to TeMV-V and 72.9-76.9% identical to bean common mosaic virus (BCMV), CABMV, PWP, SMV and watermelon mosaic virus (WMV). Here, are inserted 6 amino acids (AMAAGL) in sequence number 352.

DISCUSSION

Nucleotides of coat protein telosma mosaic virus (TeMV) are submitted to GenBank with accession number AB699343.1, AB699342.1, AB699341.1, AB699340.1,

```
       10        20        30        40        50        60
GGAAGCACTCACACAAAAGGAATGGGGCGAAAATCTCGCAACTTTTTCCATATGTATGGG
  G  S  T  H  T  K  G  M  G  R  K  S  R  N  F  F  H  M  Y  G
       70        80        90       100       110       120
GTTGAGCCAGAAAATTATACAACAATTAGGTTTGTGGATCCATTAACTGGATACACACTT
  V  E  P  E  N  Y  T  T  I  R  F  V  D  P  L  T  G  Y  T  L
      130       140       150       160       170       180
GATGAAAACCCCAGAGTGGACATCAGAATTGTTCAGGAGGAGATGGCTGAAATACGCGAT
  D  E  N  P  R  V  D  I  R  I  V  Q  E  E  M  A  E  I  R  D
      190       200       210       220       230       240
GAGATGATAGCTGAAGGAGAGTTGGACAAGCAAGCTATATACTACAAGCCAGGCATCGAA
  E  M  I  A  E  G  E  L  D  K  Q  A  I  Y  Y  K  P  G  I  E
      250       260       270       280       290       300
GCGTATTTATTTGGAAAAGGTACAGAAGAAGTGATAAAAGTTGATCTCACACCACACAAT
  A  Y  L  F  G  K  G  T  E  E  V  I  K  V  D  L  T  P  H  N
      310       320       330       340       350       360
TCAAAGGTTGTCTGCAAGAACAATGCAACAATCGCAGGGCATCCTGAAAGAGATGGTGAG
  S  K  V  V  C  K  N  N  A  T  I  A  G  H  P  E  R  D  G  E
      370       380       390       400       410       420
CTAAGACAAACTGGCATGCCACAAACACTCACAAAGAAGGAACTACCAGCATACAATGAG
  L  R  Q  T  G  M  P  Q  T  L  T  K  K  E  L  P  A  Y  N  E
      430       440       450       460       470       480
CGAGTGAAAGCGGAAAGCAAATCTGTGTATAAAGGCTTGCGTGACTACAGCGGCATTTCA
  R  V  K  A  E  S  K  S  V  Y  K  G  L  R  D  Y  S  G  I  S
      490       500       510       520       530       540
ACTCTTATTTGTCAACTTATAAATGCCTCTGATGGGCATAGTGAGTCAATTTTTGGGATT
  T  L  I  C  Q  L  I  N  A  S  D  G  H  S  E  S  I  F  G  I
      550       560       570       580       590       600
GGTTATGGTTCCTATGTAATTACAAATGGGCACTTGTTCAAGAGGAATAATGGAACCTTA
  G  Y  G  S  Y  V  I  T  N  G  H  L  F  K  R  N  N  G  T  L
      610       620       630       640       650       660
ACCATAAAATCATGGCATGGGGAGTTTGTCATCCACAATACCACTCAGATCAAGATTCAT
  T  I  K  S  W  H  G  E  F  V  I  H  N  T  T  Q  I  K  I  H
      670       680       690       700       710       720
TTCATTGAGGGTAAGGATGCAATTTTGATAAGGATGCCAAAGGATTTTCCACCATTTGCG
  F  I  E  G  K  D  A  I  L  I  R  M  P  K  D  F  P  P  F  A
      730       740       750       760       770       780
