



Molecular Characterization of the Full-length Bipartite Begomovirus Causing Pepper Yellow Leaf Curl Disease, a Common Cause of Crop Damage in Northeast and Central Thailand

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ABSTRACT

Introduction: The incidence of the yellow leaf curl disease of pepper is an important and serious threat for the production of pepper in Thailand. The disease is caused by whitefly-transmitted begomoviruses which can lead to severe economic losses of the crop.

Objective: To determine and describe the complete sequences of begomovirus important species isolated from field infected pepper plants

Methods: In this study, six diseased samples of the *Capsicum annum* plants (showing yellow mosaic and leaf curling symptom) were collected from outbreak areas during a survey in 2016 – 2019 and used to extract total DNA. The full-length viral genomes were amplified by rolling circle amplification technology (RCA), cloned and sequenced bi-directionally. The generated sequences were then assembled and characterized including their phylogenetic relationships with other selected begomoviruses.

Results: The sequences of the DNA-A genome from all six isolates showed the highest identity (97%) with the isolates of *Pepper yellow leaf curl Thailand virus* (PepYLCTHV). For the DNA-B component, it showed the closest cluster with those of PepYLCTHV (90% similarity).

Conclusion: Based on the literature review and current evidence, this is the report confirming the presence of the PepYLCTHV bipartite begomovirus from Thailand.

Keywords: *Capsicum*; *Begomovirus*; *RCA*; *Pepper yellow leaf curl*

Introduction

Chili peppers belong to the genus *Capsicum* of the Solanaceae family native in Central and South America presently dominating the world's hot spice trade [1-2]. Flesh of *Capsicum* can be used in the pharmaceutical/medicinal aspect all over the world. *Capsicum* has been used orally for upset stomach, toothache, poor circulation, fever, hyperlipidemia, and heart disease prevention [3]. Besides, its use has also been reported for relief of muscle spasms topically to treat pain associated with osteoarthritis, shingles, rheumatoid arthritis, post-herpetic neuralgia, trigeminal

neuralgia, diabetic neuropathy, fibromyalgia, and back pain [4-5]. *Capsicum* is rich in vitamin A, vitamin B complex, vitamin E and vitamin C. These vitamins and various antioxidants are active and set against the free radicals [6]. Pungency (hotness) of the *Capsicum* is due to the presence of capsaicin which is an active component of peppers producing an intense burning sensation when it comes into contact with the skin, eyes, or mucous membranes and which gives peppers their burning taste [7]. Capsaicin is the main constituent of capsaicinoid in *Capsicum*, followed by dihydrocapsaicin, nordihydrocapsaicin,

homodihydrocapsaicin, homocapsaicin and minerals levels which are beneficial to health [8-9]. *Capsicum* is susceptible to various pests and diseases including begomoviruses resulting in yield loss and becomes the most significant problem in the main production area [10]. Reduction of planting area has been observed every year due to cost of management and disease problems [11]. The begomovirus, a member of the family Geminiviridae, has been frequently reported causing disease in many vegetable crops, ornamental plant and weed species worldwide in terms of incidence and yield losses [12]. Depending on the genera, the viral genome comprises either a single DNA-A genome (monopartite) or containing DNA-A and DNA-B (bipartite) components [13]. The symptom of begomoviruses-infected plants is yellow mosaic, leaf curling, puckering and malformation. In severe scenarios, the yield loss of the infected plants could be up to 100 percent [14-16]. In Thailand, the highest incidence of disease caused by begomovirus in pepper was observed in the central part followed by the north and the northeastern range up to 90-100 percent [17]. The aim of this research was to investigate the molecular characterization and phylogenetic relationships of begomovirus infecting peppers in major production fields of the northeastern and central regions of Thailand. The updated information regarding the diversity of begomoviruses obtained from this study is expected to be useful for planning appropriate strategies in viral disease control.

Methodology

Plant materials and DNA extraction

Six infected pepper leaf samples were collected from fields in Ratchaburi, Kanchanaburi and Khon Kaen. Total genomic DNA was prepared from the diseased leaf samples using a procedure described by Dellaporta [18].

