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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 Nlb and Nla 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 Nla and Nlb 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 Nla (nts 1-1182), the Nlb (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 activity of 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 GNNSSGQP 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 μ L template cDNA, 25 pmol of each amplification primer (M4: 5'-GTT TTC CCA GTC ACG AC-3' and sprimer) (Table 2), 2 μ L dNTP mixture, 2.5 μ L 10x Ex Taq[™] Buffer and 0.1 μ L TaKaRa Ex Taq[™] in a total of 50 μ L. 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 M4: GTT TTC CCA GTC ACG AC	1,700 Chen <i>et al.</i> (2001)
TeMV-NIb	F: ACC CGA GAG GAT TGC ATG G R: CCT CTA TGT CCT TCA TAT CCC	1,176
TeMV-NIa	F: GGA AGC ACT CAC ACA AAA GG R: CCT TGA ACT CCT CCT TCG G	1,117

Table 2: DNA amplification of patchouli plant samples from Indonesia

Sample code	Origin	DNA amplification		
		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 3130xl 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-N1b and TeMV-N1a (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

N1a (nts 1-1182), the N1b (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,

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10      20      30      40      50      60
GGAAGCACTCACACAAAGGAATGGGGCGAAAATCTCGCAACTTTTCCATATGTATGGG
  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
GTTGAGCCAGAAAATTATACAACAATTAGGTTTGTGGATCCATTAAGTACACACTT
  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
GATGAAAACCCAGAGTGGACATCAGAATTGTTCCAGGAGAGATGGCTGAAATACGCGAT
  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
CGGTATTTATTGGAAAAGGTACAGAAGAAGTGATAAAAAGTTGATCTCACACCACAAAT
  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
TCAAAGGTTTCTGCAAGAACAATGCAACAATCGCAGGGCATCCTGAAAAGAGATGGTGAG
  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
CGAGTAAAAGCGGAAAGCAAATCTGTGTATAAAGGCTTGCGTGACTACAGCGGCATTCA
  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
ACTCTTATTGTCAACTTATAAATGCCTCTGATGGGCATAGTGAGTCAATTTTGGGATT
  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
GGTTATGGTTTCCTATGTAATTACAAATGGGCACTTGTTCAGAGGAATAATGGAACCTTA
  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
ACCATAAAATCATGGCATGGGGAGTTTGTTCATCCACAATACCACCTCAGATCAAGATTCAT
  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
TTCATTGAGGGTAAGGATGCAATTTTGATAAGGATGCCAAAGGATTTCCACCATTGCG
  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
AAGAGAACTTCTTCAGGAGTCCAAAGAAAGAGGAAAGAGTGTGCATGGTTGGAACGAAC