AAGAGAAACTTCTTCAGGAGTCCAAAGAAAGAGGAAAGAGTGTGCATGGTTGGAACGAAC
  K  R  N  F  F  R  S  P  K  K  E  E  R  V  C  M  V  G  T  N
      790       800       810       820       830       840
TTCCAAGAGAAGAGTCTTAGAGCCACAGTCTCAGAATCGTCAATAATAGTTCCTGAAGGG
  F  Q  E  K  S  L  R  A  T  V  S  E  S  S  I  I  V  P  E  G
      850       860       870       880       890       900
GTTGGTTCTTTTTGGATGCATTGGATTTCAACACAGGATGGATTTTGTGGACTACCATTG
  V  G  S  F  W  M  H  W  I  S  T  Q  D  G  F  C  G  L  P  L
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Fig. 1: Continue

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      910       920       930       940       950       960
GTCTCAGTAACTGATGGTTTCATTGTTGGAATACATGGTTTGACATCAAATCAATCAAGT
  V  S  V  T  D  G  F  I  V  G  I  H  G  L  T  S  N  Q  S  S
      970       980       990      1000      1010      1020
AAGAATTTCTTTGTTCCATTTACTGAGAACTTTGTCACTGATTATCTTGAAAAGGCAGAT
  K  N  F  F  V  P  F  T  E  N  F  V  T  D  Y  L  E  K  A  D
     1030      1040      1050      1060      1070      1080
GAACTATCCTGGAACAAGAATTGGTTTTGGCAGGCCATGGCCGCGGGATTACCCGAGAGG
  E  L  S  W  N  K  N  W  F  W  Q  A  M  A  A  G  L  P  E  R
     1090      1100      1110      1120      1130      1140
ATTGCATGGGGCTCACTGAATCTTGTTGATGATCAGCCGAAGGAGGAGTTCAAGGTCTCG
  I  A  W  G  S  L  N  L  V  D  D  Q  P  K  E  E  F  K  V  S
     1150      1160      1170      1180      1190      1200
AAGCTGATTTCTGATTTGTTTAGCGACACAGTGACAACACAAAGCAAGCAGGATAGGTGG
  K  L  I  S  D  L  F  S  D  T  V  T  T  Q  S  K  Q  D  R  W
     1210      1220      1230      1240      1250      1260
GTTCTGGATGCTGTAGAGGGAAATCTCAGAGCATGTGGGAAGGCTGATAGCGCTCTTGTT
  V  L  D  A  V  E  G  N  L  R  A  C  G  K  A  D  S  A  L  V
     1270      1280      1290      1300      1310      1320
ACAAAGCATGTTGTAAAGGGCAAATGCCCACATTTTGAGCAGTACTTGCATCAAGTTCCA
  T  K  H  V  V  K  G  K  C  P  H  F  E  Q  Y  L  H  Q  V  P
     1330      1340      1350      1360      1370      1380
GAGGCAGCGGCATTCTTTAAGCCCCTCATGGGATCATACCAACCAAGCAAATTGAACAAA
  E  A  A  A  F  F  K  P  L  M  G  S  Y  Q  P  S  K  L  N  K
     1390      1400      1410      1420      1430      1440
GAAGCTTTTAAGAAAGACTTCTTTAAGTACAACAAGCCAGTGGTTTTAAATGAAGTTCAC
  E  A  F  K  K  D  F  F  K  Y  N  K  P  V  V  L  N  E  V  H
     1450      1460      1470      1480      1490      1500
TTTGAATCATTTGAAAAAGCGGTTGATGGGGTCAAAATTATGATGATGGAAACCGATTTC
  F  E  S  F  E  K  A  V  D  G  V  K  I  M  M  M  E  T  D  F
     1510      1520      1530      1540      1550      1560
CATGAGTGCGTATTTGTGACTGATCCTGATGAAATCTTTGATTCATTGAATATGAAAGCA
  H  E  C  V  F  V  T  D  P  D  E  I  F  D  S  L  N  M  K  A
