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Characterization and Genetic Variation of *Sugarcane Streak Mosaic Virus*, a *Poacevirus* Infecting Sugarcane in Thailand

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Abstract

Sugarcane leaves showing yellow streak mosaic symptoms were observed in farmers' fields in Kamphaeng Saen, Nakhon Pathom province, during disease surveys conducted in 2010. Diagnosis of symptomatic leaf samples by RT-PCR for *Sugarcane mosaic potyvirus* failed, but it revealed the presence of *Sugarcane streak mosaic virus* (SCSMV). SCSMV-infected sugarcane, designated as THA-NP3, was subjected to RNA extraction and RT-PCR-based viral gene cloning and sequencing. The complete genome of THA-NP3 (JN163911) contained 9,781 nucleotides, excluding 3′ poly (A) tail which encoded a polyprotein of 3,130 amino acid residues. Protein sequence analysis indicated nine putative cleavage sites that yielded ten functional proteins namely P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and CP, and an additional frameshifted PIPO protein. Sequence alignment revealed that THA-NP3 shared 97.84% nucleotide identity with JP2 from China and 81.39-97.78% identities to other recorded SCSMV sequences. Surveys for streak mosaic disease were conducted from 2010 to 2014 at major sugarcane growing areas in five provinces, Nakhon Pathom, Kanchanaburi, Nakhon Ratchasima, Khon Kaen and Udon Thani, and among germplasm collections. The percentages of the infected samples ranged from 43.48-90.91% and 54.17-100% in collected farmers and germplasm fields, respectively. Genetic diversity based on coat protein (CP) coding sequences of 58 Thai SCSMV isolates showed 86.17-100% nucleotide identities among them and 85.70-99.29% identities to isolates from other countries. Phylogenetic analysis of CP sequences indicated two major clusters of virus variants, one in cropping fields and another in germplasm fields. Genetic variations of SCSMV isolates were consistently indicated according to recombination events detected in CP coding regions. These findings represent essential knowledge and should be utilized to improve the SCSMV resistance of sugarcane varieties.

Keywords: *Sugarcane streak mosaic virus*, *Potyviridae*, *Poacevirus*, polyprotein, coat protein sequence

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is a valuable crop for the sugar industry and is widely cultivated in Brazil, India, China, Thailand and Pakistan. In Thailand, it is largely cultivated in central, western and northeastern areas such as Nakhon Sawan, Kanchanaburi, Nakon Ratchasima, Khon Kaen and Udon Thani provinces. The major commercial sugarcane varieties cultivated in Thailand include LK92-11, K76-4, K84-200, UT1, UT3, UT8, SP50 and KK3 (Thongpaiyai, Wongpraneekul, & Chatwachirawong, 2012). Major constraints of sugarcane production in Thailand are sugarcane white leaf disease caused by phytoplasma, sugarcane red rot disease caused by *Colletotrichum falcatum*, and mosaic disease caused by *Sugarcane mosaic virus* (SCMV). During surveys conducted from 2002 to 2004, the diagnosis of symptomatic sugarcane plants showing mosaic, yellow streak, chlorotic or necrotic streak, chlorotic mild mottle or mild mosaic revealed the presence of SCMV in many sugarcane production fields (Gemechu, 2004). In 2005, a distinct virus species, namely *Sugarcane streak mosaic virus* (SCSMV) was detected in sugarcane and sorghum plants which showed typical mosaic symptoms as those caused by SCMV but failed to react with the antisera against SCMV, SrMV and JGMV (Chatenet et al., 2005).

SCSMV was reported for its devastating effects in Asia, including India (Viswanathan et al., 2008), Pakistan, Sri Lanka, Vietnam, Thailand (Chatenet et al., 2005), China (Li et al., 2011) and Indonesia (Damayanti & Putra, 2011). The identified hosts of SCSMV under natural condition are poaceae plants including sugarcane, maize, sorghum and Egyptian crowfoot grass (*Dactylactonium aegypticum*) (Hema, Joseph, Gopinath, Sreenivasulu, & Savithri, 1999; Putra, Kristini, Achadian, & Damayanti, 2014; Srinivas, Subba Reddy, Ramesh, Lava Kumar, & Sreenivasulu, 2010).

The SCSMV virion is flexuous rod, size of 890 ×15 nm, and comprises a positive sense single stranded RNA genome of 9.8 kb, characteristic of members in the family *Potyviridae* (Hema, Sreenivasulu, & Savithri, 2002). The viral genome contains a single open reading frame (ORF) which encodes a polyprotein of 3130 amino acid residues. The polyprotein is processed by the viral protease yielding ten different proteins, namely P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and CP. The first published complete genome sequence of SCSMV which was isolated from sugarcane in Pakistan, SCSMV-PAK: GQ388116, contained 9782 nucleotides (nts), excluding 3′ Poly (A) tail (Xu, Zhou, Xie, Mock, & Li, 2010). Sequence comparison and a phylogenetic tree of the complete genome revealed that SCSMV was a distinct group from those of other genus in the family *Potyviridae*, and has been recently named *Poacevirus* (Xu et al., 2010; ICTV: www.ictvonline.org). More isolates of SCSMV and their complete genome sequences were reported from China (Li et al., 2011) and India (Parameswari, Bagyalakshmi, Viswanathan, & Chinnaraja, 2013). The study on genetic variability of SCSMV has been investigated using the analysis of CP (He et al., 2013), P1 (He et al., 2013) and HC-Pro (Bagyalakshmi et al., 2012) coding regions. The comparison of these genes revealed high variation among different SCSMV isolates resulting from recombination (Bagyalakshmi et al., 2012; He et al., 2013). In Thailand, the presence and distribution of SCSMV in Thailand and genetic variations of the existing isolates still have not been recorded so far.

In this study, sugarcane leaves showing yellow streak mosaic symptom in farmers' fields were diagnosed, and the causal virus was identified. A full length genome of Thai isolate of SCSMV was reconstructed by RT-PCR amplification and its nucleotide sequence was determined for the first time. Disease surveys were conducted, samples were collected for viral gene amplification, and genetic variations among Thai SCSMV isolates were analyzed based on coat protein (CP) coding region.