  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
TTCCAAGAGAAGAGTCTTAGAGCCACAGTCTCAGAATCGTCAATAATAGTTCTCTGAAGGG
  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
GTTGGTTCTTTTGGATGCATTGGATTTCAACACAGGATGGATTTTGTGGACTACCATTG
  V G S F W M H W I S T Q D G F C G L P L

```

Fig. 1: Continue

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
 AAGAATTTCTTTGTTCCATTTACTGAGAAGCTTTGTCACTGATTATCTTGAAAAGGCAGAT
 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
 GAACTATCTTGGAAACAAGAAATGGTTTTGGCAGGCCATGGCCCGGGATTACCCGAGAGG
 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
 ATGTCATGGGGCTCACTGAATCTTGTGTGATGATCAGCCGAAGGAGGAGTTCAAGGTCTCG
 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
 AAGCTGATTTCTGATTTGTTAGCGACACAGTGACAACACAAAGCAAGCAGGATAGGTGG
 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
 GTTCTGGATGCTGTAGAGGAAATCTCAGAGCATGTGGGAAGGCTGATAGCGCTCTTGT
 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
 ACAAAGCATGTTGTAAGGGCAAATGCCACATTTTGGAGCAGTACTTGCAATCAAGTTCCA
 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
 GAGCAGCGGCATTCTTTAAGCCCTCATGGGATCATACCAACCAAGCAAATGAACAAA
 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
 GAAGCTTTTAAGAAAGACTTCTTTAAGTACAACAAGCCAGTGGTTTTAAATGAAGTTTAC
 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
 TTTGAATCATTGAAAAGCGGTTGATGGGGTCAAAATTATGATGATGGAAAACCGATTTTC
 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
 CATGAGTGCATTTTGTGACTGATCCTGATGAAATCTTTGATTCATTGAATATGAAAGCA
 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
 GCAGTTGGAGCACAGTACAAGGAAGAAGCTGAGTATTTCTTGAATGGATGAGTTT
 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
 GATAAGGAGCGCTTACTATTCTTGTGAGTTGTGAGCGGTTGTTCTATGAAAAGAGGGTTTG
 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
 TGGAAATGGCTCACTTAAAGCTGAGTTAAGACCCTTGAGAAAGTTCAGGCTAATAAGACA
 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
 AGAACATTCACAGCAGCACCCATTGATACACTTCTCGGTGCAAGGTTTGTGTGGATGAT
 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
 TTCAACAATCAATCTATAGTCTGAATCTTAAGTGTCCGTGGACAGTTGGTATGACAAAA
 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
 TTTTATGGTGGTTGGGACAAGCTTATGAGGAGTTTACCGGATGACTGGCTATATTGTTCAT
 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
 GCTGATGGATCTCAGTTTGACAGTTGTAACCTTTGTTATTAATGCAGTGTCTAGAC
 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
 ATCAGATGTTTTTTCATGGAGAATGGTGGGTTGGGCAAGAAATGCTCGCAAATTTGTAC
 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
 GCTGAGATCGTGTACACCAATATTAGCACCTGACGGCACTATATTCAAGAAGTTCAGG
 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
 GGGAAATAAGTGGGAGCCATCCACAGTTGTGGACAACACTTTGATGGTTGTAATTTCT