     1570      1580      1590      1600      1610      1620
GCAGTTGGAGCACAGTACAAAGGAAAGAAGTCTGAGTATTTCCTTGAAATGGATGAGTTT
  A  V  G  A  Q  Y  K  G  K  K  S  E  Y  F  L  E  M  D  E  F
     1630      1640      1650      1660      1670      1680
GATAAGGAGCGCTTACTATTCTTGAGTTGTGAGCGGTTGTTCTATGGAAAGAAGGGTTTG
  D  K  E  R  L  L  F  L  S  C  E  R  L  F  Y  G  K  K  G  L
     1690      1700      1710      1720      1730      1740
TGGAATGGCTCACTTAAAGCTGAGTTAAGACCACTTGAGAAAGTTCAGGCTAATAAGACA
  W  N  G  S  L  K  A  E  L  R  P  L  E  K  V  Q  A  N  K  T
     1750      1760      1770      1780      1790      1800
AGAACATTCACAGCAGCACCCATTGATACACTTCTCGGTGCAAAGGTTTGTGTGGATGAT
  R  T  F  T  A  A  P  I  D  T  L  L  G  A  K  V  C  V  D  D
     1810      1820      1830      1840      1850      1860
TTCAACAATCAATTCTATAGTCTGAATCTTAAGTGTCCGTGGACAGTTGGTATGACAAAA
  F  N  N  Q  F  Y  S  L  N  L  K  C  P  W  T  V  G  M  T  K
     1870      1880      1890      1900      1910      1920
TTTTATGGTGGTTGGGACAAGCTTATGAGGAGTTTACCGGATGACTGGCTATATTGTCAT
  F  Y  G  G  W  D  K  L  M  R  S  L  P  D  D  W  L  Y  C  H
     1930      1940      1950      1960      1970      1980
GCTGATGGATCTCAGTTTGACAGTTCGTTAACTCCTTTGTTATTAAATGCAGTGCTAGAC
  A  D  G  S  Q  F  D  S  S  L  T  P  L  L  L  N  A  V  L  D
     1990      2000      2010      2020      2030      2040
ATCAGATTGTTTTTCATGGAGAATTGGTGGGTTGGGCAAGAAATGCTCGCAAATTTGTAC
  I  R  L  F  F  M  E  N  W  W  V  G  Q  E  M  L  A  N  L  Y
     2050      2060      2070      2080      2090      2100
GCTGAGATCGTGTACACACCAATATTAGCACCTGACGGCACTATATTCAAGAAGTTCAGG
  A  E  I  V  Y  T  P  I  L  A  P  D  G  T  I  F  K  K  F  R
     2110      2120      2130      2140      2150      2160
GGGAATAATAGTGGGCAGCCATCCACAGTTGTGGACAACACTTTGATGGTTGTAATTTCT
  G  N  N  S  G  Q  P  S  T  V  V  D  N  T  L  M  V  V  I  S
     2170      2180      2190      2200      2210      2220
GTGTATTACTCATGCTTCAAGCAGGGATGGGATATGAAGGACATAGAGGAAAGACTTGTC
  V  Y  Y  S  C  F  K  Q  G  W  D  M  K  D  I  E  E  R  L  V
     2230      2240      2250      2260      2270      2280
TTTTTCGCTAATGGTGATGACATCATTTTAGCAGTCCAAGAAAGGGATGAGTGGCTTTAT
  F  F  A  N  G  D  D  I  I  L  A  V  Q  E  R  D  E  W  L  Y
     2290      2300      2310      2320      2330      2340
GACAAACTTGGGTCATCTTTCGCAGAGCTTGGGTTGAATTATGATTTCAGTGAAAGAACA
  D  K  L  G  S  S  F  A  E  L  G  L  N  Y  D  F  S  E  R  T
     2350      2360      2370      2380      2390      2400
AAGAAGCGAGAAGAGTTGTGGTTTATGTCACATCAGGCTAAAGAAGTTGATGGGTTGTAC
  K  K  R  E  E  L  W  F  M  S  H  Q  A  K  E  V  D  G  L  Y
     2410      2420      2430      2440      2450      2460
```