2. Method

2.1 Disease Survey and Sample Collection

Sugarcane disease surveys for SCSMV infection were performed from 2010 to 2014 in 5 provinces of the major sugarcane growing areas of Thailand (Nakhon Pathom, Kanchanaburi, Udon Thani, Khon Kaen and Nakhon Ratchasima). Virus-like symptomatic sugarcane samples, particularly young mosaic leaves, were collected and kept in sealed plastic bags. Samples were also collected from sugarcane germplasm collection fields, belonging to Kasetsart University, in Nakhon Pathom and Kanchanaburi provinces. More samples from the germplasm collection fields in Suphan Buri province were kindly provided by the Department of Agriculture, Ministry of Agriculture and Cooperatives.

2.2 Detection of SCSMV by Direct Antigen Coating ELISA (DAC-ELISA)

Collected sugarcane leaf tissues were diagnosed for the presence of SCSMV by DAC-ELISA, using the locally produced antiserum raised against the purified SCSMV (Kasemsin, Chiemsombat, & Hongprayoon, 2011). Briefly, one gram of leaf tissue was ground in a plastic bag containing 1 ml of the extraction buffer (PBS, pH 7.4, 0.2% sodiumdiethyldithiocarbamate). The homogenate was diluted at 1:10 in coating buffer. A 100 µl of the diluted plant extract was loaded in each well of a microtiter plate and incubated overnight at 4°C. The protocol as described by Chiemsombat, Prammanee, & Pipattanawong (2014) was followed except for SCSMV antiserum was diluted at 1:500 and incubated at 37°C for 1 h.

2.3 Viral Genome Amplification and Analysis

2.3.1 Primer Design

The specific primers used in this study (Table 1) for amplification of the SCSMV complete genome were designed based on the alignment of the complete genome sequence of SCSMV-PAK: GQ388116 and other partial SCSMV sequences recorded in GenBank (Y17738, EU650179, EF088799, EU650178, EU883391, EF088797, DQ421788, AM920686, AM920685, AB563503, GQ386845, GQ386843, GQ386844, Y17738, AY193783, AY189681). Two specific primers for amplification of the entire coat protein gene were also designed (Table 1).

Table 1. List of specific primers for amplification of SCSMV genes and the overlapping sequences of SCSMV-THA complete genome

Primer pair	Nucleotide sequence (5′ to 3′)	Product size (bp)	Annealing temp (°C)
SCS-F2	GCCAAGGCAAAGCAGATGATGAG	881	55
A-d-T-R2	TTTTTTTTTTTTTTTTTCCTCCN		
SCS-F3	AGAGACAAGCTGGGTCACACTG	1129	57
SCS-R3	CGTATTGATGCGGCCGATGAAG		
SCS-F4	CTGGAATGATGCAGTATGCGCTC	1043	57
SCS-R4	GTCCGCGTCAATGAACTTCCAG		
SCS-F5	AAGCGCCGAACACACGCTCGTG	1096	58
SCS-R5	TCACTACCCGAGCTGCCGAATG		
SCS-F6	GCCACAACTTCCAATCCCATTC	755	57
SCS-R6	TTCCGGCATTCTTCTTGGCG		
SCS-F7	AGCTACACCACCAGGAGCACG	1043	57
SCS-R7	GCGCTTCAGACGTTGACAATCG		
SCS-F8	GACGACACAACAAGGTTAGCGC	1067	55
SCS-R8	ATGAGTATACGCCCTCCGTTTG		
SCS-F9	GGAGGTGTGTTACCAGATTTGCG	1003	57
SCS-R9	CCGTCTTTCCTTGTGCGTGGTG		
SCS-F10	CAGATCCTGAACGAAATTGCACG	1102	57
SCS-R10	CTGCAATATCGGGATGATTCCTC		
SCS-F11	CGACAACACTGGTGAAGTTGAAG	1183	57
SCS-R11	CGATAGTGGTTGGCTAGCGGTG		
SCS-F12	AAATGTAAT TTCAAATTGACTAC	872	57
SCS-R12	CATTAGTTCGCATAATCACACG		
SCS-NIb-CP:F	TTGGTGGAGCAAGCACACAG	1094	61
SCS-NIb-CP:R	CGGTCAGGCAACTACCATCA		

Note. N in the A-d-T-R2 represents the regenerated bases (A, T, G and C).

2.3.2 Total RNA Extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the sugarcane leaf tissues using TLES buffer (100 mM Tris-HCl, pH 8.0, 100 ml LiCl, 10 mM EDTA, pH 8.0, 1% SDS, 0.1% sodium sulphite) according to Verwoerd, Dekker, & Hoekema (1989). The viral cDNA was synthesized using a SuperScript III cDNA synthesis kit (Invitrogen, USA) following the manufacturer's protocol. This cDNA was added to PCR reactions for synthesis of the 11 overlapping fragments covering the whole genome of SCSMV, using our designed 11 primer pairs (Table1).

PCR reaction consisted of 1X PCR buffer, 0.4 mM dNTP mix, 2mM $MgSO_4$, 10 pmol of each primer, 1 U of Hi-Fidelity *Taq* (Invitrogen, USA), 1 µl of cDNA and RNase-free water to adjust the total volume to 25 µl. The reaction was started with the initial step of denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 98°C for 30 sec, annealing for 1 min at temperature according to the annealing temperature of each primer pair (Table 1), extension at 68°C for 1-2 min according to the length of each overlapped sequence (1 kb/1 min) and 1 cycle of the final extension at 68°C for 7 min. RT-PCR products were analyzed on 0.8% agarose gel electrophoresis.

2.3.3 Viral Gene Cloning and Sequencing

The RT-PCR products obtained from each primer were purified using a PCR/Gel purification kit following the manufacturer's protocol (Favorgen Biotech Corp, Taiwan) and separately cloned into the pGEM-T cloning vector (Promega, USA). The selected clones with a viral gene insert were subjected for sequencing in both directions at BioDesign, Thailand.

2.3.4 Viral Genome Sequence Assembly and Analysis

The presence of sections of the SCSMV was verified using nucleotide blast on sequenced inserts (www.ncbi.nlm.nih.gov/BLAST) and then assembled from 5′ to 3′ in BioEdit, version 7.2.5 (http://www.mbio.ncsu.edu/). The full length nucleotide sequences of the complete genome were initially confirmed by searching for an ORF by using ORFinder (http://www.ncbi.nlm.nih.gov). The putative cleavage sites of the deduced proteins on the polyprotein were determined by comparing the potential cleavage sites with those of SCSMV isolates, PAK: GQ388116, TPT: GQ246187, ID: JF488066, JP1: JF488064, JP2: JF488065 and IND671: JN941985.