 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
 GTGTATTACTCATGCTTCAAGCAGGATGGGATATGAAGGACATAGAGGAAAGACTTGT
 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
 TTTTCGCTAATGGTGTGATGACATCATTTTAGCAGTCCAAGAAAGGATGAGTGGCTTTAT
 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
 GACAACTTGGGTCATCTTTCGAGAGCTTGGGTTGAATTATGATTTCAAGTGAAGAACA
 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
 AAGAAGCGAGAAGAGTTGGTTTATGTACATCAGGCTAAGAAGTTGATGGGTTGTAC
 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

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ATCCAAAGCTTGAACCTGAAAGAATTGTTCAATTCTAGAATGGGACAGAAGCAAGGAA
I P K L E P E R I V S I L E W D R S K E
2470      2480      2490      2500      2510      2520
TTCATGCATAGGACAGAGGCAATCTGCGCTGCGATGATAGAAGCATGGGGGCACACTGAG
F M H R T E A I C A A M I E A W G H T E
2530      2540      2550      2560      2570      2580
CTTCTCACAGAAATCGAAAAGTTCTATTTATGGCTGCTTCAAAAAGATGAATTCAAACAG
L L T E I R K F Y L W L L Q K D E F K Q
2590      2600      2610      2620      2630      2640
CTCGCAGCGAAGGAAAGACACCCCTACATTGCAGAGAGCGCACTTAAGAAATGTACACA
L A A E G K T P Y I A E S A L K K L Y T
2650      2660      2670      2680      2690      2700
GATAAGGATGTCAGGATGGATGAGTTGCAAGCATATTTGAATGTTCTGGATTTGAATAT
D K D V R M D E L Q A Y L N V L D F E Y
2710      2720      2730      2740      2750      2760
ATCGAAGGATGTGGGAGTCAGTTTCTTACAACTCAAGTGAAGTGAAGGATGTTGATGCT
I E G C G E S V S L Q S S E V K D V D A
2770      2780      2790      2800      2810      2820
GGGAATCCCAACAAAGATAAAAAGAAAGGGTTGAGCCTTCCCAACACCCAAATGAAAAAC
G N P N K D K K K G V E P S Q H P N E N
2830      2840      2850      2860      2870      2880
AAAGCAGTTGCTATTCCAGACAAAGATGTTGGCAAAGCTCAAAAAGAAATTTGTGCCA
K A V A I P D K D V G K S S K G N I V P
2890      2900      2910      2920      2930      2940
AGGTTGCAAAAGATTACGAAGAAAATGAATCTGCCCATGGTGAAGGTAAAGTAATACTT
R L Q Q I T K K M N L P M V K G K V I L
2950      2960      2970      2980      2990      3000
GACTTGGATCATTGATTGATTACAAACCTAACCAACAGATTTGTTTAATACAAGAGCA
D L D H L I D Y K P N Q T D L F N T R A
3010      3020      3030      3040      3050      3060
ACTAAGCAGCAGTTTGATTTCATGGTACAATGCTGTCAAGGCTGAGTATGAGTTGGACGAC
T K Q Q F D S W Y N A V K A E Y E L D D
3070      3080      3090      3100      3110      3120
ACTCAAGTGAACGTCGTTATGAATGGTTTTATGGTCTGGTGCATTGAGAATGGAACCTCA
T Q V N V V M N G F M V W C I E N G T S
3130      3140      3150      3160      3170      3180
CCAGATGTTAATGGCGTGTGGGTGATGATGGACGGAGAAGAACAAGTTGAATACCCACTG
P D V N G V W V M M D G E E Q V E Y P L
3190      3200      3210      3220      3230      3240
