GENETIC DIVERSITY AND RECOMBINATION EVENT OF BEGOMOVIRUSES INFECTING PUMPKIN IN THAILAND

Wiwat Piyatassee[1,2] and Ratchanee Hongprayoon[1,2,3*]
[1]Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
[2]Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
[3]Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
* Corresponding author: agrrat@ku.ac.th.

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ABSTRACT

Pumpkin-infecting begomoviruses in Thailand were characterized and their genetic diversity was studied based on DNA-A. Seventy-five pumpkin samples associated with begomovirus disease symptoms were collected from 2016 to 2018 and initially screened using six polyclonal antibodies specific to cucurbit-infecting viruses. Sixteen samples were positive in ELISA test and the genomic DNA of fifty-nine negative samples were further amplified using rolling circle amplification for the presence of begomovirus and nine samples showed positive results. Nucleotide sequence analysis showed that six sample isolates, namely KN44, KN47A, KN49A, KN52A, CRI136 and SNK152 were closely related to the squash leaf curl China virus (SLCCNV) isolate [Thailand]. Two samples (KN51A and NMA7) were closely related to SLCCNV isolate [Wax gourd: Nakhon Pathom] and KN7 was closely related to the squash leaf curl Yunnan virus (SLCuYV) isolate [Thailand]. Phylogenetic reconstruction revealed that eight isolates of SLCCNV were clustered in the SLCCNV clade whereas the SLCuYV isolate KN7 was clustered in the SLCuYV clade. Analysis of the recombination events found no recombination evidence in any of the SLCCNV isolates. Although we found positive results of the recombination events in the SLCuYV isolate KN7 from four out of nine statistical methods, it was still not accepted based on the recombination event criteria. Future study should investigate the possible threat generated by recombination and reassortment which may cause greater diversity and symptom severity. Our current findings provide useful information for begomovirus detection, disease surveillance and breeding programs for begomovirus-resistance.

Key words: SLCuYV, SLCCNV, ToLCNDV, cucurbit, DNA-A

INTRODUCTION

Begomovirus is the largest genus of plant viruses, containing 388 species reported by the International Committee on Taxonomy of Viruses (ICTV) (Zerbini et al. 2017). Devastation of plants affected by these viruses has been reported in a wide range of dicotyledonous plants in tropical and subtropical regions, especially in the family Cucurbitaceae (Brown et al. 2012). In 2019, the Office of Agricultural Economics (2019) reported on the export ranking of vegetable seed from Thailand which placed watermelon, cucumber and pumpkin seeds in the third, fifth and sixth ranks, respectively, among the 385.71 t with total revenue of approximately USD 43 million. However, a major problem to the industrial seed production is the serious threat posed by begomovirus infection that has an economical effect on cucurbit plants as the infection can cause substantial yield losses in these crops. These viruses

can be transmitted by white flies with high-rate distribution (Vincent 2013). These are responsible for a considerable amount of damage in cucurbits in Thailand, including three species of begomoviruses, namely *Squash leaf curl China virus* (SLCCNV) (Ito et al. 2008a), *Squash leaf curl Yunnan virus* (SLCuYV) (Junpetch et al. 2017) and *Tomato leaf curl New Delhi virus* (ToLCNDV) (Ito et al. 2008b). Infected plants show a wide range of symptoms including leaf curling, stunting, green-yellow mosaic/mottle, interveinal yellowing, yellow spots and vein swelling in dicotyledonous plants (Inoue-Nagata et al. 2016).

Begomoviruses belong to the family *Geminiviridae*. The virus genome is single-stranded, closed and circular DNA encapsidated in non-enveloped, icosahedral, twinned particles. There are two types of begomoviruses: those with a monopartite genome called DNA-A and those with a bipartite genome referred to as DNA-A and DNA-B. The genomic DNA of the monopartite genome and the DNA-A of the bipartite genome have similar genome organization comprising six open reading frames (ORFs); AV1, AV2, AC1, AC2, AC3 and AC4 which encode coat protein (CP), pre-coat protein, Rep protein, transcriptional transactivator protein (TrAP), replication enhancer protein (REn protein), and pathogenicity function and disease symptom, respectively (Marwal et al. 2014). The main function of DNA-B is its involvement in virus transportation in cell-to-cell movement and virus infection mechanisms in a wide host range. It contains two ORFs (BV1 and BC1) encoding a nuclear shuttle protein (NSP) and a movement protein (MP), respectively (Nawaz-ul-Rehman and Fauquet 2009).