2.4 Amplification, Cloning and Sequencing of the CP Coding Region

The CP coding region of SCSMV was amplified by RT-PCR using a CP specific primer pair, SCS-NIb-CP: F and SCS-NIb-CP: R (Table 1). The viral cDNA was synthesized from total RNA using ReverTraAce (TOYOBO, Japan) following the manufacturer's protocol.

The PCR reaction consisted of 1X PCR buffer, 0.4 mM dNTP mix (TOYOBO, Japan), 2mM $MgSO_4$, 10 pmol of each primer, 1 U of KOD-Plus-Neo (TOYOBO, Japan), 1 µl of cDNA and RNase-free water to adjust the total volume to 25 µl. The amplification cycle was the same as described above (2.3.2) except that the annealing temperature was 61°C for 1 min. The RT-PCR products were analyzed by 0.8% agarose gel electrophoresis and submitted to direct sequencing in both directions (SolGent, South Korea). Some selected purified products were cloned into pGEM-T cloning vectors (Promega, USA), and the plasmids containing gene inserts were sequenced in both directions (SolGent, South Korea).

2.5 Sequence Analysis and Phylogenetic Tree of the CP Coding Regions

The nucleotide (nt) and amino acid (aa) sequences of the CP coding regions among SCSMV isolates used in this study (Table 2) were analyzed by using clustalW in the CLC program package (http://www.clcbio.com). Pairwise comparisons were also created using the CLC program. The phylogenetic relationships were analyzed by using MEGA6 program (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

2.6 Recombination Analysis of the CP Coding Regions

The recombination events of SCSMV isolates used in this study (Table 2) were detected using RDP4 (Martin, Murrell, Golden, Khoosal, & Muhire, 2015). The detection algorithms used in this study were the automated RDP, GENECONV, Chimaera, MaxChi, BOOTSCAN, SISCAN, 3Seq and LARD which are implemented in the RDP4 program (version 4.50) with default setting.

2.7 Networks Analysis Using Splitstree

Phylogenetic networks of the CP coding regions among SCSMV isolates (Table 2) were created by using SplitsTree4.11 (Huson & Bryant, 2006). The alignment file obtained from clustalW was used for construction of the phylogenetic network using median network in SplitsTree4.11.

3. Results

3.1 Incidence of SCSMV in Surveyed Sugarcane Fields

In this survey, diseased sugarcane plants showed typical symptoms of yellow streak mosaic, especially on young leaves (Figure 1a-b), while older leaves showed mild symptoms. Two hundred and thirty-three sugarcane leaf samples were collected from 14 farmers' fields of which 153 samples tested positive by DAC-ELISA. Some sample obtained from older leaves had positive reactions by DAC-ELISA but the absorbance (A_{405}) values (0.277-0.339) were lower than in younger leaves. Therefore, the presence of SCSMV in some selected samples was confirmed by RT-PCR. This survey indicated that SCSMV is widespread and was present in all collected fields in Nakhon Pathom, Kanchanaburi, Udon Thani, Khon Kaen and Nakhon Ratchasima provinces (Figure 2). The percentage of the infected samples of all collected fields ranged from 43.48-90.91% (Figure 2).

In three fields containing germplasm collections, 138 samples from 73 sugarcane varieties were collected. Of these, 91 samples obtained from 50 varieties indicated positive reactions by DAC-ELISA. The percentage of positive reaction within varieties ranged from 54.17-100% (Figure 2). In a subsequent study, we selected 36 isolates from different farmers' fields in 5 provinces and 22 isolates from fields containing germplasm collections to examine the genetic variation based on sequence analysis of the viral CP gene. All selected isolates yielded the expected 1094 bp RT-PCR product, from which an isolate from Kamphaeng Saen District, Nakhon Pathom Province, designated as THA-NP3 (Figure 1a) was subjected to full length genome sequencing.

Table 2. The CP gene of Thai SCSMV isolates from different surveyed areas used for comparison and phylogenetic tree analysis

No.	Isolates name	Surveyed areas	Collection year	Sugarcane varieties	GenBank accession no.
1	FDT1	Kanchanaburi	2012	Unknown	KP987806
2	FKB1	Kanchanaburi	2012	Unknown	KP987848
3	FKB13	Kanchanaburi	2012	Unknown	KP987807
4	FKB6	Kanchanaburi	2012	Unknown	KP987839

5	FSC1	Kanchanaburi	2012	Unknown	KP987820
6	FSC2	Kanchanaburi	2012	Unknown	KP987821
7	FSC7	Kanchanaburi	2012	Unknown	KR057207
8	FSC8	Kanchanaburi	2012	Unknown	KP987822
9	FKPS10	Nakhon Pathom	2012	Unknown	KR057206
10	FKPS19	Nakhon Pathom	2012	Unknown	KP987811
11	FKPS22	Nakhon Pathom	2012	Unknown	KP987812
12	FNP5	Nakhon Pathom	2012	Unknown	KP987813
13	FNP-KPS	Nakhon Pathom	2012	Unknown	KP987814
14	FNS5	Nakhon Pathom	2012	Unknown	KP987819
15	FNS4	Nakhon Pathom	2012	Unknown	KP987840
16	THA-NP3	Nakhon Pathom	2010	Unknown	JN163911
17	FUD9-3	Udon Thani	2012	Unknown	KP987824
18	FUD9-5	Udon Thani	2012	Unknown	KP987825
19	FUD10-7	Udon Thani	2012	Unknown	KR057211
20	FUD10-4	Udon Thani	2012	Unknown	KP987826
21	FUD10-12	Udon Thani	2012	Unknown	KP987827
22	FUD11-1	Udon Thani	2012	Unknown	KR057212
23	FUD11-2	Udon Thani	2012	Unknown	KP987828
24	FUD12-3	Udon Thani	2012	Unknown	KP987829
25	FUD12-6	Udon Thani	2012	Unknown	KR057213
26	FUD12-8	Udon Thani	2012	Unknown	KP987830
27	FUD12-9	Udon Thani	2012	Unknown	KP987831
28	FUD12-10	Udon Thani	2012	Unknown	KP987804
29	FKH8	Khon Khan	2012	Unknown	KP987805
30	FKH4-8	Khon Kaen	2012	Unknown	KP987808
31	FKH5-1	Khon Kaen	2012	Unknown	KP987809
32	FKH5-2	Khon Kaen	2012	Unknown	KP987810
33	FNR3-1	Nakon Ratchasima	2012	Unknown	KP987815
34	FNR3-4	Nakon Ratchasima	2012	Unknown	KP987816
35	FNR3-6	Nakon Ratchasima	2012	Unknown	KP987817
36	FNR3-9	Nakon Ratchasima	2012	Unknown	KP987818
37	GK88-65	Nakhon Pathom	2014	K88-65	KP987836
38	GK88-87	Nakhon Pathom	2014	K88-87	KP987837
39	GUT6	Nakhon Pathom	2014	UT6	KP987842
40	GUT4	Nakhon Pathom	2014	UT4	KP987832
41	GUT5	Nakhon Pathom	2014	UT5	KP987841
42	GK76-4	Nakhon Pathom	2014	K76-4	KP987847
43	GRT2007-091	Kanchanaburi	2014	RT2007-091	KP987843
44	GRT2003-639	Kanchanaburi	2014	RT2003-639	KP987844
45	GEhaew	Kanchanaburi	2014	Ehaew	KP987802
46	G03208	Kanchanaburi	2014	03208	KP987803
47	GUT3	Kanchanaburi	2014	UT3	KP987834
48	G519	Kanchanaburi	2014	519	KP987835
49	GROC7	Kanchanaburi	2014	ROC7	KP987846
50	G99-2-294	Kanchanaburi	2014	99-2-294	KR057205
51	G02-483	Kanchanaburi	2014	02-483	KR057208
52	G94-2-106	Kanchanaburi	2014	94-2-106	KR057209
53	G95-2-213	Kanchanaburi	2014	95-2-213	KR057210
54	GUT10	Kanchanaburi	2014	UT10	KR057214
55	G03041	Suphan Buri	2012	03041	KP987845
56	GSP50-2	Suphan Buri	2012	SP50-2	KP987823
57	GUT8	Suphan Buri	2012	UT8	KP987833
58	GK92-80	Suphan Buri	2012	K92-80	KP987838