Fig. 1: Continue

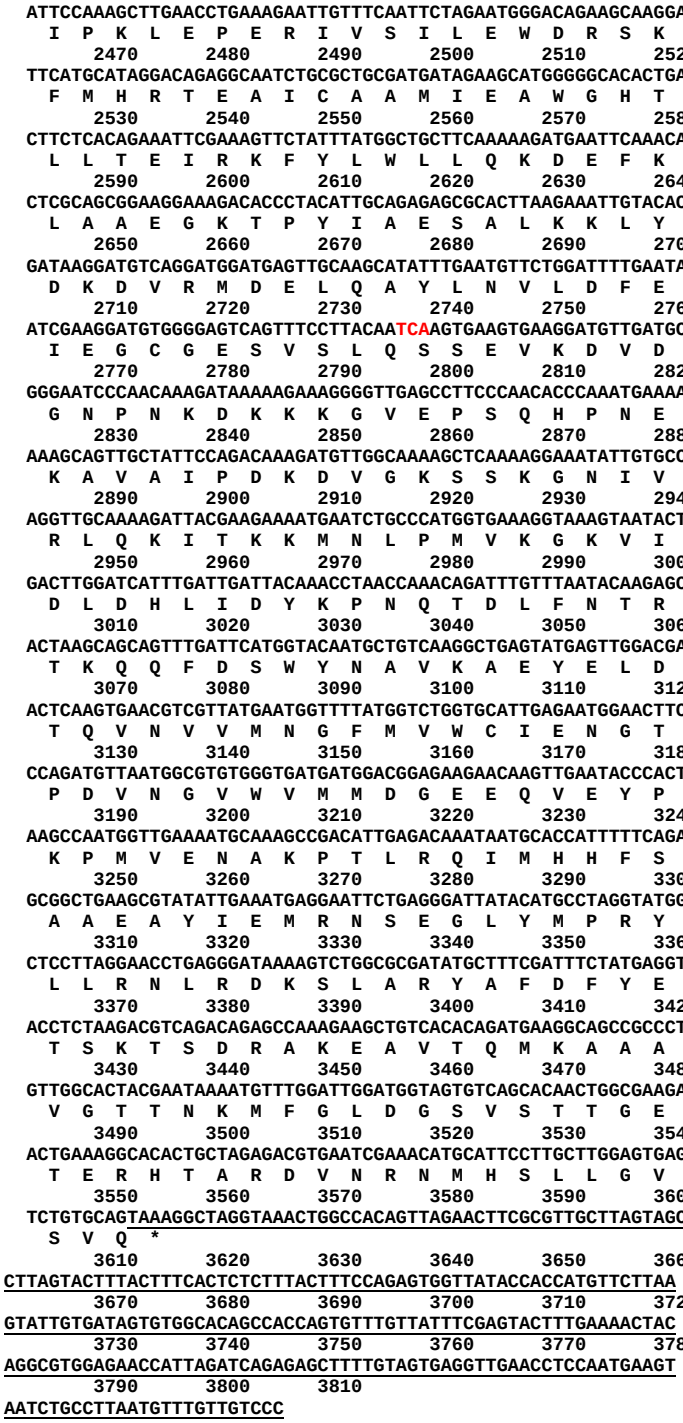

Fig. 1: Nucleotides sequence of the NIa (1182 nt), NIb (1550 nt), CP (815 nt) and 3'-UTR (253 nt) encoding gene of TeMV-I and deduced aa sequence. Thick underline and asterisk indicate 3'-UTR region and stop codon, respectively

AB699339.1, AB699338.1 and AB699131.1 (Noveriza *et al*., 2012a, b) but that nucleotides of coat protein were not complete yet. So need to find the full length coat protein of those sequences for complete information of that species in Indonesia. This study is conducted using cloning technique to find genome structure of TeMV from patchouli in Indonesia.