RCA and cloning of full-length *PepYLCV* segmented DNA-A and DNA-B

Full-length begomovirus genomes (DNA-A and DNA-B) were amplified from the extracted DNA using rolling circle amplification (RCA) based TempliPhi™ DNA amplification kit (GE Healthcare, USA) as instructed by the manufacturer's protocol. The reaction was undertaken by transferring 0.5 µl (10-20 ng) of template DNA to a 5 µl sample buffer. The amplification reaction used was denaturation at 95°C of template DNA for 3 minutes, and then cooled down to room temperature or 4°C. To this mixture, 5 µl of reaction buffer and 0.2 µl of enzyme mix were added, and the reaction was run for 4-18 hours at 30°C. The reaction was then terminated by incubating the mixture at 65°C for 10 minutes, and then cooled down to 4°C. The

product of the TempliPhi reaction was monomerized by restriction digestion with *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, *Sal*I and *Xba*I (Thermo Fisher Scientific, USA) for DNA-A and DNA-B. Digested products were resolved on 1% agarose gel electrophoresis and the bands with the size of ~2.8 kbp were purified using GeneJET gel extraction kit (Thermo Fisher Scientific, USA). The components were cloned into the respective site of the pUC19 vector to produce the recombinant plasmids.

Sequencing and bioinformatic analysis

Monomeric full-length clones were purified using GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, USA) using the manufacturer's protocol. The DNA samples were then sent to the 1st BASE Laboratories (Malaysia) for DNA sequencing. The recombinant clone was bidirectionally sequenced using M13 primers (M13 Forward-GTTTTCCTCAGTCACGAC; M13 Reverse-CAGGAAACAGCTATGAC). Primer walking technique using the newly generated sequences (AV 5'-CCATCTGCAGGCCACAT-3'; AC 5'-AATACTGCAGGCTT-3' for segment DNA-A and 10-1F 5'-GGGACCAATTGGCAATTGCC-3'; 10-1R 5'-ACGGTTGCCAATCCTCCTCCC-3' for DNA-B) was also carried out to complete the gap of the begomovirus genomes. The DNA sequence data of each isolate were manually assembled and edited using the SeqMan program (Lasergene, USA). Multiple sequence alignments were then aligned with these closely related begomovirus DNA sequences and phylogenetic trees were generated by MEGA software version X with 1,000 bootstrap replications [19].

Results

Genome amplification and cloning of circular DNAs

The complete genome of *PepYLCTHV* isolate was amplified from the infected pepper leaves samples as circular DNA. Restriction of RCA amplicons with *Pst*I (DNA-A) and *Bam*HI (DNA-B) generated a DNA fragment of ~2.8 kb product by cleaving the sequence at one position. These restricted fragments were then cloned into PUC-19 vectors. In contrast, there were two restricted DNA fragments when using *Eco*RI, *Hind*III, *Sal*I and *Xba*I indicating multi-recognition sites (data not shown). Seven full-length amplicons were then cloned and sequenced. Based on the Blast analysis the DNA sequences showed highest similarity with *PepYLCTHV* segment DNA-A (97% similarity) and with *PepYLCTHV* segment DNA-B (90% similarity). The DNA sequences obtained were designated as *PepYLCTHV*-KKN601, *PepYLCTHV*-KKN603, *PepYLCTHV*-KKN604, *PepYLCTHV*-KRI155, *PepYLCTHV*-KRI157, *PepYLCTHV*-RBI-24, DNA-A isolates and *PepYLCTHV*-KKN601 DNA-B isolate (table 1).