Note. Isolate No. 1-36 were obtained from farmers field and isolate No. 37-58 were obtained from germplasm collection fields.

Figure 1. Yellow streak mosaic symptoms on SCSMV infected plants. Sugarcane leaves obtain from the farmer's field in Kamphaeng Saen, Nakhon Pathom, isolate THA-NP3 (a), sugarcane cv. UT8 (b), the inoculated plants, sorghum cv. UT325B at 15 days post-inoculation, dpi (c) and the commercial corn, cv. Tender58 at 5 dpi (d)

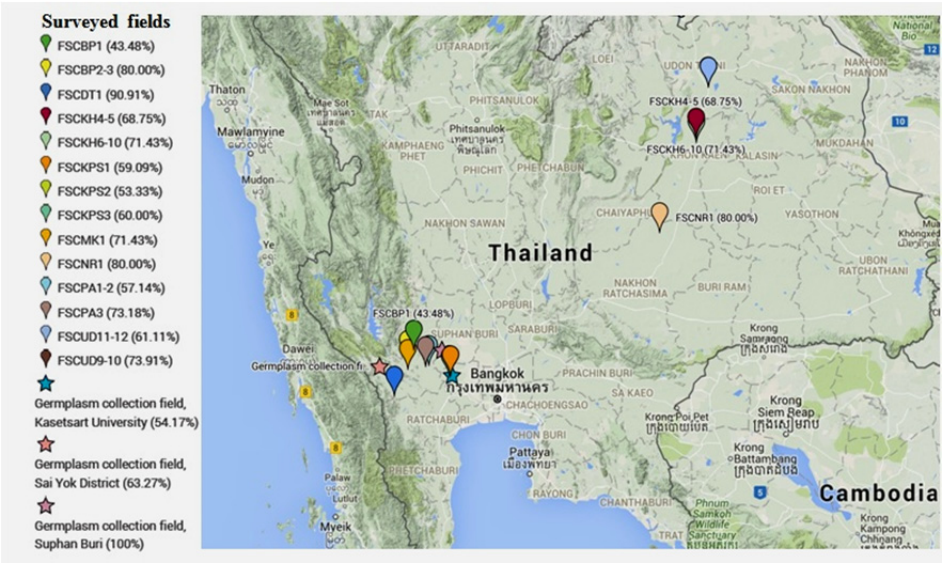

Figure 2. The survey map of streak mosaic disease incidence in farmers (F) and germplasm collection (G) sugarcane fields during 2010 to 2014 in five provinces, including Kanchnaburi (FSCPA1-2, FSCPA3, FSCBP1, FSCBP2-3, FSCDT1, FSCMK1), Khon Kaen (FSCKH4-5, FSCKH6-10), Nakhon Pathom (FSCKPS1, FSCKPS2, FSCKPS3), Nakhon Ratchasima (FSCNR1), and Udon Thani (FSCUD9-10, FSCUD11-12). The percentages of the infection rates are shown in brackets for each surveyed fields

3.2 Genome Characterization of THA-NP3

The genome of isolate THA-NP3 (JN163911) consisted of 9781 nts, excluding the 3′ poly (A) tail. Blast analysis of the complete genome revealed that this virus isolate was homologous to SCSMV isolates recorded in the database. Sequence analysis revealed that the 5′ untranslated region (UTR) consisted of 199 nts. The initiation

codon AUG (nts 200-202) of the long polyprotein ORF was ended by the termination codon UGA (nts 9591-9593) and was followed by a 3′ UTR of 189 nts (Figure 3). The coding region, consisted of 9393 nts, encoded a polyprotein of 3130 amino acid residues with a calculated Mr of 356.53 kDa. This polyprotein had extensive amino acid sequence homology to those of SCSMV polyprotein isolates.

Nine putative cleavage sites of the polyprotein were identified in comparison to the putative sites of SCSMV-PAK polyprotein (Xu et al., 2010) and some others (Figure 3). All cleavage sites of THA-NP3 proteins as well as the positions of the amino acids were similar to those of the SCSMV isolates, PAK, ID, JP1, JP2 and TPT but not IND671. The amino acid sequences of the conserved motifs were slightly different among SCSMV isolates (Figure 3). Thus, the putative 10 functional proteins of THA-NP3 are P1 (41.47 kDa), HC-Pro (54.41 kDa), P3 (37.39 kDa), 6K1 (5.43 kDa), CI (74.82 kDa), 6K2 (5.55 kDa), NIa-VPg (22.47 kDa), NIa-Pro (26.64 kDa), NIb (57.43 kDa) and CP (31.07 kDa).