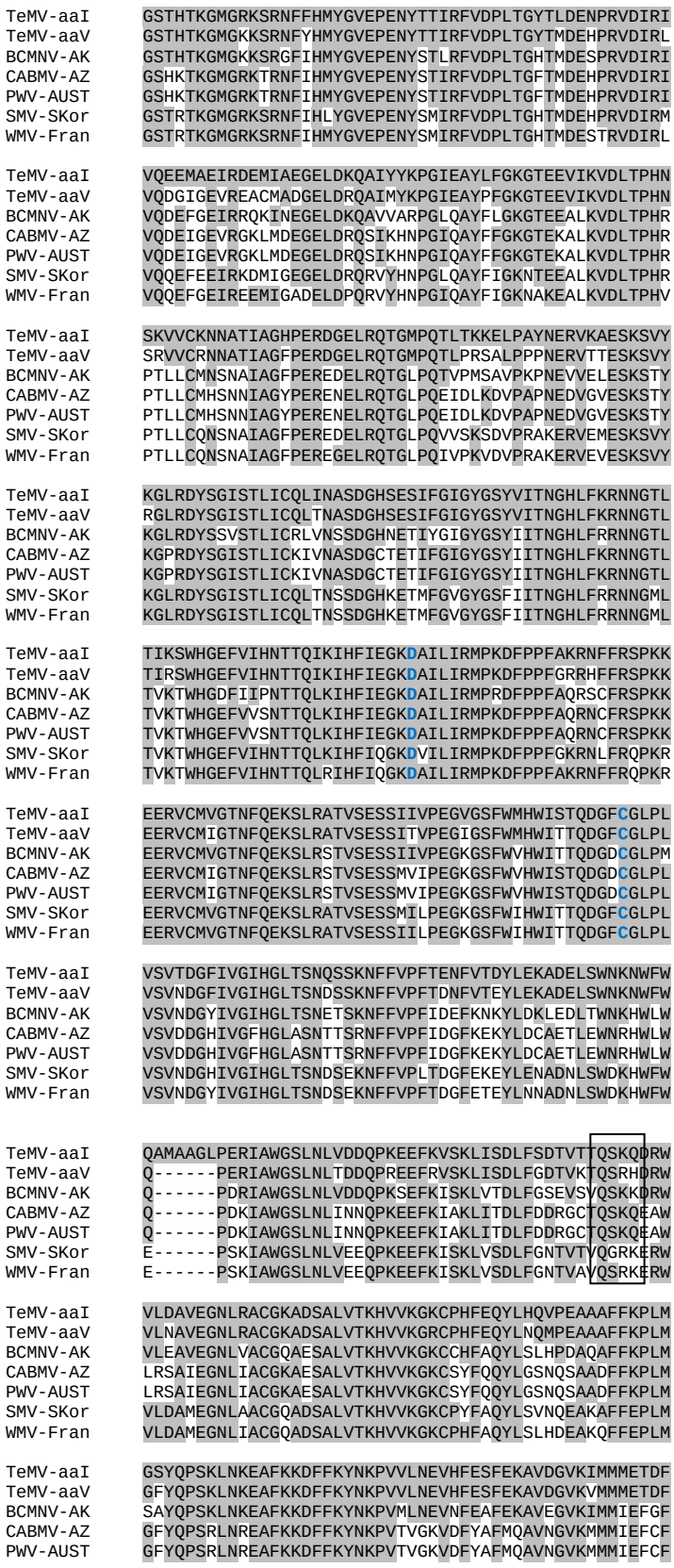

Fig. 2: Continue

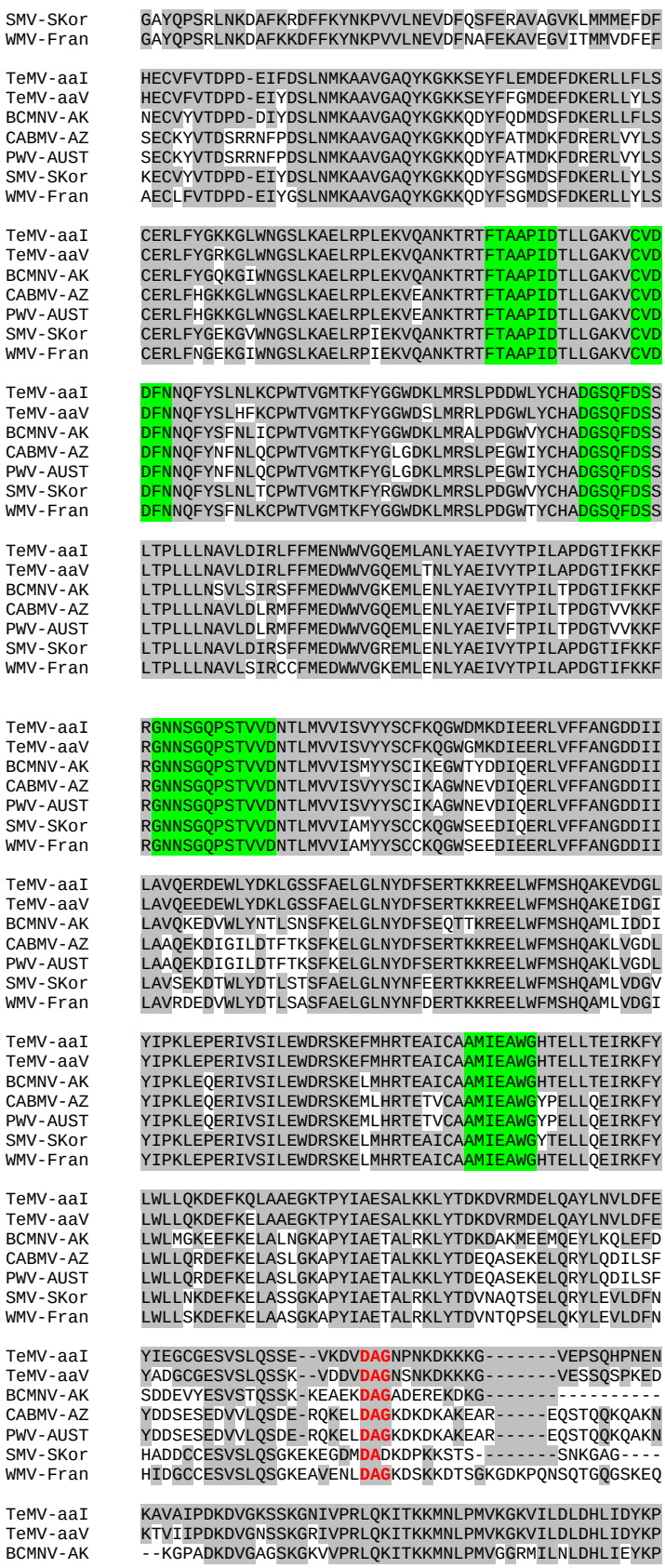

Fig. 2: Continue

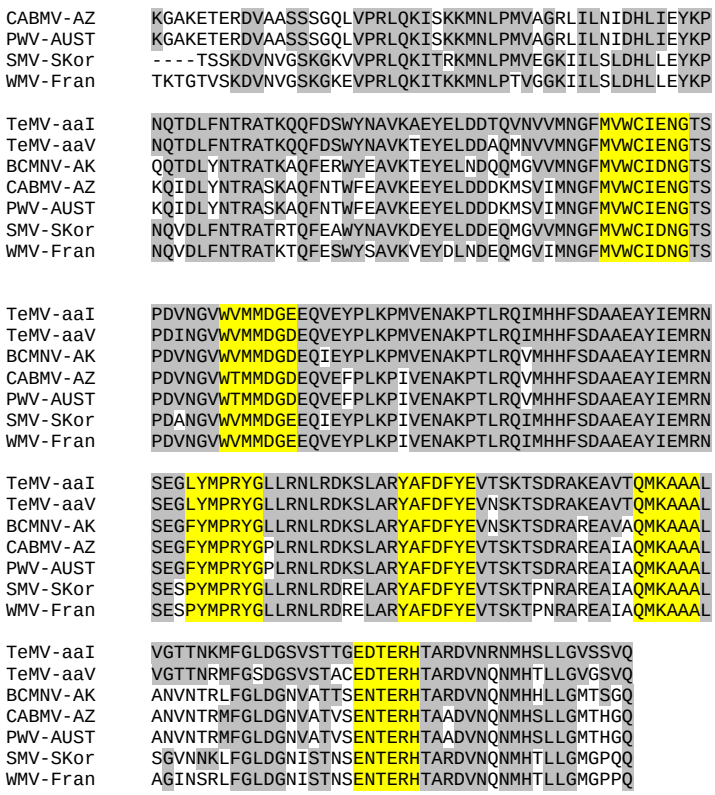

Fig. 2: Comparison of the aa sequence of TeMV-I poplyprotein (1183 aa; NIa, NIb and CP) with those of other potyvirus. Abbreviations for the viruses and sources of sequences are as described in the references 3082 aa, TeMV-aaV, Ha *et al.* (2008); 3169 aa, BCMNV-AK, Larsen *et al.* (2005); 3053 aa, CABMV-AZ, Mlotshwa *et al.* (2002); 3086 aa, PWV-AUST, Wylie and Jones (2011); 3067 aa, SMV-SKor, Seo *et al.* (2009) and 3217 aa, WMV-Fran, Desbiez and Lecoq (2004). Identical aa residues with TeMV-aaI are indicated by a black highlight and different aa, when compared to TeMV-aaI, are denoted. Hyphens are used to indicate deletions introduced for best alignment. Yellow and green highlight are used to indicate a conserve sites in CP and NIb Potyvirus genome, respectively