In general, geminiviruses have the capacity to rapidly evolve via mutation and genetic recombination which can significantly contribute to geminivirus evolution in which a high rate of recombination may contribute to the emergence of new geminivirus diseases. The geminiviruses are insect-transmitted viruses that have emerged, over the past 25 years, as one of the most economically important (Brown et al. 2012; Rocha et al. 2013). Genetic diversity of these viruses may be caused by mutation, base substitution, nucleotide homologous exchange (recombination) and genome exchange (reassortment) (Padidam et al. 1999). These events might increase the efficiency of virus distribution in various host plants and of symptom virulence (Idris et al. 2008). This research sought to study the genetic diversity and molecular characterization of begomoviruses infecting pumpkins in Thailand. The information obtained from this research will benefit virus detection, plant breeding programs for disease resistance, disease surveillance and crop management.

MATERIALS AND METHODS

Collection and initial detection of virus diseases. Pumpkin samples showing begomovirus-like symptoms including mosaic, yellow mosaic, yellow leaf curl, leaf curl, leaf stunt and leaf distortion were collected from planting areas in central, northern and northeastern Thailand. All samples were collected following the stratified random technique (Delp et al. 1986), with 10 samples/type of symptom in a 400 m^2 area.

Viruses infecting the pumpkin samples were initially detected using indirect plate-trapped antigen enzyme-linked immunosorbent assay (indirect PTA-ELISA) with six polyclonal antibodies specific to cucurbit-infecting viruses, namely zucchini yellow mosaic virus (ZYMV) (Chantajorn 2017), cucumber green mottle mosaic virus (CGMMV) (Phoomsuk 2008), papaya ring spot virus (PRSV) (Kositsakulchai et al. 2013), watermelon silver mottle virus (WSMoV), tomoto leaf curl New Delhi virus (ToLCNDV) (Chantajorn 2017) and melon yellow spot virus (MYSV) (Parnsa-ard et al. 2009).

Cloning and sequencing of begomovirus genome. Plant tissue of 0.5 g was used for total DNA extraction using a CTAB method modified from Doyle and Doyle (1990). Rolling circle amplification (RCA) of the genomic DNA was performed using *Phi*29 polymerase following the protocol for the Illustra™ TempliPhi 100 Kit (GE Healthcare UK). The full-length of begomovius DNA-A was obtained

using randomized restriction enzyme (RE) digestion with eight types of REs consisting of *Hin*dIII, *Bam*HI, *Sma*I, *Sal*I, *Sac*I, *Pae*I, *Pst*I and *Kpn*I (Fermentas, USA) (using one type of REs/reaction). Polymerase chain reaction (PCR) was conducted using BEGEN-A degenerate primers, with the BEGEN-A-forward primer: 5' SCMGATATYMTCATTTCHACDCCC 3' and the BEGEN-A-reverse primer: 5' TGGWCGCTTCGMCATARTTC 3' (Piyatassee 2020). The amplifications were conducted for 30 cycles of: denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 3 min.

These primer sequences were designed based on 35 begomovirus sequence accessions in the GenBank database and reported throughout Asia consisting of SLCCNV, SLCuYV, ToLCNDV and squash leaf curl Philippines virus (SLCuPV). The full-length DNA-A sequences was cloned into the pQE80-L expression vector/pJET1.2 blunt cloning vector and transformed into *Escherichia coli* strain DH5α using heat shock transformation (Sambrook and Russel, 2001) and the nucleotide sequence was analyzed using the Sanger dideoxy nucleotide sequencing method (Sanger and Coulson 1975) by Apical Scientific (Selangor, Malaysia).

Determination and characterization of begomovirus DNA-A using GenBank database. The complete sequence of DNA-A starting with the conserved nonanucleotide sequence was converted to FASTA format. The identities of the complete sequences were searched for using basic local alignment search tools (BLAST) available as online software in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). The locations and sizes of begomovirus ORFs were predicted using the ORF finder available online (https://www.ncbi.nlm.nih.gov/orffinder/). These complete sequences were then deposited in the GenBank database.

Pairwise alignment and phylogenetic reconstruction analysis. The dataset consisted of begomovirus occurrences in Thailand. The dataset sequences were aligned using the MUSCLE option in the MEGA software version 7.0.26 (Kumar et al. 2016). The MUSCLE format file was performed with pairwise alignment to calculate identities for all pairs of sequences in the dataset using the Sequence Demarcation Tool (SDT) software version 1.2 (http://www.cbio.uct.ac.za/SDT). The complete begomovirus sequences were introduced into the MEGA software for phylogenetic reconstruction and aligned using the ClustalW option. A neighbor-joining tree was generated using the MEGA software using the Kimura 2-parameter model to correct transition and transversion substitution rates for 1,000 bootstrap values (Saitou and Nei 1987).

Begomovirus recombination event. The dataset was compared within begomovirus occurrences in Thailand and Asia using the ClustalW option in the MEGA software and exported to the Recombination Detection Program version 4.16 (RDP4) (Martin et al. 2015). The dataset was analyzed using nine methods consisting of: RDP (Martin and Rybicki 2000), GENECONV (Padidam et al. 1999), Bootscan (Martin et al. 2005), Maxchi (Maynard 1992), Chimera (Posada and Crandall, 2001), SiScan (Gibbs et al. 2000), 3Seq (Boni et al. 2007), LARD (Holmes et al. 1999) and PhylPro (Weiller 1998). Positive results of the recombination event criteria were determined by the number of positive reactions based on three out of five methods or five out of seven methods or seven out of nine methods.