AAGCCAATGGTTGAAAATGCAAAGCCGACATTGAGACAAAATAATGCACCATTTTTCAGAT
K P M V E N A K P T L R Q I M H H F S D
3250      3260      3270      3280      3290      3300
GCGGCTGAAGCGTATATTGAAATGAGGAATCTGAGGGATTATACATGCCCTAGGTATGGT
A A E A Y I E M R N S E G L Y M P R Y G
3310      3320      3330      3340      3350      3360
CTCCTTAGGAACCTGAGGGATAAAAAGTCTGGCGGATATGCTTTCGATTTCTATGAGGTA
L L R N L R D K S L A R Y A F D F Y E V
3370      3380      3390      3400      3410      3420
ACCTCTAAGACGTCAGACAGAGCCAAGAAGCTGTACACAGATGAAGGCAGCCGCCCTC
T S K T S D R A K E A V T Q M K A A A L
3430      3440      3450      3460      3470      3480
GTTGGCACTACGAATAAAAATGTTTGGATTGGATGGTAGTGTGCAGCACAACTGGCGAAGAT
V G T T N K M F G L D G S V S T T G E D
3490      3500      3510      3520      3530      3540
ACTGAAAGGCACACTGCTAGAGACGTGAATCGAAACATGCATTCTTGGGAGTGAGC
T E R H T A R D V N R N M H S L L G V S
3550      3560      3570      3580      3590      3600
TCTGTGCAGTAAAGGCTAGGTAAACTGGCCACAGTTAGAACTTCGCGTTGCTTAGTAGCC
S V Q *
3610      3620      3630      3640      3650      3660
CTTAGTACTTTACTTTCACTCTTTACTTTCCAGAGTGGTTATACCACCATGTTCTTAA
3670      3680      3690      3700      3710      3720
GTATTGTGATAGTGTGGCACAGCCACCAGTGTGTTTATTTTCGAGTACTTTGAAAACACTAC
3730      3740      3750      3760      3770      3780
AGGCGTGGAGAACCATTAGATCAGAGAGCTTTTGTAGTGAGGTTGAACCTCCAATGAAGT
3790      3800      3810
AATCTGCCTTAATGTTTGTGTCCT

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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.

TeMV-aaI GSTHTKGMGRKSRNFHMYGVEPENYTTIRFVDPLTGYTLDENPRVDIRI
 TeMV-aaV GSTHTKGMGKSRNFYHMYGVEPENYTTIRFVDPLTGYTMDEHPRVDIRL
 BCMNV-AK GSTHTKGMGKSRGFIHMYGVEPENYSTLRFVDPLTGHTMDESPRVDIRI
 CABMV-AZ GSHKTKGMGRKTRNFIHMYGVEPENYSTIRFVDPLTGFTMDEHPRVDIRI
 PWV-AUST GSHKTKGMGRKTRNFIHMYGVEPENYSTIRFVDPLTGFTMDEHPRVDIRI
 SMV-SKor GSTRTKGMGRKSRNFIHLYGVEPENYSMIRFVDPLTGHTMDEHPRVDIRM
 WMV-Fran GSTRTKGMGRKSRNFIHMYGVEPENYSMIRFVDPLTGHTMDESTRVDIRL

TeMV-aaI VQEEAEIRDEMI AEGELDKQAIYYKPGIEAYLFGKGTTEEVIKVDLTPHN
 TeMV-aaV VQDGI GEVREACMADGELDRQAIMYKPGIEAYPFGKGTTEEVIKVDLTPHN
 BCMNV-AK VQDEFGEIRRQKINEGELDKQAVVARPGLQAYFLGKGTTEEALKVDLTPHR
 CABMV-AZ VQDEIGEVRGKLMDEGELDRQSIKHNPQIAYFFGKGTTEKALKVDLTPHR
 PWV-AUST VQDEIGEVRGKLMDEGELDRQSIKHNPQIAYFFGKGTTEKALKVDLTPHR
 SMV-SKor VQQEFEBIRKDMIGEELDRQRVYHNPGLQAYFIGKNTEEALKVDLTPHR
 WMV-Fran VQQEFGEIREEMI GADELDPQRVYHNPQIAYFIGKNAEALKVDLTPHV

TeMV-aaI SKVVCNNATIAGHPERDDELRTGMPQTLTKKELPAYNERVKAESKSVY
 TeMV-aaV SRVVCRRNATIAGFPERDDELRTGMPQTLPRALPPNERVTTESKSVY
 BCMNV-AK PTLLCMNSNAIAGFPEREDELRTGLPQVTPMSAVPKPNEVVELESKSTY
 CABMV-AZ PTLLCMHSNNAIAGYPERENELRTGLPQEIIDLKDVPAFNEVDVGESKSTY
 PWV-AUST PTLLCMHSNNAIAGYPERENELRTGLPQEIIDLKDVPAFNEVDVGESKSTY