The genome sequence of THA-NP3 was analyzed for the presence of Pretty Interesting Potyviridae ORF (PIPO) in the P3 gene with a highly conserved motif, $G_{1\text{-}2}A_{6\text{-}7}$ similar to the previous report of potyviruses (Chung, Miller, Atkins, & Firth, 2008). The result revealed that the conserved motif, GGAAAAAAA was found at the nucleotide position 3085-3093 which is similar to that of SCSMV-PAK reported by Xu et al. (2010). The deduced 139 aa of PIPO of THA-NP3 was obtained from 420 bp in the +1 frame at the nucleotide position 3091-3510 as reported by Chandran and Gajjeraman (2015).

The motif scan, using the NCBI-CDD database, of the THA-NP3 polyprotein revealed 19 motifs. The P1 of this virus isolate contained a serine peptidase at the amino acid position 208-312. The peptidase_C6 conserved motif, which is contained in the HC-Pro protein, was found at the amino acid position 684-810. The conserved motif, C-71-X-H (aa 715-787), was found at the C-terminal region of the HC-Pro protein while the conserved motifs associated with aphid transmission were not found. The CI protein was the largest protein among ten functional proteins and contained RNA helicases of superfamily II at the positions 1451-1583. The THA- NP3 VPg protein contained a conserved tyrosine for linking VPg to the 5′-terminus of the viral RNA in the motif L-Y-D-L-D (aa 1988-1992). The NIa protein contained cysteine protease at the amino acid position 2242-2274. The NIb protein contained the conserved motif, D-G-S-R-Y-D (aa 2589-2594) which represented the function for RNA dependent RNA polymerase. The potyvirus coat protein conserved motif was found in the CP protein at the amino acid position 2912-3072. Moreover, the conserved motifs such as Y-X-P-17X-W (aa 2915-2935) and A-X-P-2X-R-2X-M-6X-A (aa 3012-3027) and D-F (aa 3062-3063) were also found in the CP protein, but the DAG motif involved in aphid transmission was not found.

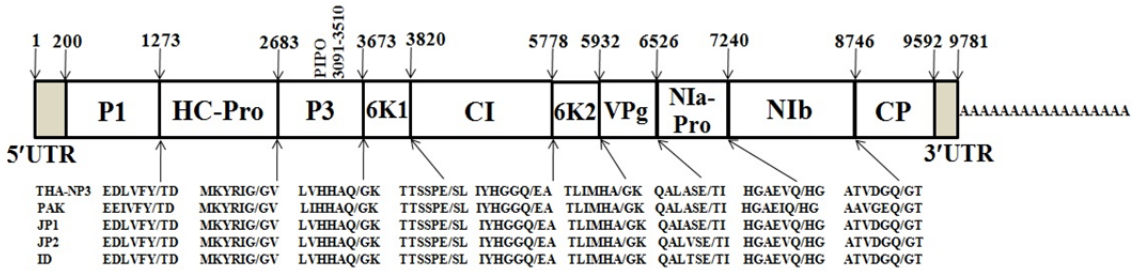

Figure 3. Schematic represents genome organization of *Sugarcane streak mosaic virus*, an isolate THA-NP3 (JN163911). The 5′UTR (199 nts) and the 3′UTR (189 nts) regions are shown as shaded boxes. The boxes show each of 10 functional proteins. Numbers above the box indicate the nucleotide positions including protein initial codons. The cleavage sites for viral proteinases of each complete genome isolate are indicated below. The PIPO protein is encoded in +1 frame at the nucleotide position 3091-3510. The 3′ poly (A_{17}) obtain from the RT-PCR product using the first primer pair (SCS-F2, A-d-T-R2)

3.3 Comparison of THA-NP3 Complete Genome with Other Genome Isolates

The comparisons of the complete genome sequences showed that THA-NP3 was very similar to JP2, JP1, ID and TPT isolates with 97.84%, 97.78%, 97.73% and 94.80% nucleotide identities, respectively. It was less similar to PAK and IND671 isolates with 81.83% and 81.39% nucleotide identities, respectively (Table 3). The comparison of the polyprotein among seven SCSMV isolates showed that THA-NP3 shared 98.98% identities with three isolates (JP1, JP2, ID) and 94.38-98.40% identities with two Indian isolates (TPT, IND671) while it shared 95.27% identity with PAK. These results suggest that THA-NP3 is clustered in the same group with JP1, JP2, ID and TPT isolates which separated from PAK and IND671 isolates. Moreover, THA-NP3 polyprotein showed 52.40%

nt and 50.38% aa identities with TriMV.

The NIb protein was more conserved protein among poaceviruses, the similarity showed that about 94.82-99.00% identities among eight SCSMV isolates and 64.62% identity with TriMV (Table 3). The CP protein of SCSMV-THA showed 94.72-98.93% identities among eight SCSMV isolates while it showed only 48.48% identity with TriMV.

The comparison of THA-NP3 with potyviruses causing mosaic diseases in sugarcane (SCMV, SrMV, MDMV, JGMV) showed less similarity with 29.01-29.38% (nt) and 17.49-18.33% (aa) identities (Table 3). Thus, THA-NP3 was a distinct virus genus separated from those of genus *Potyvirus* infecting sugarcane.

3.4 Analysis of the Complete CP Gene

The RT-PCR products using the primers, SCS-NIb-CP: F and SCS-NIb-CP: R revealed 1094 bp of the partial sequence of the polyprotein and 3′UTR. Sequence analysis revealed that the complete CP gene contained 846 nucleotides which encoded 281 amino acid residues. In this study, we investigated the genetic variability of the complete CP region (846 nts, 281 aa) of the 58 Thai SCSMV isolates; 36 isolates of which, were obtained from 14 farmers' fields; and 22 isolates of which, were obtained from three germplasm collection fields (Table 2). A nucleotide sequence comparison showed that the 36 isolates from different farmers' fields shared 86.17-100% identities while they shared 86.05-99.29% identities to SCSMV isolates from other countries. The 22 isolates from germplasm shared 86.52-100% identities and 85.70-99.29% identities to the isolates from other countries. The CP gene of Thai SCSMV isolates shared only 60.28-63.71% identities to the isolate of TriMV (NC_012799) which was used as an outgroup for construction of a phylogenetic tree.

The amino acid sequences revealed 96.09-100% identities among the 36 isolates from collected farmers' fields while they shared 94.31-100% identities to the isolates from other countries. The 22 germplasm isolates from collected germplasm shared 94.66-100% identities among each other, while they shared 93.24-100% identities to the isolates from other countries. In addition, the CP gene of Thai SCSMV isolates shared 47.69-48.40% identities when compared to the isolate of TriMV. The amino acid sequence alignment of all isolates from Thailand and other isolates from other countries revealed more variation at the amino acid position 1-31 of N-terminal region and the core region, including C-terminus, were more conserved.