RESULTS AND DISCUSSION

Collection and initial detection of virus disease. Total of seventy-five samples of pumpkin were collected; 19 from the central region, 13 from the northern region and 43 from the northeastern region. The results showed 16 positive samples with specific antibodies of 6 MYSV-infected samples (8%), 3 WSMoV-infected samples (4%), 2 PRSV-infected samples (2.7%), 2 ToLCNDV-infected samples (2.7%), 2 ZYMV-infected samples (2.7%) and 1 CGMMV-infected sample (1.33%).

The remaining 59 negative samples exhibited some begomovirus-infecting symptoms such as yellow mosaic, yellow leaf curl, mosaic and leaf distortion. These samples were further screened for possible begomovirus infection using the RCA technique in the next step.

Cloning and sequencing of begomovirus DNA-A. Out of the 59 negative samples, TempliPhi products were obtained from 9 isolates (KN7, KN44, KN47A, KN49A, KN51A, KN52A, CRI136, NMA7 and SNK152). Two isolates (KN7 and KN44) could be cut giving a piece of DNA at approximately 2,700 bp which is the approximate size of the begomovirus DNA-A. The isolate KN7 was cut with *Hin*dIII at nucleotide position 71 while KN44 was cut by *Bam*HI at nucleotide position 124 within the AV2 gene. There was some difficulty when single digestion was applied as seven out of nine isolates could not be cut to give a single fragment. This might have been due to there being no restriction site specific to the tested enzymes on their genome. Therefore, an attempt was made to obtain the full-length DNA from the virus genome using a PCR method which allowed the full-length DNA-A to be amplified. The seven remaining samples (KN47A, KN49A, KN51A, KN52A, CRI136, NMA7 and SNK152) were amplified using PCR with BEGEN-A degenerate primers, giving a piece of DNA at approximately 2,700 bp from all samples, which were then cloned and sequenced.

Determination and characterization of begomovirus DNA-A sequences. The DNA-A sequences from nine isolates were analyzed by comparison with the GenBank database. The results showed that two distinct begomovirus species were detected in nine isolates of pumpkin, namely SLCCNV isolate [Thailand] DNA-A (Accession no. AB330078.1) (Ito et al. 2008a) between 97.95-99.09% nucleotide identity (eight isolates) and SLCuYV isolate [Thailand] DNA-A (Accession no. KX388157.1) (Junpetch et al. 2017) at 98.91% nucleotide identity (Table 1).

Characterization of the obtained DNA-A (based on the number of ORFs) differentiated them into two groups. The first group (SLCCNV) contained five ORFs (AV1, AV2 on the virion-sense strand and AC1, AC2, AC3 on the complementary-sense strand). The second group (SLCuYV) with only isolate KN7 contained six ORFs (AV1, AV2 on the virion-sense strand and AC1, AC2, AC3, AC4 on the complementary-sense strand). Both groups encompassed a conserved region at TAATATT/AC called a nonanucleotide which is the origin of begomovirus replication (Laufs et al., 1995).

Three begomoviruses reported here are commonly found in cucurbit plants (Charoenvilaisiri et al. 2020; Junpetch et al. 2017; Ito et al. 2008a; Ito et al. 2008b; Nagendran et al. 2017; Xie and Zhou 2003). Among these begomoviruses, SLCCNV is a predominant species in cucurbits and the result agrees with Charoenvilaisiri et al. (2020) in which SLCCNV was found in all pumpkin samples they collected from 2015 to 2017. Begomoviruses were detected in tomatoes, peppers and cucurbits in which the predominant species were different depending on the location and host plant such as tomato yellow leaf curl Thailand virus (TYLCTHV) for tomato, pepper yellow leaf curl Thailand virus (PepYLTHV) for pepper, ToLCNDV for luffa, bitter gourd, cucumber and melon, and SLCCNV for pumpkin and wax gourd.

Pairwise alignment and phylogenetic reconstruction analysis. The pairwise sequence alignment generated by the Sequence Demarcation Tool version 1.2 showed that eight isolates of SLCCNV DNA-A shared greater than 95.6% pairwise identity with the other relevant SLCCNV sequences used in this analysis. The SLCuYV isolate KN7 shared greater than 98.4% pairwise identity with other relevant SLCuYV sequences. All pairwise identities from the nine isolates had cut-off values greater than 94% for demarcation of the begomovirus strain using demarcation criteria standardization (Brown et al. 2015), as shown in Table 2.