Table 1 List of begomovirus complete sequences used for phylogenetic analysis

Begomovirus species (isolate)	Host	Location	Abbreviation	Type	Reference length	GenBank Accession no.
<i>Eggplant golden mosaic virus</i>	<i>Solanum melongena</i>	Thailand	EGMV	DNA-A	2742	KU569586
<i>Pepper golden mosaic virus</i>	<i>Cucumis sativus</i>	Mexico	PepGMV	DNA-B	2597	MN013411
<i>Pepper huasteco yellow vein virus</i>	<i>Capsicum annuum</i>	Mexico	PHYVV	DNA-B	2582	LN848927
<i>Pepper huasteco yellow vein virus</i>	<i>Capsicum annuum</i>	Mexico	PHYVV	DNA-B	2596	MG582069
<i>Pepper leaf curl virus</i>	<i>Capsicum annuum</i>	Malaysia: Klang	PepLCV	DNA-A	2739	AF414287
<i>Pepper leaf curl virus</i>	<i>Capsicum annuum</i>	Thailand	PepLCV	DNA-A	2744	NC000882
<i>Pepper yellow leaf curl Indonesia virus</i>	<i>Pseuderanthemum reticulatum</i>	Indonesia	PepYLCIV	DNA-B	2739	MN094865
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-A	2742	KT322143
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-A	2742	KT322146
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-B	2732	MK946435
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-A	2742	MK946436
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-A	2742	NC028989
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Bemisia tabaci</i>	Thailand: Suphanburi	PepYLCThV	DNA-B	2732	NC040186
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-B	2731	KX885224
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCThV	DNA-B	2730	KX885225
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Bemisia tabaci</i>	Thailand: Suphanburi	PepYLCThV	DNA-A	2742	KX943290
<i>Tomato yellow leaf curl Kanchanaburi virus</i>	<i>Solanum lycopersicum</i>	Viet Nam	TYLCKaV	DNA-B	2740	DQ169055
<i>Tomato yellow leaf curl Kanchanaburi virus</i>	<i>Solanum lycopersicum</i>	Thailand: Kanchanaburi	TYLCKaV	DNA-B	2752	NC005811
<i>Tomato yellow leaf curl Thailand virus</i>	<i>Solanum lycopersicum</i>	Thailand	TYLCThV	DNA-B	2737	NC000870
Begomovirus identified in This study						
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen- Thailand	PepYLCThV	DNA-A	2733	MW715819
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen - Thailand	PepYLCThV	DNA-B	2745	MW715820
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen- Thailand	PepYLCThV	DNA-A	2733	MW715821
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen - Thailand	PepYLCThV	DNA-A	2733	MW715822
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Ratchaburi- Thailand	PepYLCThV	DNA-A	2742	MW715823
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Kanchanaburi- Thailand	PepYLCThV	DNA-A	2742	MW715824
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Kanchanaburi- Thailand	PepYLCThV	DNA-A	2742	MW715825
Out group						
<i>Chili vinal mottle virus</i>						NC005778

Diversity and phylogenetic analysis

The generated nucleotide sequences were used for genetic diversity and phylogenetic relationship analysis with the sequences of selected begomoviruses by using MEGA X software. As shown in figure 1, a phylogenetic analysis of DNA-A was constructed, and it revealed that all DNA sequences obtained from this study were grouped with PepYLCTHV two sub-clades comprising the isolates identified in this study. The isolates of DNA-A from PepYLCTHV-KKN601, PepYLCTHV-KKN603 and PepYLCTHV-KKN604 grouped in a well-supported clade (bootstrap 100%). The other 3 isolates PepYLCTHV- RBI24, PepYLCTHV- KRI155 and PepYLCTHV- KRI157 were placed in the different clade containing EGMV isolate TH12WE12 (KU569586), PepYLCTHV isolate BRM103, WF-SPN-Pep2015 and SPN-PG1 (MK946436, KX943290 and KT322143). Within the DNA-B clade, the isolates PepYLCTHV-KKN601 identified in this study grouped with a clade comprising PepYLCTHV isolate BRM103 (MK946435), KON-KG5B (KX885224), WF-SPN-Pep2015 (NC040186) and TMK-KR8B (KX885225) in a well-supported clade (bootstrap 100%) and distance from *Pepper yellow leaf curl Indonesia virus* (PepYLCIV, MN094865) reported from Indonesia (figure 2).