3.5 Phylogenetic Relationships of SCSMV Based on the CP Sequences

Phylogenetic relationships of the CP gene (846 nts) from 58 Thai SCSMV isolates (Table 2) and 27 SCSMV isolates from other countries were determined using a maximum-likelihood method. The CP sequences from Thai SCSMV isolates clustered in four well defined variant groups (Figure 4). Two sub-groups, which were designated as sub-groups 1A and 2A, contained 38 isolates from Thailand, 9 isolates from China, 2 isolates from Japan, 1 isolate from Indonesia, 2 isolates from India and the unique variant from the isolate GK76-4 (Figure 4). The second major group consisted of 2 sub-groups, 1B and 2B which represented the isolates from germplasms in Thailand, India and China (Figure 4). The second sub-group, 2B contained only the isolates from collected germplasm in Thailand (Figure 4). These results suggested that Thai SCSMV isolates from different farmers' fields were more closely related to the isolates from China while the isolates from collected germplasm were closely related to the isolates from India and Pakistan (Figure 4).

Table 3. Identity percentages of complete genome sequence comparison between THA-NP3 isolate and the isolates from other countries, including potyviruses causing mosaic diseases in sugarcane

protein name \ virus isolate	PAK	JP1	JP2	ID	TPT	IND671	AP	TriMV	SCMV	SrMV	MDMV	JGMV
whole genome (9781nt[a])	81.83	97.78	97.84	97.73	94.80	81.39	na[c]	52.40	29.24	29.01	29.38	29.17
Polyprotein (3130aa[b])	95.27	98.98	98.98	98.98	98.40	94.38	na	50.38	18.33	17.49	18.14	17.65
P1 (358 aa)	95.25	98.32	98.60	98.60	98.04	88.37	na	38.92	9.84	9.84	11.17	10.11
HC-Pro (470 aa)	90.85	98.51	98.51	98.72	98.30	90.64	na	45.11	14.95	15.15	15.76	14.34
P3 (330 aa)	95.15	99.09	99.09	99.39	98.18	96.06	na	34.94	9.81	9.81	9.26	10.90
6K1 (49 aa)	93.88	100	100	100	87.50	91.84	na	50.00	13.04	10.14	15.94	8.06
CI (656 aa)	96.95	99.24	99.09	99.24	97.71	96.95	na	59.15	24.26	23.96	24.70	21.88
6K2 (48 aa)	100	100	100	97.92	92.16	100	100	49.02	8.57	10.00	8.57	7.86
NIa-VPg (198 aa)	96.97	98.48	98.48	98.48	94.12	97.98	95.45	22.27	22.01	20.57	na	22.49
NIa-Pro (238 aa)	97.48	100	100	100	93.70	94.96	95.80	44.12	18.42	16.17	19.55	16.23
NIb (502 aa)	95.02	99.00	99.00	98.80	97.04	94.82	97.01	64.62	32.29	32.66	31.38	33.64
CP (281 aa)	95.73	98.58	98.93	98.58	95.47	94.72	96.80	48.48	14.91	12.86	12.99	14.41

Note. [a]nts: nucleotides; [b]aa: amino acid residues; [c]na: not available.

GenBank accession no. of the virus isolates are the followings: SCSMV-PAK: GQ388116 (Pakistan),

SCSMV-JP1: JF488064 (China), SCSMV-JP2: JF488065 (China), SCSMV-ID: JN163911 (China), SCSMV-TPT: GQ246187 (India), SCSMV-IND671: JN941985 (India), SCSMV-AP: Y17738 (India), TriMV: NC_012799 (USA), SCMV: NC_003398 (China), SrMV: NC_004035 (China), MDMV: NC_003377 (Bulgaria), JGMV: NC_003606 (unknown; direct submission).

3.6 Recombination Analysis of the CP Gene

Using RDP seven algorithms, four recombinant isolates, namely GK76-4, M55, CB671-1 and GROC7 were detected (Table 4). The recombinant isolate GK76-4 had two recombination sites, nt 1-196 and nt 694-846 (Table 4). The recombinant isolate GK76-4 was distributed from the major parental isolate (FUD12-10) and the minor parental isolate (GRT2007-091). Another recombinant isolate, namely GROC7 (Table 4) had only one recombination site (nt 562-798) which was contributed by the major parental isolate (GRT2007-091) and the minor parental isolate (FUD12-8).

The recombinant isolate (M55) from China was distributed from the major parental isolate (GRT2007-091) and the minor parental isolate (PAK) while the recombinant isolate (CB671-1) from India was distributed from the major parental isolate from Thailand (FUD10-7) and the minor parental isolate from India (IND671). The recombination sites of M55 and CB671-1 were similar (Table 5). These results confirmed that the recombination occurred in the CP coding region among SCSMV isolates from different geographical regions, and sugarcane varieties in the presence of four recombinant isolates, GK76-4, GROC7, M55 and CB671-1 (Table 4).

3.7 Phylogenetic Networks of Thai SCSMV Isolates

The splits networks based on the alignment of the CP gene of 58 Thai SCSMV isolates revealed that the recombination events occurred among Thai SCSMV isolates that divided Thai SCSMV isolates into two major network groups (Figure 5). The recombinant isolate GK76-4 shared with these two network groups suggested that recombination occurred between the virus isolates from the collected farmers and germplasm fields. Nine SCSMV isolates (AP, TPT, IND671, JP1, JP2, ID, M55, CB671-1 and PAK) with likely to be the recombinant were selected for splits network analysis with 58 Thai SCSMV isolates. The splitstree based on the selected 67 isolates exhibited two major network groups (Figure 5). JP1, JP2, ID, TPT and AP isolates shared the same network group with the collected farmer isolates. The second network group consisted of 4 isolates, CB671-1, M55, IND671 and PAK that shared the same network with the collected germplasm isolates (Figure 5).

Table 4. Recombination events detected in the CP coding region of SCSMV isolates by using RDP4 (version 4.5)

Event no.	Recombinant isolate	Recombination site (nt)	Major parent	Minor parent	Detection method*						
					R	G	B	M	C	S	T
1	GK76-4	1-196, 694-846	FUD12-10	GRT2007-091	-	+	+	+	+	+	+
2	M55	99-831	GRT2007-091	PAK	-	-	-	+	+	+	+
3	CB671-1	59-820	FUD10-7	IND671	+	-	+	+	+	+	+
4	GROC7	562-798	GRT2007-091	FUD12-8	+	+	+	+	+	-	+

Note. *The methods used for recombination detection are the followings, R: RDP, G: GENECONV, B: Bootscan, M: MaxChi, C: Chimaera, S: SiScan and T: 3Seq, + represents the recombination was found and - represents the recombination was not found.