 SMV-SKor PTLLCQNSNAIAGFPEREDELRTGLPQVVSXSDVPRAKERVEMESKSVY
 WMV-Fran PTLLCQNSNAIAGFPEREDELRTGLPQIVPKVDVPRAKERVEVESKSVY

TeMV-aaI KGLRDYSGISTLICQLINASDGHSESIFGIGYGSYVITNGHLFRKNNGTL
 TeMV-aaV RGLRDYSGISTLICQLTNASDGHSESIFGIGYGSYVITNGHLFRKNNGTL
 BCMNV-AK KGLRDYSVSTLICRLVNSSDGHNETIYGIYGSYIITNGHLFRKNNGTL
 CABMV-AZ KGPRDYSGISTLICIVNASDGCETETIFGIGYGSYIITNGHLFRKNNGTL
 PWV-AUST KGPRDYSGISTLICIVNASDGCETETIFGIGYGSYIITNGHLFRKNNGTL
 SMV-SKor KGLRDYSGISTLICQLTNSSDGHKETMFGVYGSYIITNGHLFRKNNGML
 WMV-Fran KGLRDYSGISTLICQLTNSSDGHKETMFGVYGSYIITNGHLFRKNNGML

TeMV-aaI TIKSWHGEFVIHNTTQIKIHFIEGKDAILIRMPKDFPPFAKRNFRRSPKK
 TeMV-aaV TIRSWHGEFVIHNTTQIKIHFIEGKDAILIRMPKDFPPFGRHRFRSPKK
 BCMNV-AK TVKTWHGDFIIPNTTQIKIHFIEGKDAILIRMPKDFPPFAQRNCFRSPKK
 CABMV-AZ TVKTWHGEFVVSNTTQIKIHFIEGKDAILIRMPKDFPPFAQRNCFRSPKK
 PWV-AUST TVKTWHGEFVVSNTTQIKIHFIEGKDAILIRMPKDFPPFAQRNCFRSPKK
 SMV-SKor TVKTWHGEFVIHNTTQIKIHFIEGKDAILIRMPKDFPPFGRNLRFRQPKR
 WMV-Fran TVKTWHGEFVIHNTTQLRIHFIEGKDAILIRMPKDFPPFAKRNFRRQPKR

TeMV-aaI EERVCMVGTNFQEKSLRATVSESSIIVPEGKGSFWMHWISTQDGF CGLPL
 TeMV-aaV EERVCMIGTNFQEKSLRATVSESSIIVPEGIGSFWMHWITQDGF CGLPL
 BCMNV-AK EERVCMVGTNFQEKSLRATVSESSIIVPEGKGSFWMHWITQDGD CGLPM
 CABMV-AZ EERVCMIGTNFQEKSLRATVSESSMV IPEGKGSFWMHWISTQDGD CGLPL
 PWV-AUST EERVCMIGTNFQEKSLRATVSESSMV IPEGKGSFWMHWISTQDGD CGLPL
 SMV-SKor EERVCMVGTNFQEKSLRATVSESSMILPEGKGSFWMHWITQDGF CGLPL
 WMV-Fran EERVCMVGTNFQEKSLRATVSESSIILPEGKGSFWMHWITQDGF CGLPL

TeMV-aaI VSVTDGFI VGIHGLTSNQSSKNFVVPFTENFVTDYLEKADELSWNKNWF
 TeMV-aaV VSVNDGFI VGIHGLTSNDSSKNFVVPFTDNFVTEYLEKADELSWNKNWF
 BCMNV-AK VSVNDGYI VGIHGLTSNETSKNFVVPFIDEFKNKYLDKLEDLTKNKHVLW
 CABMV-AZ VSVDDGHI VGFHGLASNTSRNFVVPFIDGFKEKYLDCAETLEWNRHWLW
 PWV-AUST VSVDDGHI VGFHGLASNTSRNFVVPFIDGFKEKYLDCAETLEWNRHWLW
 SMV-SKor VSVNDGHI VGIHGLTSNDSEKNFVVPFTDGFETEYLENADNLSWDKHWFW
 WMV-Fran VSVNDGYI VGIHGLTSNDSEKNFVVPFTDGFETEYLENADNLSWDKHWFW

TeMV-aaI QAMAAGLPERI AWGSLNLDVDDQPKKEEFKVKSLISDLFSDTVTQSKQDRW
 TeMV-aaV Q-----PERI AWGSLNLDVDDQPREEFVSKSLISDLFGDTVKQSRHRDRW
 BCMNV-AK Q-----PDR IAWGSLNLDVDDQPKSEFKISKLVTDLFGSEVSYQSKKDRW
 CABMV-AZ Q-----PDK IAWGSLNLDVDDQPKKEEFKIAKLI TDLFDDRGCTQSKQFAW
 PWV-AUST Q-----PDK IAWGSLNLDVDDQPKKEEFKIAKLI TDLFDDRGCTQSKQFAW
 SMV-SKor E-----PSK IAWGSLNLDVEEQPKKEEFKISKLVSDLFGNTVTVQGRKFRW
 WMV-Fran E-----PSK IAWGSLNLDVEEQPKKEEFKISKLVSDLFGNTVAVQSRKFRW

TeMV-aaI VLDAVEGNLRACGKADSALVTKHVVKGKCPHFQYQLHQVPEAAAFKPLM
 TeMV-aaV VLNAVEGNLRACGKADSALVTKHVVKGRCRPHFQYQLNQMPPEAAAFKPLM
 BCMNV-AK VLEAVEGNLVACGQAESALVTKHVVKGKCCFAQYQLSLHPDAQAFFKPLM
 CABMV-AZ LRSALIEGNL IACGKAESALVTKHVVKGKCSYFQYQLGNSQSAADF KPLM
 PWV-AUST LRSALIEGNL IACGKAESALVTKHVVKGKCSYFQYQLGNSQSAADF KPLM