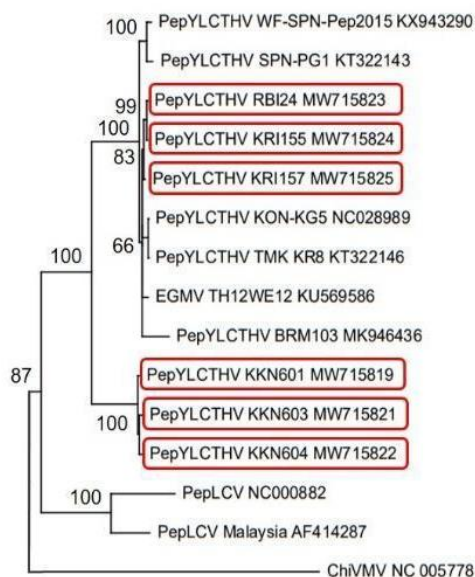


Figure 1 Phylogenetic trees constructed from aligned complete nucleotide sequences of PepYLCTHV DNA-A obtained from this study (red boxes) with other begomoviruses using the neighbor-joining algorithm. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values are numbered along branches

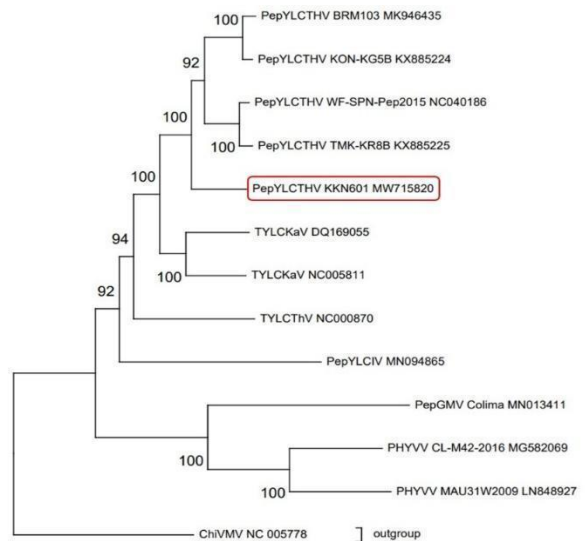


Figure 2 Phylogenetic trees constructed from aligned complete nucleotide sequences of PepYLCTHV DNA-A obtained from this study (red boxes) with other begomoviruses using the neighbor-joining algorithm. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values are numbered along branches

Discussion

Begomoviruses can infect a wide range of cultivated crops and huge economic losses worldwide [20-21]. The epidemiological surveys of viral disease in pepper major production areas in Thailand from recent studies demonstrate that the evidence of begomovirus on various varieties of pepper has gradually been increased consistently throughout the country after the first report in 2018 [17,22]. In this study, full genome sequences of DNA-A and DNA-B were amplified from field infected pepper samples. Based on sequence analyses, begomovirus isolates obtained from this study showed genome-wide pairwise identities above the 91% taxonomic threshold for inclusion in the same species [23]. This information concluded that the virus identified from this study is PepYLCTHV bipartite begomovirus and all isolates were suggested to originate from Thailand.

Conclusion

This information concluded that the virus identified from this study is PepYLCTHV bipartite begomovirus and all isolates were suggested to originate from Thailand. The generated information was obtained from full-length genome amplification of DNA-A and DNA-B from field surveyed samples. This information regarding the knowledge of the distribution and diversity of interesting relationships between the genus Begomovirus in different locations is very crucial for developing and deploying suitable resistant cultivars for pepper crop and strategies for the development of

effective integrated disease management against viruses.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Author contributions

AL carried out all the experiments, interpreted data, and prepared the first draft. SN suggested the revised manuscript, SM assisted with the DNA sequence analysis, EC reviewed and edited the manuscript.