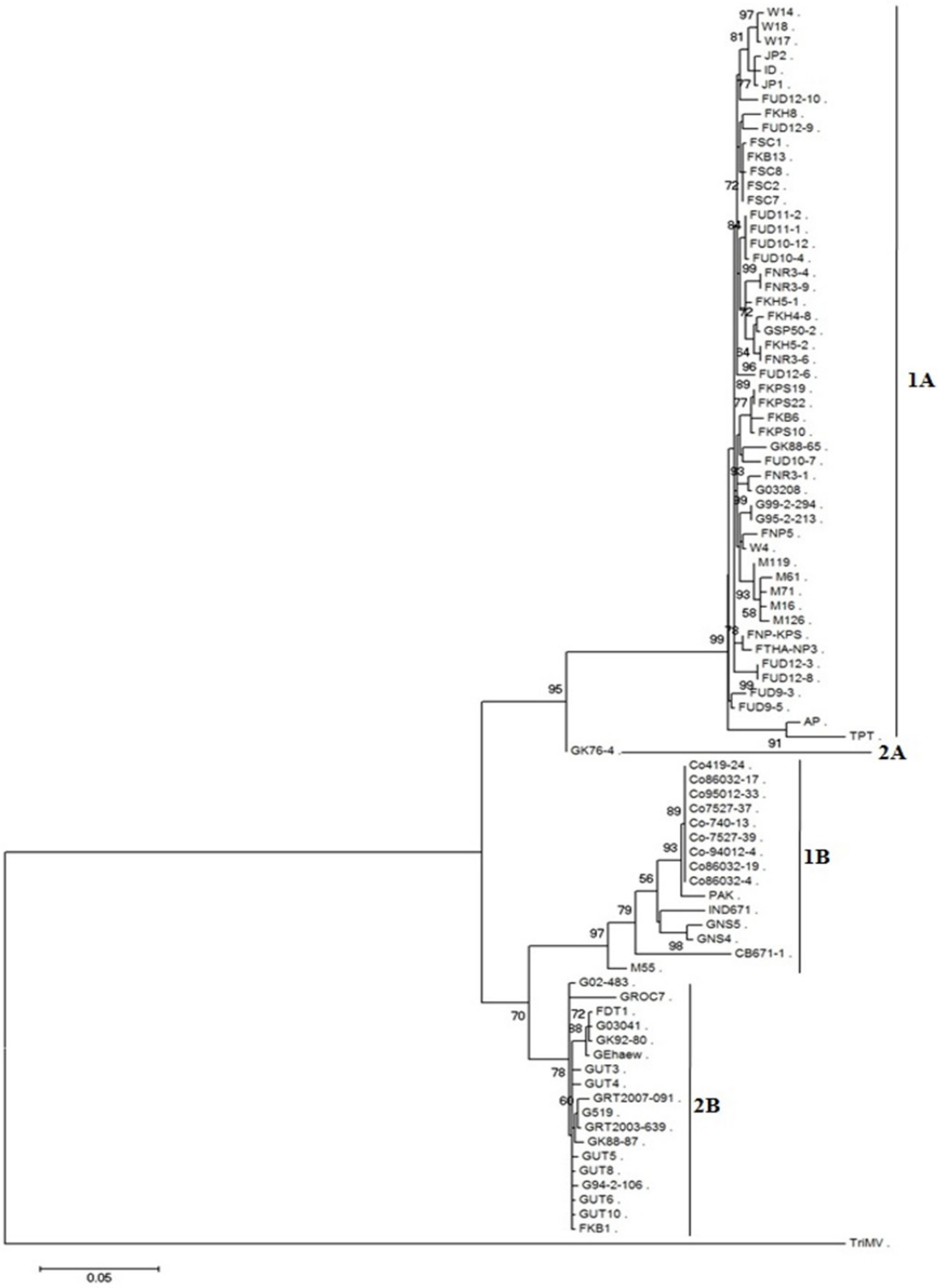

Figure 4. Maximum-likelihood tree based on the aligned CP nucleotide sequences of the 58 Thai SCSMV isolates and the 27 SCSMV isolates from other countries

The 13 SCSMV isolates from China are the following: M16 (JQ954718), M126 (JQ954701), M119 (JQ954719), M55 (JQ954717), M61 (JQ954716), M71 (JQ954714), W14 (JQ954700), W17 (JQ954699), W18 (JQ954698), W4 (JQ954720), ID (JF488066), JP1 (JF488064) and JP2 (JF488065). The 13 SCSMV isolates from India are the followings: Co419-24 (AM749404), Co7527-37 (AM749409), Co7527-39 (AM749410), Co740-13 (AM749398), Co86032-17 (AM920684), Co86032-19 (AM920685), Co86032-4 (AM920678), Co94012-4 (AM920678), Co95012-33 (AM749407), CB671-1 (DQ421788), IND671 (JN941985), AP (Y17738), TPT

(GQ246187), one SCSMV isolate from Pakistan (GQ388116) and an outgroup, TriMV (NC_012799).