 SMV-SKor VLDAVEGNL IACGQADSALVTKHVVKGKCPYFAQYQLSVNQEAKAFFEPLM
 WMV-Fran VLDAVEGNL IACGQADSALVTKHVVKGKCPHFAQYQLSLHDEAKQFFEPLM

TeMV-aaI GSYQPSKLNKEAFKKDFFKYKPKVVLNEVHFESFEKAVDGVKIMMETDF
 TeMV-aaV GFYQPSKLNKEAFKKDFFKYKPKVVLNEVHFESFEKAVDGVKIMMETDF
 BCMNV-AK SAYQPSKLNKEAFKKDFFKYKPKVMLNEVNF EAFKAVDGVKIMMIEFDF
 CABMV-AZ GFYQPSRLNREAFKKDFFKYKPKVTVGKVDYAFMQAVNGVKMMMIEFCF
 PWV-AUST GFYQPSRLNREAFKKDFFKYKPKVTVGKVDYAFMQAVNGVKMMMIEFCF

Fig. 2: Continue

SMV-SKor	GAYQPSRLNKDAFKRDFFKYNKPVVLNEVDFQSFERAVAGVKLMMMEFDF
WMV-Fran	GAYQPSRLNKDAFKKDFFKYNKPVVLNEVDFNAFEKAVEGVITMMVDFEF
TeMV-aaI	HECVFVTDPPD-EIFDSLNMKAAVGAQYKGGKSEYFLEMDEFDKERLLFLS
TeMV-aaV	HECVFVTDPPD-EIYDSLNMKAAVGAQYKGGKSEYFGMDEFDKERLLYLS
BCMNV-AK	NECVYVTDPPD-DIYDSLNMKAAVGAQYKGGKQDYFQDMDSFDKERLLFLS
CABMV-AZ	SECKYVTDSSRRNFPDSLNMKAAVGAQYKGGKQDYFATMDKFDRELRVYLS
PWV-AUST	SECKYVTDSSRRNFPDSLNMKAAVGAQYKGGKQDYFATMDKFDRELRVYLS
SMV-SKor	KECVYVTDPPD-EIYDSLNMKAAVGAQYKGGKQDYFSGMDSFDKERLLYLS
WMV-Fran	AECLEFVTDPPD-EIYGSLSNMKAAVGAQYKGGKQDYFSGMDSFDKERLLYLS
TeMV-aaI	CERLFYGGKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
TeMV-aaV	CERLFYGRKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
BCMNV-AK	CERLFYGGKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
CABMV-AZ	CERLFYGGKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
PWV-AUST	CERLFYGGKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
SMV-SKor	CERLFYGGKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
WMV-Fran	CERLFYGGKGLWNGSLKAE LRPLEKVQANKTRTFTAAPIDTLLGAKVCVD
TeMV-aaI	DFNNQFYSLNLCPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
TeMV-aaV	DFNNQFYSLHFKPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
BCMNV-AK	DFNNQFYSLNLCPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
CABMV-AZ	DFNNQFYSLNLCPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
PWV-AUST	DFNNQFYSLNLCPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
SMV-SKor	DFNNQFYSLNLCPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
WMV-Fran	DFNNQFYSLNLCPCWTVGMTKFGYGGWDLKMRSLPDDWLYCHADGSGQFDSS
TeMV-aaI	LTPLLLLNAVLDIRLFFMENWVWGQEMLANLYAEIVYTPILAPDGTIFKFF
TeMV-aaV	LTPLLLLNAVLDIRLFFMEDWVWGQEMLANLYAEIVYTPILAPDGTIFKFF
BCMNV-AK	LTPLLLLNAVLDIRLFFMEDWVWGQEMLANLYAEIVYTPILAPDGTIFKFF
CABMV-AZ	LTPLLLLNAVLDIRLFFMEDWVWGQEMLANLYAEIVYTPILAPDGTIVKFF
PWV-AUST	LTPLLLLNAVLDIRLFFMEDWVWGQEMLANLYAEIVYTPILAPDGTIVKFF
SMV-SKor	LTPLLLLNAVLDIRLFFMEDWVWGQEMLANLYAEIVYTPILAPDGTIFKFF
WMV-Fran	LTPLLLLNAVLDIRLFFMEDWVWGQEMLANLYAEIVYTPILAPDGTIFKFF
TeMV-aaI	RGNNSGQPSTVVDNNTLMVVISVYYSCFKQGWDMKDIERLVFFANGDDII
TeMV-aaV	RGNNSGQPSTVVDNNTLMVVISVYYSCFKQGWDMKDIERLVFFANGDDII
BCMNV-AK	RGNNSGQPSTVVDNNTLMVVISVYYSCFKQGWDMKDIERLVFFANGDDII