Nucleotide sequence of 3′ terminal of RNA genome obtained and compared with sequences available in the GenBank indicated that this sequence belongs to the genus Potyvirus and species of telosma mosaic virus (TeMV), because of having 85% nucleotide identity over the whole genome of TeMV from Vietnam (Ha *et al.*, 2008). This virus also reported in Thailand, it cause mosaic and fruit woodiness disease on passion fruit (Chiemsombat *et al.*, 2014). According to King *et al.* (2011) that the current species demarcation criterion is either 85% nucleotide identity over the whole genome or 85% amino acid identity over the full length coat protein.

For further discussion, 3′ terminus of RNA genome of TeMVI (length 3,805 nts or 1,183 aa) was sequenced. These sequences consist of a part of NIa gene (394 aa), NIb gene (517 aa), CP gene (272 aa) and 3′ UTR (353 nts). According to Zheng *et al.* (2008), there were 17 conserved sites in genome of Potyvirus while four of them were identified by earlier worker. Of the previously identified sites, NIIENGV found by Nicolas and Laliberte (1991) ("ATNIIENG") ranked third, QMKAAA found by Langeveld *et al.* (1991) and Pappu *et al.* (1993) ranked 5th, GNNSGQ site identified by Gibbs and Mackenzie (1997) (PV2) in 1997 ranked 7th and the MVWCIE/DNG site identified by Langeveld *et al.* (1991) and Pappu *et al.* (1993) ("WCIEN") ranked 12th. The amino acid sequences of CP gene of TeMV-I and TeMV-V have 6 conserved sites, EN/DTERH, QMKAAA, YAFDFYE, MVWCIE/DNG, WV/TMMDGD/E/N and P/R/AYMPRYG (Fig. 2), which are generally owned by the Potyvirus group. On NIb gene of TeMV-I and TeMV-V genome, there were 6 conserved sites, CVDDFN, A/SMI/VES/AWG, GNNSGQ, GQPSTVVD, FTAAPL/ID/E and DGSQ/RFDA (Fig. 2).

When the amino acid sequence of the 3 terminal of RNA genome of TeMV was compared with 6 species in the genus

Potyvirus, it was proved that there was a mutation in NIa gene (Nia-protease) of TeMV-I at QAMAAGLP (1115-1133 nt, Fig. 1). There were six additional amino acids at the end of NIa gene (AMAAGL, Fig. 2). The NIa-Pro is a trypsin-like cysteine proteinase responsible for the remaining of seven cleavage events in the C-terminal which was two thirds of the polyprotein (Guo *et al*., 2011). So NIa-protease gene (Nia-pro) plays an important role in multiplication of the virus. If the gene is isolated, it can be used in resistance to viral gene. Though TeMV is relatively a new reported virus, the occurrence of this virus in distinct countries like Vietnam, Indonesia and Thailand. In this regards, the sequence analysis and development of specific primers for TeMV in this study will be of great help in the detection and protection of this virus.

CONCLUSION

Telosma mosaic virus (TeMV) Indonesian isolate was clone and sequence using newly primer set for NIa and Nib region. It was determined a sequence of 3805 nucleotides (nt) or a polypeptide chain of 1193 amino acid (aa) at the 3′-terminal region of the genome of TeMV. This contained the 3′-terminal part of the NIa (nts 1-1182), the NIb (nts 1183-2733), the coat protein (nts 2734-3549) and the 3′-UTR (nts 3550-3805). The stop codon (TAA) was followed by a 3′-untranslated region (3′-UTR) of 253 nucleotides. The sequence analysis and development of specific primers for TeMV in this study will be of great help in the detection and protection of this virus.

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