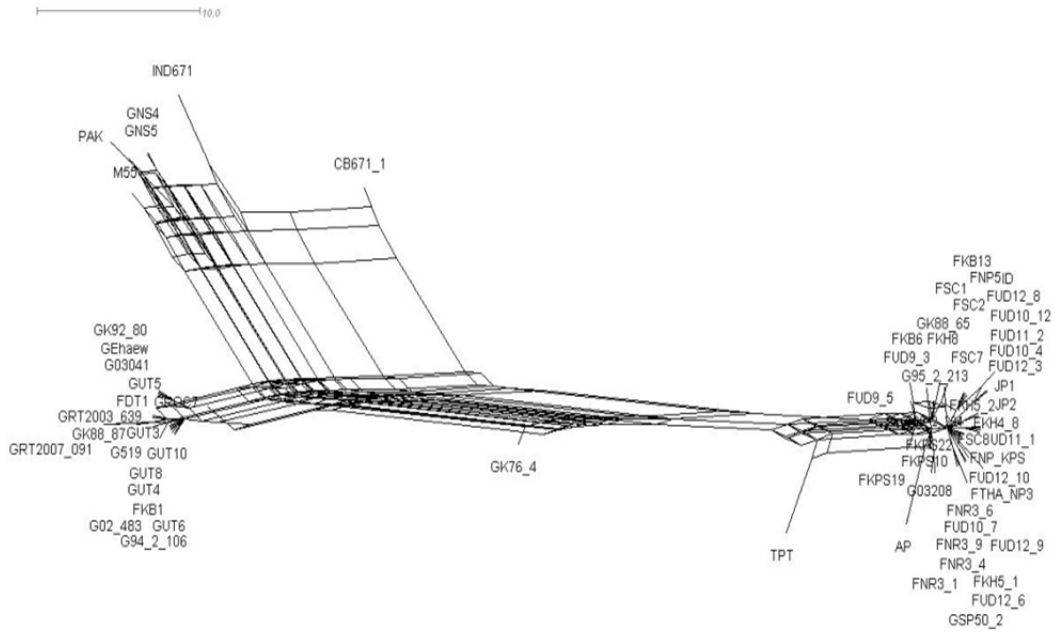

Figure 5. Phylogenetic networks of SCSMV isolates based on analysis of the CP coding sequences

The CP coding sequences from the 67 SCSMV isolates (58 Thai isolates and 9 isolates from other countries) were analyzed using the median network in SplitsTree4. The 9 SCSMV isolates are the followings: ID (JF488066), JP1 (JF488064), JP2 (JF488065), PAK (GQ388116), IND671 (JN941985), AP (Y17738), TPT (GQ246187), M55 (JQ954717), CB671-1 (DQ421788).

4. Discussion

Yellow streak mosaic is a typical symptom of streak mosaic disease in sugarcane caused by *Sugarcane streak mosaic virus* (SCSMV). In addition, the mosaic symptoms in sugarcane are associated with several viruses such as *Sugarcane mild mosaic virus* (SCMMV), *Sugarcane striate mosaic associated virus* (SCSMaV), *Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus* (SrMV). The typical symptoms and host ranges are similar among these viruses (Chen, Chen, & Adams, 2002). In this study, host range tests on plant species in genus *Poaceae* including sorghum cv. UT325B and the commercial corn cv. Tender58 were investigated by mechanical inoculation. The typical symptoms of yellow streak mosaic were also exhibited on the inoculated sorghum and corn at 15 and 5 dpi, respectively (Figure 1c-d). These exhibited streak mosaic symptoms on the inoculated sorghum and corn were similar to those symptoms caused by SCMV that reported as potyvirus causing mosaic diseases in sugarcane, corn and sorghum in Thailand (Gemechu, 2004). These infected plants also confirmed for the presence of SCSMV infection by RT-PCR and the results revealed the presence of SCSMV-CP gene. Thus, we strongly confirmed that yellow streak mosaic symptom in these sugarcane leaves was caused by SCSMV, as previously reported by Chatenet et al. (2005).

The disease surveys from 2010 to 2014 revealed that the incidence of SCSMV was widespread across the major sugarcane growing areas in 5 provinces and the germplasm collection fields. The sugarcane variety groups maintained at the germplasm collection fields such as UT3, UT4, UT5, UT6, UT8 and UT10 were found to be more frequently infected with SCSMV. Than other sugarcane varieties such as K76-4, K88-65 and K88-87. More widespread observation of SCSMV in many sugarcane fields might be facilitated by mechanical transmission such as cutting knives but insect vectors are still uncertain. Our survey suggested that the commercial sugarcane varieties including germplasm collections were widely infected with SCSMV.

In this study, we selected the virus isolate THA-NP3 derived from sugarcane with unknown variety for complete genomic sequencing (Figure 1a). The complete genome sequence of THA-NP3 was successfully assembled from 11 overlapping sequences by using a set of primers designed in this study (Table 1). All cleavage sites of THA-NP3 proteins and the position of their amino acids were almost identical to those of SCSMV isolates PAK,

ID, JP1, JP2 and TPT except for the isolate, IND671 which contained 3131 amino acid residues of the polyprotein. Nucleotide sequence comparison among seven complete genome isolates (THA-NP3, PAK, ID, JP1, JP2, IND671 and TPT) revealed more genetic variations in the P1, HC-Pro and CP genes. These three proteins revealed more genetic variation among SCSMV isolates originally from different sugarcane varieties (Bagyalakshmi et al., 2012; He et al., 2013).

Based on gene sequence variability, Thai SCSMV isolates were divided into two distinct groups (Figure 4), which were the group containing isolates from farmers' fields and another group of isolates from germplasm collection fields. However, some virus isolates obtained from farmers' fields were clustered in the same group with germplasm isolates. These results suggested that the variation of the CP gene occurred among various sugarcane varieties but was not associated with the geographical origin of the isolate.

Network analysis of the 58 Thai SCSMV isolates also confirmed that the recombination events occurred in the CP coding region among the virus isolates from different fields and germplasms (Figure 5). More evidence from the recombination detection by RDP4 revealed two recombinant isolates, GK76-4 and GROC7 (Table 4). A previous study reported that the recombinant isolate from China, CB671-1, was distributed from the parents, W23×IND671, and three recombinant isolates (CB740, CB9217-1 and S-8) were distributed from the same parents, THA-NP3×CB671-1 (He et al., 2013). In this study, we found that two recombinant isolates from China (CB671-1 and M55) were distributed from the parents, FUD10-7×IND671 and GRT2007-091×PAK, respectively. These results suggest that the recombination events occurred in the CP gene among the virus isolates from Thailand, China and India. More results have been reported that the recombination events occurred throughout the HC-Pro gene of SCSMV but not in the P1 gene (Bagyalakshmi et al., 2012; He et al., 2013). Recombination events have been reported as the evolutionary history of single-stranded RNA genome such as *Turnip mosaic virus* (TuMV), in the P1, HC-Pro, P3, CI, 6K2, VPg, NIa-Pro, NIb and CP genes, except for 6K1 gene (Ohshima et al., 2007). The recombinant isolate (GK76-4) obtained in this study was found to have two recombination sites which occurred in the variable N-terminal region and the conserved sequence at the C-terminal regions of the CP gene (Table 4).

In conclusion, our research indicates that the genetic base of hosts, including biological background, was an important factor for viral genetic variation and differentiation in SCSMV populations. This is the first report on the incidence of SCSMV in the commercial sugarcane varieties and the germplasm collections in Thailand. These results will assist sugarcane varieties improvement, screening and breeding for the virus resistant varieties.

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