Table 1. Analysis of DNA-A sequences obtained from nine pumpkin isolates compared with GenBank database.

Isolate	Province	Symptom	Virus name	Size (bp)	%Nucleotide identity	%Query cover	Acc.no.
KN7	Kanchanaburi	YLC	SLCuYV isolate [Thailand] DNA-A	2,740	98.91	100	MN563794
KN44	Kanchanaburi	YM	SLCCNV isolate [Thailand] DNA-A	2,736	97.95	100	MK978176
KN47A	Kanchanaburi	YM	SLCCNV isolate [Thailand] DNA-A	2,736	98.06	100	MN365019
KN49A	Kanchanaburi	YM	SLCCNV isolate [Thailand] DNA-A	2,736	98.90	100	MN437658
KN51A	Kanchanaburi	LD	SLCCNV isolate [Wax gourd: Nakhon Pathom] DNA-A	2,739	98.10	100	MN437657
KN52A	Kanchanaburi	M	SLCCNV isolate [Thailand] DNA-A	2,736	98.72	100	MN437659
CRI136	Chiang Rai	YM	SLCCNV isolate [Thailand] DNA-A	2,736	99.09	100	MN437662
NMA7	Nakhon Ratchasima	YM	SLCCNV isolate [Wax gourd: Nakhon Pathom] DNA-A	2,739	98.01	100	MN437660
SNK152	Sakon Nakhon	YM, LD	SLCCNV isolate [Thailand] DNA-A	2,736	98.43	100	MN437661

Note: YM = yellow mosaic, YLC = yellow leaf curl, M = mosaic, LD = leaf distortion.

Table 2 Percentage of pairwise alignment of nine isolates within the dataset of begomoviruses found in Thailand using Sequence Demarcation Tool software version 1.2 (Matrix)

	AB368447.1 ToLCNDV	AB330079.1 ToLCNDV	AB368448.1 ToLCNDV	AF102276.1 ToLCNDV	KU992383.1 ToLCNDV	MN437657 KN51*	MN437660 NMA7*	EU543562.1 SLCCNV**	MN437662 CRI136*	AB330078.1 SLCCNV**	MN437658 KN49*	MK978176 KN44*	MN365019 KN47*	MN437659 KN52*	MN437661 SNK152*	MN563794 KN7*	KX388157.1 SLCuYV**	MK040597.1 SLCuYV	AY514632.1 TYLCTHV	AY514630.1 TYLCTHV	DQ871222.1 TYLCTHV	AY514631.1 TYLCTHV
AB368447.1 ToLCNDV		99.7	99.5	97.3	97.1	86.2	86.3	85.9	85.7	85.7	85.9	85.7	85.9	85.8	85.8	70.1	70.1	69.9	72.7	72.5	73.3	73
AB330079.1 ToLCNDV	99.7		99.6	97.5	97.3	86.4	86.4	86.1	85.9	85.9	86.1	85.9	86	86	86	70.1	70.2	70	72.8	72.7	73.4	73.1
AB368448.1 ToLCNDV	99.5	99.6		97.4	97.1	86.2	86.3	85.9	85.8	85.7	86	85.8	85.9	85.9	85.9	70.3	70.4	70.2	72.8	72.6	73.4	73.4
AF102276.1 ToLCNDV	97.3	97.5	97.4		96.1	86.2	86.3	86.1	85.8	85.7	85.9	85.8	85.8	85.8	85.8	70	70.2	70	73.3	73.3	73.3	73.6