CABMV-AZ	RGNNSGQPSTVVDNNTLMVVISVYYSCFKAGWNEVDIQLERLVFFANGDDII
PWV-AUST	RGNNSGQPSTVVDNNTLMVVISVYYSCFKAGWNEVDIQLERLVFFANGDDII
SMV-SKor	RGNNSGQPSTVVDNNTLMVVISVYYSCFKQGWSEEDIQLERLVFFANGDDII
WMV-Fran	RGNNSGQPSTVVDNNTLMVVISVYYSCFKQGWSEEDIQLERLVFFANGDDII
TeMV-aaI	LAVQERDEWLYDKLGSSFAELGLNYDFSERTKKREELWFMSHQAKEVDGL
TeMV-aaV	LAVQEEDEWLYDKLGSSFAELGLNYDFSERTKKREELWFMSHQAKEIDGI
BCMNV-AK	LAVQKEDVWLYDNTLSNSFKELGLNYDFSERTKKREELWFMSHQAMLIDDI
CABMV-AZ	LAAQEKDIGILDFTKSFKELGLNYDFSERTKKREELWFMSHQAKLVGDL
PWV-AUST	LAAQEKDIGILDFTKSFKELGLNYDFSERTKKREELWFMSHQAKLVGDL
SMV-SKor	LAVSEKDTWLYDNTLSNSFAELGLNYDFSERTKKREELWFMSHQAMLVDGV
WMV-Fran	LAVRDEWVWLYDNTLSNSFAELGLNYDFSERTKKREELWFMSHQAMLVDGI
TeMV-aaI	YIPKLEPERIVSILEWDRSKEFMHRTEAICAAMIEAWGHTELLTEIRKFF
TeMV-aaV	YIPKLEPERIVSILEWDRSKEFMHRTEAICAAMIEAWGHTELLTEIRKFF
BCMNV-AK	YIPKLEQERIVSILEWDRSKEFMHRTEAICAAMIEAWGHTELLTEIRKFF
CABMV-AZ	YIPKLEQERIVSILEWDRSKEMLHRTETVCAAMIEAWGYPELLQEIRKFF
PWV-AUST	YIPKLEQERIVSILEWDRSKEMLHRTETVCAAMIEAWGYPELLQEIRKFF
SMV-SKor	YIPKLEPERIVSILEWDRSKEFMHRTEAICAAMIEAWGHTELLTEIRKFF
WMV-Fran	YIPKLEPERIVSILEWDRSKEFMHRTEAICAAMIEAWGHTELLTEIRKFF
TeMV-aaI	LWLLQKDEFKQLAAEGKTPYIAESALKKLYTDKDVDMDELQAYLNVLDLFE
TeMV-aaV	LWLLQKDEFKQLAAEGKTPYIAESALKKLYTDKDVDMDELQAYLNVLDLFE
BCMNV-AK	LWLMGKEEFKELALNGKAPYIAETALRKLTYTDKDAKMEEMQEYKQLEFD
CABMV-AZ	LWLLQRDEFKELASLGKAPYIAETALRKLTYTDEQASEKELQRYLQDILSF
PWV-AUST	LWLLQRDEFKELASLGKAPYIAETALRKLTYTDEQASEKELQRYLQDILSF
SMV-SKor	LWLLNKDEFKELASSGKAPYIAETALRKLTYDVNAQTSSELQRYLEVLDLFDN
WMV-Fran	LWLLSKDEFKELASSGKAPYIAETALRKLTYDVNTQPSSELQRYLEVLDLFDN
TeMV-aaI	YIEGCGESVSLQSSE--VKDVDAGNPNKDKKKG-----VEPSQHPNEN
TeMV-aaV	YADGCGESVSLQSSK--VDDVDAGNSNKDKKKG-----VSSQSPKED
BCMNV-AK	SDDEVYVESVTSQSSK-KEAEKADGADEREKDKG-----
CABMV-AZ	YDSESEDDVVLQSDE-RQKELDAGKDKDAKAKEAR----EQSTQQQAKN
PWV-AUST	YDSESEDDVVLQSDE-RQKELDAGKDKDAKAKEAR----EQSTQQQAKN
SMV-SKor	HADDCCESVSLQSKEKEGDMADKDKPKKSTS-----SNKGAG----
WMV-Fran	HIDGCCESVSLQSKEAVENLDAGKDSKDKTSKGGDKPKQNSQTGQGSKEQ
TeMV-aaI	KAVAIIPDKDVGKSSKGNIVPRLQKITKKMNLPMVKGKVIILDLHLIDYKP
TeMV-aaV	KTVIIPDKDVGNSKGRIVPRLQKITKKMNLPMVKGKVIILDLHLIDYKP
BCMNV-AK	--KGPADKDVGAGSKGVVPRQLKITKKMNLPMVGGRMILNLDHLIEYKP

Fig. 2: Continue

CABMV-AZ	KGAKETERDVAASSSGQLVPRLQKISKMMNLPVAGRLILNIDHLIEYKP
PWV-AUST	KGAKETERDVAASSSGQLVPRLQKISKMMNLPVAGRLILNIDHLIEYKP
SMV-SKor	----TSSKDVNVGSKGKVPRLQKITRKMNLPVVEGKIILSLDHLEEYKP
WMV-Fran	TKTGTVSKDVNVGSKGKEVPRLQKITRKMNLPVGGKIIILSLDHLEEYKP
TeMV-aaI	NQTDLFNTRATKQQFDSWYNAVKAIEYELDDTQVNVVMNGFMVWCIDNGTS
TeMV-aaV	NQTDLFNTRATKQQFDSWYNAVKTEYELDDAQMNVMNGFMVWCIDNGTS
BCMNV-AK	QQTDLYNTRATKAQFERWYEAVKTEYELNDQQMGVVMNGFMVWCIDNGTS
CABMV-AZ	KQIDLYNTRASKAQFNTWFEAVKEEYELDDDKMSVIMNGFMVWCIDNGTS
PWV-AUST	KQIDLYNTRASKAQFNTWFEAVKEEYELDDDKMSVIMNGFMVWCIDNGTS