References

- [1] Gupta D. An overview of capsicum. International Journal of Pharmaceutical and Biological Science Archive. 2016. 3(4).
- [2] Bhutia KL, Khanna VK, Meetei TNG, Bhutia ND. Effects of climate change on growth and development of chilli. Agrotechnology. 2018. 7(180).
- [3] Qin Y, Ran L, Wang J, Yu L, Lang HD, Wang X L, et al. Capsaicin Supplementation Improved Risk Factors of Coronary Heart Disease in Individuals with Low HDL-C Levels. Nutrients. 2017. 9(9), 1037.
- [4] Mason L, Moore RA, Derry S, Edwards JE, McQuay HJ. Systematic review of topical capsaicin for the treatment of chronic pain. BMJ. 2004 Apr; 24;328 (7446):991.
- [5] Gagnier JJ, van Tulder MW, Berman BM, Bombardier C. Herbal medicine for low back pain. Cochrane Database of Systematic Reviews 2007. 32: 82-92.
- [6] Tzubentula J, Kaushal, KJ. Entrepreneurial behaviour of king chilli growers in Peren district, Nagaland, India. International Journal of Current Microbiology and Applied Sciences. 2020. 9(3): 1783-1791.
- [7] Arimboor R, Natarajan RB, Menon KR, Chandrasekhar LP, Moorkoth V. Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: Analysis and stability-a review. Journal of Food Science and Technology. 2015. 52(3): 1258-1271.
- [8] Mori A, Lehmann S, O'Kelly J. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53mutant prostate cancer cells. Cancer Research. 2006. 66(6): 3222-3229.
- [9] McCarty MF, DiNicolantonio JJ, O'Keefe JH. Capsaicin may have important potential for promoting vascular and metabolic health. Open Heart. 2015. 2(1).
- [10] Charoenvilaisiri S, Seepiban C, Phironrit N, Phuangrat B, Yoohat K, Deeto R, et al. Occurrence and distribution of begomoviruses infecting tomatoes, peppers and cucurbits in Thailand. Crop Protection. 2020. 127, 104948.
- [11] Department of Agriculture Extension (DOAE). The production of hot pepper in Thailand. 2020. 37-38.
- [12] Varma A, Mandal B, Singh, MK. Global Emergence and Spread of Whitefly (*Bemisia tabaci*) Transmitted Geminiviruses. In W. Thompson (Ed), The Whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants. Springer. 2011.
- [13] Gilbertson RL, Batuman O, Webster CG, Adkins S. Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. The Annual Review of Virology. 2015. 2, 67-93.
- [14] Khan MS, Raj SK, Singh R. First report of Tomato leaf curl New Delhi virus infecting chilli in India. Plant Pathology. 2006. 55(2): 289-289.
- [15] Kumar S, Kumar R, Kumar S, Singh AK, Singh M, Rai AB, et al. Incidence of leaf curl disease on Capsicum germplasm under field conditions. Indian Journal of Agricultural Science. 2011. 8, 187-189.
- [16] Senanayake DMJB, Mandal B, Lodha S, Varma A. First report of Chilli leaf curl virus affecting chilli in India. Plant Pathology. 2007. 56(343).
- [17] Laprom A, Nilthong S, Chukeatirote E. (2019). Incidence of viruses infecting pepper in Thailand. Bio Molecular Concepts. 2019. 10(1): 184-193.
- [18] Dellaporta SL, Wood J, Hicks JB. A plant DNA miniprep: version II. Plant Molecular Biology Reporter. 1983. 1: 19-21.
- [19] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 2013. 30: 2725-2729.
- [20] Briddon RW, Markham PG. Universal primers for the PCR amplification of dicot-infecting geminiviruses. Molecular Biotechnology. 1994. 1(2): 202-205.
- [21] Gutierrez C. DNA replication and cell cycle in plants: Learning from geminiviruses. EMBO Journal. 2000. 19: 792-799.

- [22] Chiemsombat P, Yule S, Srikamphung B. Begomoviruses associated to pepper yellow leaf curl disease in Thailand. *Journal of Agricultural Research*. 2018. 3(7): 1-11.
- [23] Brown JK, Zerbini FM, Navas-Castillo J. Revision of begomovirus taxonomy based on pairwise sequence comparisons. *Archives of Virology*. 2015. 160(6): 1593-1619.