KU992383.1 ToLCNDV	97.1	97.3	97.1	96.1		85.7	85.8	85.5	85.7	85.6	85.8	85.6	85.6	85.6	85.6	70.3	70.3	70	72.7	72.6	73.1	73.2
MN437657 KN51*	86.2	86.4	86.2	86.2	85.7		98	98.1	96	96.2	96.2	96.3	96.3	96.2	96.1	71.1	71	71.2	73.1	72.5	73.3	73.2
MN437660 NMA7*	86.3	86.4	86.3	86.3	85.8	98		98.8	95.8	96	95.8	95.6	95.7	95.8	95.6	70.5	70.6	70.7	73.2	72.9	73.3	73.3
EU543562.1 SLCCNV**	85.9	86.1	85.9	86.1	85.5	98.1	98.8		95.7	95.8	95.6	95.6	95.7	95.8	95.6	70.6	70.7	70.6	73.2	72.9	73.4	73.2
MN437662 CRI136*	85.7	85.9	85.8	85.8	85.7	96	95.8	95.7		99.1	98.4	98.1	98.2	98.5	98.2	71.2	71.3	71.1	73	72.7	73.1	73.6
AB330078.1 SLCCNV**	85.7	85.9	85.7	85.7	85.6	96.2	96	95.8	99.1		98.9	98	98.1	98.7	98.4	70.6	70.8	70.7	73.5	72.9	73.4	73.7
MN437658 KN49*	85.9	86.1	86	85.9	85.8	96.2	95.8	95.6	98.4	98.9		98.7	98.7	99.1	98.8	70.9	70.9	70.8	73.3	72.9	73.4	73.7
MK978176 KN44*	85.7	85.9	85.8	85.8	85.6	96.3	95.6	95.6	98.1	98	98.7		99.5	98.9	99	71.3	71.1	71.1	73.1	72.8	73.3	73.6
MN365019 KN47*	85.9	86	85.9	85.8	85.6	96.3	95.7	95.7	98.2	98.1	98.7	99.5		99.4	98.8	71	71.2	71.1	73.4	72.8	73.2	73.6
MN437659 KN52*	85.8	86	85.9	85.8	85.6	96.2	95.8	95.8	98.5	98.7	99.1	98.9	99.4		98.9	71	71.1	71.1	73.3	72.9	73.4	73.7
MN437661 SNK152*	85.8	86	85.9	85.8	85.6	96.1	95.6	95.6	98.2	98.4	98.8	99	98.8	98.9		71.2	71.1	71.1	73.1	72.7	73.3	73.7
MN563794 KN7*	70.1	70.1	70.3	70	70.3	71.1	70.5	70.6	71.2	70.6	70.9	71.3	71	71	71.2		98.9	98.4	83.1	85.5	86.6	85.1
KX388157.1 SLCuYV**	70.1	70.2	70.4	70.2	70.3	71	70.6	70.7	71.3	70.8	70.9	71.1	71.2	71.1	71.1	98.9		99.3	82.9	85.5	86.6	85.3
MK040597.1 SLCuYV	69.9	70	70.2	70	70	71.2	70.7	70.6	71.1	70.7	70.8	71.1	71.1	71.1	71.1	98.4	99.3		83	85.5	86.7	85.3
AY514632.1 TYLCTHV	72.7	72.8	72.8	73.3	72.7	73.1	73.2	73.2	73	73.5	73.3	73.1	73.4	73.3	73.1	83.1	82.9	83		94.7	93.1	91.4
AY514630.1 TYLCTHV	72.5	72.7	72.6	73.3	72.6	72.5	72.9	72.9	72.7	72.9	72.9	72.8	72.8	72.9	72.7	85.5	85.5	85.5	94.7		96.7	95
DQ871222.1 TYLCTHV	73.3	73.4	73.4	73.3	73.1	73.3	73.3	73.4	73.1	73.4	73.4	73.3	73.2	73.4	73.3	86.6	86.6	86.7	93.1	96.7		95.7
AY514631.1 TYLCTHV	73	73.1	73.4	73.6	73.2	73.2	73.3	73.2	73.6	73.7	73.7	73.6	73.6	73.7	73.7	85.1	85.3	85.3	91.4	95	95.7	