SMV-SKor	NQVDLFNTRATRTQFEAWYNAVKDEYELDDEQMGVVMNGFMVWCIDNGTS
WMV-Fran	NQVDLFNTRATKTQFESWYSAVKVEYDLNDEQMGVIMNGFMVWCIDNGTS
TeMV-aaI	PDVNGVWVMDGDEQVEYPLKPMVENAKPTLRQIMHHFSDAAEAYIEMRN
TeMV-aaV	PDINGVWVMDGDEQVEYPLKPMVENAKPTLRQIMHHFSDAAEAYIEMRN
BCMNV-AK	PDVNGVWVMDGDEQIEYPLKPMVENAKPTLRQVMHHFSDAAEAYIEMRN
CABMV-AZ	PDVNGVWVMDGDEQVEFPLKPIVENAKPTLRQVMHHFSDAAEAYIEMRN
PWV-AUST	PDVNGVWVMDGDEQVEFPLKPIVENAKPTLRQVMHHFSDAAEAYIEMRN
SMV-SKor	PDANGVWVMDGDEQIEYPLKPIVENAKPTLRQIMHHFSDAAEAYIEMRN
WMV-Fran	PDVNGVWVMDGDEQVEYPLKPIVENAKPTLRQIMHHFSDAAEAYIEMRN
TeMV-aaI	SEGLYMPRYGLLRNLRDKSLARYAFDFYEVTSKTSDRAKEAVTQMKAAL
TeMV-aaV	SEGLYMPRYGLLRNLRDKSLARYAFDFYEVNSKTSDRAKEAVTQMKAAL
BCMNV-AK	SEGFYMPRYGLLRNLRDKSLARYAFDFYEVNSKTSDRAREAVAQMKAAL
CABMV-AZ	SEGFYMPRYGPLRNLRDKSLARYAFDFYEVTSKTSDRAREAIAQMKAAL
PWV-AUST	SEGFYMPRYGPLRNLRDKSLARYAFDFYEVTSKTSDRAREAIAQMKAAL
SMV-SKor	SESPYMPRYGLLRNLRDELARYAFDFYEVTSKTPNRAREAIAQMKAAL
WMV-Fran	SESPYMPRYGLLRNLRDELARYAFDFYEVTSKTPNRAREAIAQMKAAL
TeMV-aaI	VGTTNKMFGLDGSVSTTGEDTERHTARDVNRNMHSLLGVS SVQ
TeMV-aaV	VGTTNRMFGSDGSVSTACEDTERHTARDVNQNMHTLLGVGSVQ
BCMNV-AK	ANVNTRLFGLDGNVATTSENTERHTARDVNQNMHLLGMTSGQ
CABMV-AZ	ANVNTRMFGLDGNVATVSENERHTAADVNQNMHSLLGMTHGQ
PWV-AUST	ANVNTRMFGLDGNVATVSENERHTAADVNQNMHSLLGMTHGQ
SMV-SKor	SGVNNKLFGLDGNISTNSENERHTARDVNQNMHTLLGMGPQQ
WMV-Fran	AGINSRLFGLDGNISTNSENERHTARDVNQNMHTLLGMGPQQ

Fig. 2: Comparison of the aa sequence of TeMV-I poplyprotein (1183 aa; Nla, Nlb 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-aal are indicated by a black highlight and different aa, when compared to TeMV-aal, 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 Nlb 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 TeMV-I (length 3,805 nts or 1,183 aa) was sequenced. These sequences consist of a part of Nla gene (394 aa), Nlb 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, QMKAAL 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) ("WCIE") ranked 12th. The amino acid sequences of CP gene of TeMV-I and TeMV-V have 6 conserved sites, EN/DTERH, QMKAAL, YAFDFYE, MVWCIE/DNG, WV/TMMDGD/E/N and P/R/AYMPRYG (Fig. 2), which are generally owned by the Potyvirus group. On Nlb 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 Nla gene (Nia-protease) of TeMV-I at QAMAAGLP (1115-1133 nt, Fig. 1). There were six additional amino acids at the end of Nla gene (AMAAGL, Fig. 2). The Nla-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 Nla-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 Nla 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 Nla (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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