Notes: Full length DNA-A of nine isolates of begomoviruses from this study = (*). Reference sequence = (**).

Phylogenetic reconstruction analysis generated using the neighbor-joining method showed that eight isolates of SLCCNV DNA-A were clustered with a close relationship to the SLCCNV isolates from Nakhon Pathom, Thailand: AB330078.1 (Ito et al. 2008a) and Wax gourd-Nakhon Pathom: EU543562.1 (Sawangjit 2009), as shown in Fig. 1. All isolates were clustered within the SLCCNV clade from continental Asia and other relevant countries in subcontinental Asia.

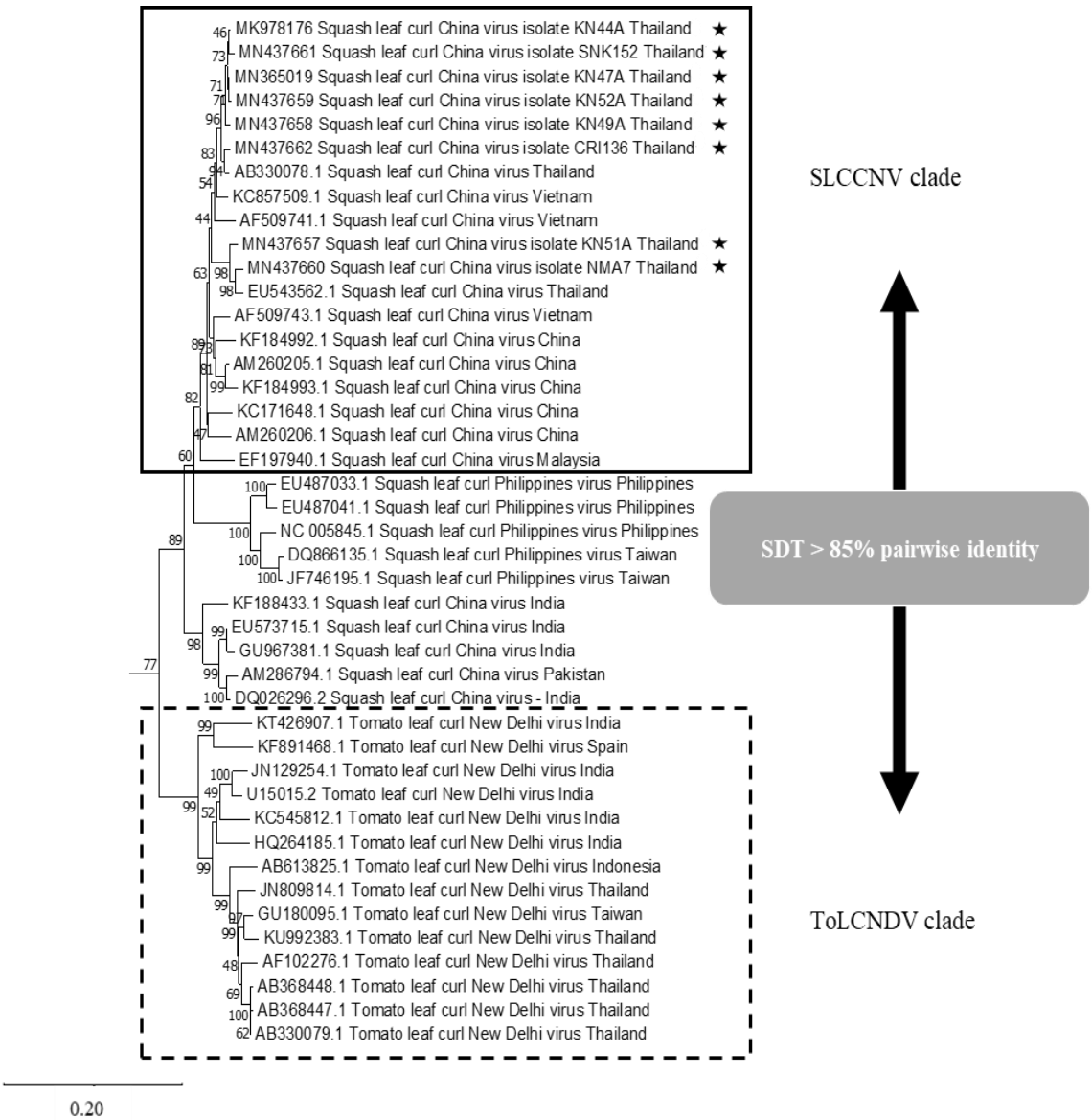

Fig. 1 Evolutionary genetic relation analysis based on complete nucleotide sequences of DNA-A belonging to eight isolates of SLCCNV compared with begomovirus DNA-A sequences in GenBank database. Evolutionary distances were computed using Kimura 2-parameter method (units are number of base substitutions per site). Molecular evolutionary genetic analysis was conducted using MEGA version 7.0.26 software.

Notes: Eight isolates of SLCCNV from this study = star, SLCCNV clade = solid line, ToLCNDV clade = dashed line. Comparison between SLCCNV and ToLCNDV clade by SDT = grey box

The SLCuYV isolate KN7 showed a close relationship with the SLCuYV isolate from Nakhon Pathom that included the SLCuYV isolate Thailand: KX388157.1 (Junpetch et al. 2017) and isolate PK-2018 Nakhon Pathom, Thailand: MK040597.1 (Butnut et al. 2019). KN7 was clustered within the SLCuYV clade from continental Asia which was closely related to the TYLCTHV clade from Thailand (Fig. 2) and this correlated with the first report of SLCuYV in squash (Xie and Zhou 2003). However, the virus shared only 84% pairwise identity with TYLCTHV.

Begomovirus recombination event. The full-length DNA-A of nine isolates were further analyzed using the RDP4 program following the recombination event criteria based on nine statistical methods to detect a recombination event (Martin et al. 2015). The results showed no recombination evidence among the nine isolates based on the recombination event criteria. Although there was no recombination evidence found, KN7 still shared the highest possible recombination event to TYLCTHV as a major parent. This result was correlated with phylogenetic tree analysis using the neighbor-joining method that showed a close relationship between SLCuYV and TYLCTHV. KN7 shared a partial recombination with the SLCCNV isolate CRI136 as a minor parent (Fig. 3A-B) within the AC1 gene in the prediction of the breakpoint at positions 1,846 to 1,944. The unknown fragments as shown in KN7 (Fig. 3A) were unidentified with the recombination event and had a *p*-value from the RDP method of 4×10^{-11} (Fig. 3B).

Due to the recombination event of SLCuYV sharing a major parent with TYLCTHV and minor parent with SLCCNV, it may affect KN7 and create interspecies between TYLCTHV and SLCCNV. As in the previous report, recombination events of three distinct TYLCTHV strains provided evidence of recombination in the AC1 sequences which revealed a common hotspot for recombination in geminiviruses (Sawangjit et al. 2005). The pumpkin infected with KN7 observed in this study displayed yellow leaf curl symptom similar to that infected with TYLCTHV but different from SLCCNV isolates (yellow mosaic disease). Owing to low number of samples, further study is required to investigate the possible threat generated by recombination and reassortment which may cause greater diversity and symptom severity.

CONCLUSIONS

From the pumpkin samples explored, eight isolates of SLCCNV were sequenced and determined for their genetic diversity. All the SLCCNV isolates were not distinct. However, low number of the SLCuYV isolates, reported so far, may not represent a suitably large number for genetic diversity study. The possible threat of SLCuYV adaptation generated by recombination and reassortment may generate virus diversity, an increased host range and symptom severity therefore it should be studied as a priority. These findings provide useful information to support the effective management which involves developing specific methods for early detection and disease management including breeding for begomovirus-resistance.

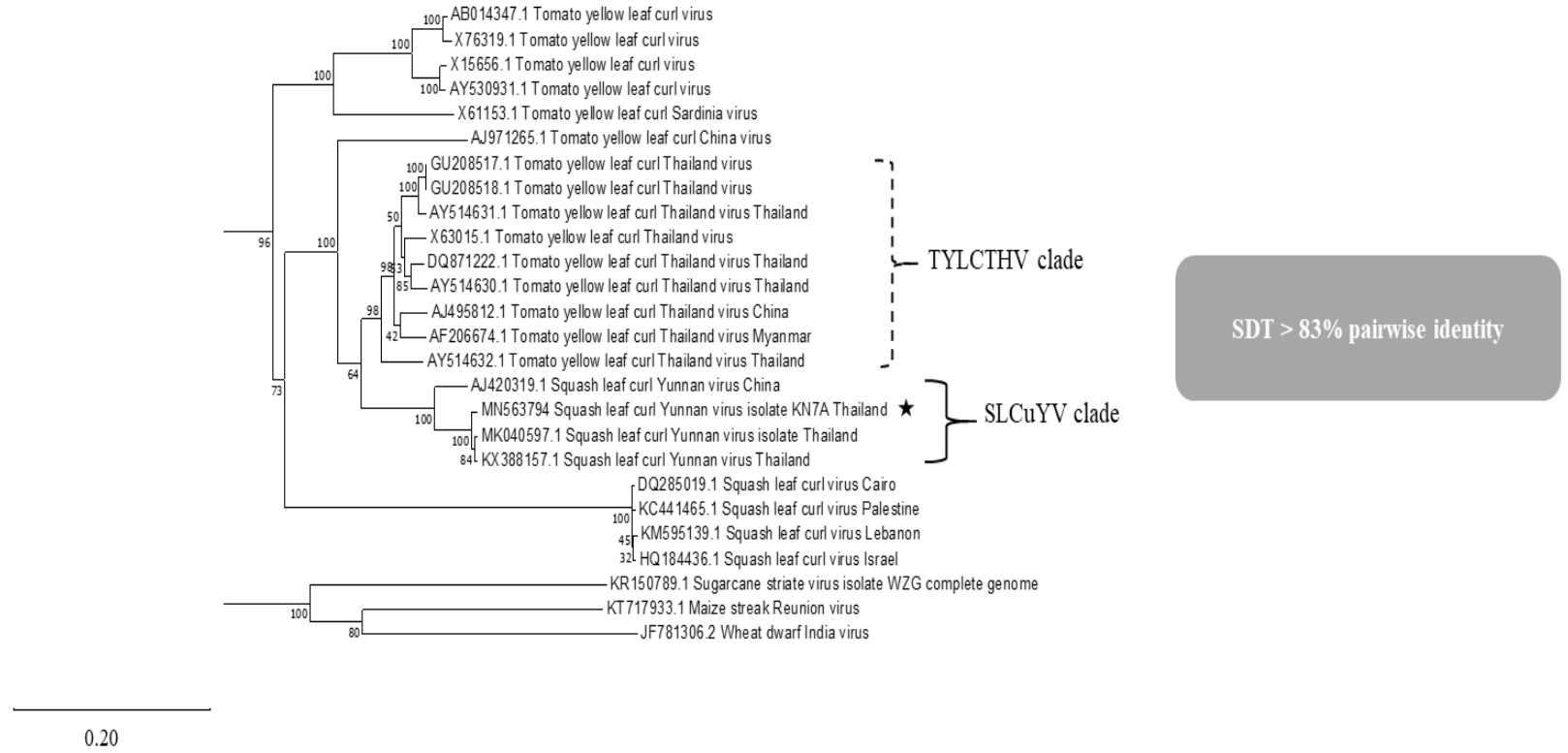

Fig. 2 Evolutionary genetic relation analysis based on complete nucleotide sequences of DNA-A belonging to one isolate of SLCuYV compared with begomovirus DNA-A sequences in GenBank database. Evolutionary distances were computed using Kimura 2-parameter method (units are number of base substitutions per site). Molecular evolutionary genetic analysis conducted using MEGA version 7.0.26 software.

Notes: One isolate of SLCuYV from this study = star, SLCuYV clade = solid bracket, TYLCTHV clade = dashed bracket. Comparison between SLCuYV and TYLCTHV clades by SDT = grey box.

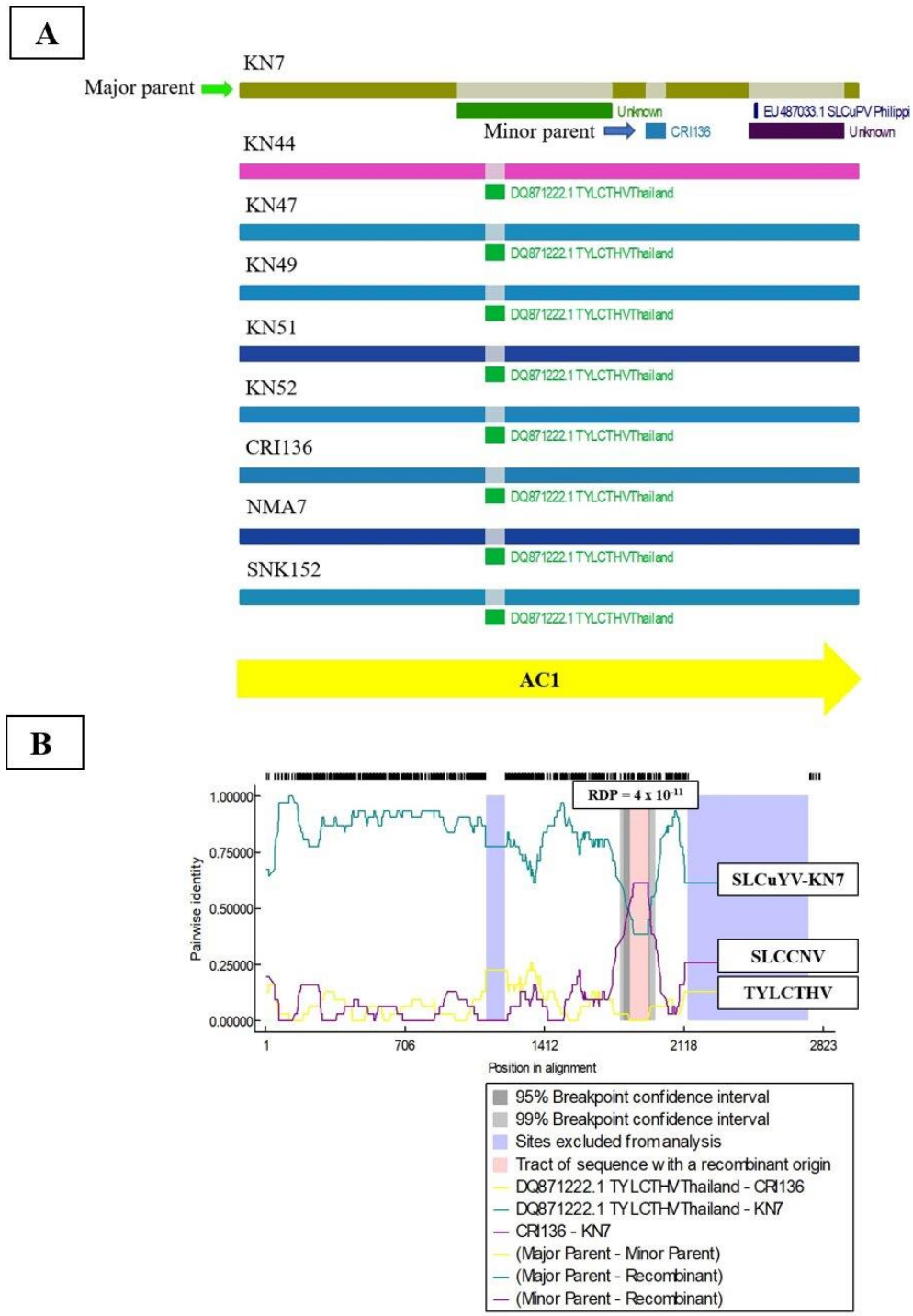

Fig. 3 Analysis of possible recombination in full-length segments of begomovirus isolates from Thailand and Asia using RDP method. (A) Genome map indicates possible recombination events detected in AC1 gene (yellow arrow). Major parent = TYLCTHV (green arrow) and minor parent = SLCCNV isolate CRI136 (blue arrow). (B) Graphic presentation of prediction breakpoint at positions 1,846 to 1,944 as detected in SLCuYV isolate KN7.

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